THE 14TH INTERNATIONAL SYMPOSIUM ON
FISH NUTRITION & FEEDING

Program & Abstracts

May 31–June 4, 2010, Qingdao, China
PREFACE

The 14th International Symposium on Fish Nutrition and Feeding continues a series of scientific meetings that began 25 years ago. At that time, fish nutrition was an active area of research in Europe, North America and Japan but there was little exchange of information among research groups from these areas other than scientific exchanges. The Symposium was the first opportunity for researchers from different countries to meet together for a conference dedicated solely to fish nutrition. The 1984 conference was attended by about 125 people. Since then, the conference has grown to its current size of about 600 scientists and is the most important meeting for aquatic nutritionists to attend.

The ISFNF is managed by an International Scientific Committee that oversees the scientific program and provides continuity from year to year, and the Local Organizing Committee that is comprised of members from the host country. This committee is responsible for choosing the venue and for all details associated with hosting the meeting. Both committees chose the theme of the 14th ISFNF meeting which is “Quality, Safety and Sustainability.”

Aquaculture is expected to achieve a milestone this year by providing half of all fisheries products that are consumed around the world. This rapid increase in aquaculture production could not have been realized without the efforts of fish nutrition researchers. Looking forward 10 years, aquaculture production must continue to increase to supply the increased demand for fisheries products that is expected to accompany population growth, increasing incomes in developing countries and shifts
in eating habits in developed countries. This is a huge challenge for producers but also a huge challenge for fish nutrition researchers given the finite global supplies of fish meal and fish oil that up to now have been the mainstay of fish feed formulations for many important species. As fish feed production increases in step with aquaculture production, feed formulations must utilize higher levels of alternate proteins and lipid sources to become sustainable. As feed formulations shift away from fish meal and oil, new information will be needed on use of alternative ingredients and on the requirements of fish species for essential dietary nutrients. Quality and safety of farmed products will also require more scientific scrutiny.

The International Scientific and Local Organizing Committee selected session topics in scientific program of the 14th ISFNF to address these important issues. The eleven session topics include those addressing sustainability including Alternative Feed Protein and Lipid Sources, Feed Processing and Availability and Nutrition and the Environment. The other themes of the meeting are addressed in three sessions, Nutrition and Quality, Nutrition and Health and Feed Additives. The remaining topics were chosen because of their importance to understanding the nutritional requirements of fish and shellfish and how to formulate feeds to produce fast growing, healthy fish for the consumer in an environmentally-sustainable fashion.

On behalf of the Local Organizing Committee and the International Scientific Committee, we welcome the participants to Qingdao, China, for the 14th International Symposium on Fish Nutrition and Feeding.

Dr. Ronald W. Hardy
Chairman, International Scientific Committee
欢迎

Welcome to Qingdao, China!

On behalf of the Local Organizing Committee, I welcome you to the 14th International Symposium on Fish Nutrition & Feeding and Qingdao, China. This symposium would be successful due to the great assistances of you. It has gathered more than 600 participants from more than 30 countries from the Africa, Americas, Asia, Europe and Oceania.

Qingdao is the host city for the water sports of 2008 Olympic Games. It looks like a shining pearl enshring in the east coast of China with its beautiful scenery and European style architecture. It is well known by natural scene as well as many world-famous products, such as Tsingtao Beer, Haier. June is a golden season in Qingdao and the climate is comfortable. We hope that you will not only benefit from the scientific programme that is the focus of the symposium but also enjoy the social events, tourist attractions and the beauty of Qingdao.

We are pleased to see you and hope that you will take away with your happy memories of the symposium and your stay here. Finally, remember that Qingdao is also famous for its Tsingtao Beer and some fine wine, please enjoy them, Cheers!

Dr. Kangsen Mai
Academician, Chinese Academy of Engineering
Chairman, Organizing Committee
14th International Symposium on Fish Nutrition & Feeding
International Scientific Committee

Dr. Hardy R. (Chair) (University of Idaho, USA)
Dr. Lall S.P. (National Research Council, Canada)
Dr. Izquierdo M.S. (GIA Grupo de Investigacion en Acuicultura, Spain)
Dr. Shiau S.Y. (National Taiwan Ocean University, Keelung)
Dr. Lie Ø. (National Institute of Nutrition and Seafood Research, Norway)
Dr. Corraze G. (UMR 1067- INRA, Nutrition, Aquaculture & Genomics, France)
Dr. Koshio S. (Kagoshima University, Japan)
Dr. Glencross B. (CSIRO Marine & Atmospheric Research, Australia)
Dr. Cruz-Suárez L.E. (Universidad Autonoma de Nuevo Leon, México)
Dr. Mai K. (Ocean University of China, Qingdao)

Local organizing committee

Dr. Mai K. (Chair) (Ocean University of China)
Dr. Xie S. (Institute of Hydrobiology, Chinese Academy of Sciences)
Dr. Chen L. (East China Normal University)
Dr. Ye Y. (Soochow University)
Dr. Tan B. (Guangdong Ocean University)
Dr. Dong S. (Ocean University of China)
Dr. Chen R. (Ocean University of China)
Dr. Luo Y. (Ocean University of China)

Secretariat

Dr. Zhang W. (Ocean University of China)
Dr. Wang X. (Ocean University of China)
ACKNOWLEDGEMENTS

The Local Organizing Committee of the 14th International Symposium on Fish Nutrition & Feeding would like to gratefully acknowledges the following organizations for sponsorship of the symposium:

**Gold Sponsors:**

Ocean University of China  
Qingdao Municipal Government  
National Renderers Association, Inc, USA  
Guangdong Haid Group Co., Ltd  
The 111 project of China (B08049)

**Silver Sponsors:**

National Natural Science Foundation of China  
EWOS Innovation AS  
Diamond V Mills, Inc  
DSM Nutritional Products (China)  
Beijing Enhalor Bio-Tech Co., Ltd  
Guangdong Yuehai Feed Group Co., Ltd

**Cooperate Sponsors:**

Qingdao Master Biotechnology Co., Ltd  
Novus International, Inc.  
K.C.Wong Education Foundation  
Tongwei Co., Ltd  
Wuxi Hanove Animal Health Products Co., Ltd  
Qingdao Great Seven Bio-Tech Co., Ltd  
Weihai Gold Feed Co., Ltd  
Feed Industry Association of Qingdao

**Media Supports:**

Feed Industry of China  
Aquatic Living Resources
14th International Symposium on Fish Nutrition & Feeding

Programme & Abstracts

Table of Contents

| Programme of Events       | 7 |
| Schedule of Events        | 8 |
| Oral Presentation Abstracts | 46 |
| Poster Presentation Abstracts | 152 |
| Author Index for Presentations | 512 |

Contact information of Secretariat

<table>
<thead>
<tr>
<th>Departments</th>
<th>Phone No. in Hotel</th>
<th>Name</th>
<th>Mobile No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secretariat (Room 201)</td>
<td>6201</td>
<td>Zhang Wenbing</td>
<td>13625320236</td>
</tr>
<tr>
<td>Hotel Count (Huanghai)</td>
<td>61125, 61126</td>
<td>Wang Xiaojie</td>
<td>13325003167</td>
</tr>
<tr>
<td>Ticket Service Center</td>
<td>6627</td>
<td>Ma Hongming</td>
<td>15615121025</td>
</tr>
<tr>
<td>Dispensary</td>
<td>6229</td>
<td>Zhang Yanjiao</td>
<td>13045029229</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Li Yan</td>
<td>15064227815</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cheng Zhenyan</td>
<td>13589356706</td>
</tr>
<tr>
<td>Date Time</td>
<td>May 31st (Monday)</td>
<td>Date Time</td>
<td>June 1st (Tuesday)</td>
</tr>
<tr>
<td>------------</td>
<td>--------------------</td>
<td>-----------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>08:00-12:00</td>
<td>Registration</td>
<td>08:30-09:00</td>
<td>Welcome and opening address</td>
</tr>
<tr>
<td>12:00-13:30</td>
<td>Lunch Registration</td>
<td>09:00-09:30</td>
<td>Invited presentation (Kangsen Mai)</td>
</tr>
<tr>
<td>13:30-18:00</td>
<td>Registration &amp; poster installation</td>
<td>09:30-10:00</td>
<td>Taking photo</td>
</tr>
<tr>
<td>18:00-21:00</td>
<td>Welcome cocktails &amp; finger supper</td>
<td>Registration &amp; poster installation</td>
<td>10:00-12:00</td>
</tr>
<tr>
<td>21:00-24:00</td>
<td>Registration &amp; poster installation</td>
<td>12:00-13:30</td>
<td>Lunch break</td>
</tr>
<tr>
<td>13:30-15:30</td>
<td>Alternative feed protein and lipid sources</td>
<td>13:30-14:30</td>
<td>Broodstock and larval nutrition</td>
</tr>
<tr>
<td>15:30-15:45</td>
<td>Coffee break</td>
<td>14:30-15:00</td>
<td>Invited presentation (D Allen Davis)</td>
</tr>
<tr>
<td>15:45-17:00</td>
<td>Physiological metabolism</td>
<td>15:00-15:30</td>
<td>Crustacean nutrition/metabolism</td>
</tr>
<tr>
<td>17:00-18:00</td>
<td>Poster session</td>
<td>15:30-15:45</td>
<td>Coffee break</td>
</tr>
<tr>
<td>18:00-19:30</td>
<td>Buffet dinner</td>
<td>15:45-17:15</td>
<td>Crustacean nutrition/metabolism</td>
</tr>
</tbody>
</table>

**14th International Symposium on Fish Nutrition & Feeding (Programme of Events)**
14th International Symposium on Fish Nutrition & Feeding

Schedule of Events

Venue: The Huanghai Hotel

May 31, 2010

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>08:00-12:00</td>
<td>Registration (Beside the Reception)</td>
</tr>
<tr>
<td>12:00-13:30</td>
<td>Lunch (2nd Floor, Zhonghua Hall) Registration (Beside the Reception)</td>
</tr>
<tr>
<td>13:30-18:00</td>
<td>Registration (Beside the Reception) Poster installation (The Conference Centre, 3rd Floor, No. 1 and No. 2 Meeting Room)</td>
</tr>
<tr>
<td>18:00-21:00</td>
<td>Welcome cocktails &amp; finger supper (2nd Floor, Zhonghua Hall) Registration (Beside the Reception) Poster installation (The Conference Centre, 3rd Floor, No. 1 and No. 2 Meeting Room)</td>
</tr>
<tr>
<td>21:00-24:00</td>
<td>Registration (Beside the Reception) Poster installation (The Conference Centre, 3rd Floor, No. 1 and No. 2 Meeting Room)</td>
</tr>
</tbody>
</table>

ORAL PRESENTATION

June 1, 2010

<table>
<thead>
<tr>
<th>Time</th>
<th>Session: Alternative feed protein and lipid sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>09:00-09:30</td>
<td>Invited Presentation</td>
</tr>
<tr>
<td>09:30-10:00</td>
<td>Taking photo/Coffee break</td>
</tr>
<tr>
<td>10:00-10:15</td>
<td>M Øverland, L T Mydland, O H Romarheim, T Landsverk, A Skrede</td>
</tr>
<tr>
<td>10:15-10:30</td>
<td>M Sprague, J Walton, J Dick, F. Strachan, D R Tocher, JG Bell</td>
</tr>
<tr>
<td>10:30-10:45</td>
<td>Bundit J, Taweesin A.</td>
</tr>
</tbody>
</table>

Session: Alternative feed protein and lipid sources
Chairpersons: Drs. Ron Hardy & Kangsen Mai

Invited Presentation
Kangsen Mai
Achievements and prospects of aquaculture and aquafeed industry in China

Taking photo/Coffee break

M Øverland, L T Mydland, O H Romarheim, T Landsverk, A Skrede
Microbes – a potential sustainable aquafeed resource

M Sprague, J Walton, J Dick, F. Strachan, D R Tocher, JG Bell
Effects of feeding a northern, southern fish oil or DHA-rich algal meal replacement feed on the persistent organic pollutant (POP) levels in diets and flesh of Atlantic salmon

Bundit J, Taweesin A.
Effects of replacing soybean meal with Moringa oleifera leaf meal on growth performance, protein digestibility, haematology and antioxidant action against lipid peroxidation in juvenile tilapia Oreochromis niloticus
<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Speaker(s)</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:45-11:00</td>
<td>O5</td>
<td>Odd H Romarheim, Margareth Øverland, Liv T Mydland, Anders Skrede, Thor Landsverk</td>
<td>Bacterial meal counteracts intestinal inflammation caused by soybean meal in Atlantic salmon</td>
</tr>
<tr>
<td>11:00-11:15</td>
<td>O6</td>
<td>Ole Torrissen and Reidar Toresen</td>
<td>Sustainable feed for Atlantic salmon; the fish in feed to fish produced</td>
</tr>
<tr>
<td>11:15-11:30</td>
<td>O7</td>
<td>Mengqing Liang, Hongbo Yao, Keke Zheng, Qing Chang, Jialin Wang</td>
<td>The effects of size-fractionated fish hydrolysate in the diet on growth, and feed utilization of turbot (Scophthalmus maximus)</td>
</tr>
<tr>
<td>11:30-11:45</td>
<td>O8</td>
<td>Chunfang Cai, Wenjuang Wang, Wei Zhang, A. Krogdahl, Yuantu Ye</td>
<td>The effect of soybean meal (SBM) and its four chemical factors on growth performance and intestinal morphology of juvenile allognogenetic silver crucian carp (Carassius auratus gibelio♀×Cyprinus carpio♂)</td>
</tr>
<tr>
<td>11:45-12:00</td>
<td>O9</td>
<td>Katerina Kousoulaki, Sissel Albrektsen, Eyolf Langmyhr, Hanne Jorun Olsen, Paddy Campbell, Anders Aksnes</td>
<td>The water soluble fraction in fish meal (stickwater) stimulates growth in Atlantic salmon (Salmo salar L.) given high plant protein diets</td>
</tr>
<tr>
<td>12:00-13:30</td>
<td>Lunch</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13:30-13:45</td>
<td>O10</td>
<td>Kenan Engin, O Tufan Eroldoğan, Ilgın Özşahinoğlu, O Taşbozan, H. Asuman Yılmaz, Mustafa Yıldız, Pınar Mumoğullarında</td>
<td>Effects of fish oil replacement by increasing levels of cotton seed oil (CSO) on ammonia and urea excretion rates in the juvenile European sea bass, Dicentrarchus labrax</td>
</tr>
<tr>
<td>13:45-14:00</td>
<td>O11</td>
<td>Mohammed Aliyu-Paiko, Roshada Hashim, Alexander A S Chong</td>
<td>Effects of partial substitution of dietary Fish Oil (FO) with Crude Palm Oil (CPO) and Palm Fatty Acid Distillate (PFAD) on growth, feed efficiency, Muscle Fatty Acid Composition, Biological Indices and the Activities of Hepatic Lipogenic Enzymes in Snakehead (Channa striatus, Bloch 1793) Fingertling</td>
</tr>
<tr>
<td>14:00-14:15</td>
<td>O12</td>
<td>Kaushik, SJ, Corraze, G, Bell, JG, Tocher, DR, Perez-Sanchez, J, Csengeri, I, Giri, SS, Mohanty, S, Torstensen, B, Berntsson, M, Lie, O</td>
<td>Reduced reliance on marine wild fishery resources: progress in combined replacement of fish meal and fish oil in fish feeds under the EU-Aquamax project</td>
</tr>
</tbody>
</table>

**Session: Physiological metabolism**  
Chairpersons: Drs. Santosh Lall & Cunming Duan

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Speaker(s)</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:15-14:30</td>
<td>O13</td>
<td>Ramez Alhazzaa, Andrew Bridle, Peter Nichols and Chris Carter</td>
<td>Thermal regulation of lipid metabolism in barramundi, Lates calcarifer</td>
</tr>
<tr>
<td>14:30-14:45</td>
<td>O14</td>
<td>Nopadon Pirarat, Takayuki katagiri, Kunihiko Futami, Makoto Endo, Masashi Maita</td>
<td>Distribution of melamine related crystal in walking catfish (Clarias batrachus) organs</td>
</tr>
<tr>
<td>14:45-15:00</td>
<td>O15</td>
<td>Ashild Krogdahl and Anne Sundby</td>
<td>Why do fish have limited capacity for carbohydrate utilization?</td>
</tr>
<tr>
<td>15:00-15:15</td>
<td>O16</td>
<td>Pirozzi, I and Booth, MA</td>
<td>The effect of temperature, body weight and plane of nutrition on the routine metabolic rate and postprandial metabolic response in mulloway,</td>
</tr>
<tr>
<td>Time</td>
<td>Session</td>
<td>Speaker(s)</td>
<td>Topic</td>
</tr>
<tr>
<td>------------</td>
<td>---------</td>
<td>---------------------------------------------------------------------------</td>
<td>-----------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>15:30-15:45</td>
<td></td>
<td></td>
<td>Coffee break</td>
</tr>
<tr>
<td>15:45-16:00</td>
<td>O18</td>
<td>Tibiábin Benítez-Santana, Mónica Betancor, M José Caballero, Eyad Atalah, Eduardo Juárez-Carrillo, Silvia Torrecillas, Carmen M. Hernández-Cruz and Marisol Izquierdo</td>
<td>Family-specific responses to dietary fish oil replacement by vegetable oil in Atlantic salmon: microarray analysis of liver transcriptome</td>
</tr>
<tr>
<td>16:00-16:15</td>
<td>O19</td>
<td>Yuanyou Li, Liang Zhang, Suqi Wang, Shude Xu, Changbo Hu, Yijun Zheng</td>
<td>Studies on HUFA biosynthesis in a marine teleost Siganus canaliculatus</td>
</tr>
<tr>
<td>16:15-16:30</td>
<td>O20</td>
<td>Lu Zhang, Kangsen Mai, Qinghui Ai, Chunxiao Zhang, Huitao Li, Junli Wan, Ji Dancing Zhang, Shixuan Zheng</td>
<td>Effect of different dietary vitamin D levels on growth and calcium and phosphorus metabolism in Japanese seabass, Lateolabrax japonicus C</td>
</tr>
<tr>
<td>16:30-16:45</td>
<td>O21</td>
<td>Elvis M Chikwati, Inger Rudshaug, Gunn C Östby, Fredrik F Venold, Thor Landsverk, Ståle Refstie, Anne Marie Bakke, Michael H Penn, Åshild Krogdahl</td>
<td>Intestinal epithelial cell turnover is an adaptive response to diet-induced enteritis in Atlantic salmon (Salmo salar L)</td>
</tr>
<tr>
<td>16:45-17:00</td>
<td>O22</td>
<td>F Médale, M Dupont-Nivet, S. Rimoldi, HA Yilmaz, F Terrier, G Corraze, E Quillet, I Geurden</td>
<td>Responses of rainbow trout clonal lines to plant-based feed evidence genotype x diet interactions</td>
</tr>
<tr>
<td>17:00-18:00</td>
<td></td>
<td></td>
<td>Poster session</td>
</tr>
<tr>
<td>18:00-19:30</td>
<td></td>
<td></td>
<td>Buffet dinner</td>
</tr>
</tbody>
</table>

**Workshop: Alternative feed protein sources**

Chairpersons: Drs. Delbert M Gatlin III & Peng Li

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Speaker(s)</th>
<th>Topic</th>
</tr>
</thead>
<tbody>
<tr>
<td>19:00-19:15</td>
<td>O23</td>
<td>Sergio F. Nates*, Kent Swisher</td>
<td>The Role of Animal by-Products in Aquaculture Feeds</td>
</tr>
<tr>
<td>19:15-19:30</td>
<td>O24</td>
<td>Dominique Bureau</td>
<td>Assessment of the Nutritive Value of Processed Animal Proteins for Fish</td>
</tr>
<tr>
<td>19:30-19:45</td>
<td>O25</td>
<td>John Halver</td>
<td>Is ruminant meat and bone meal a vector of BSE transmission through aquaculture feeds?</td>
</tr>
<tr>
<td>19:45-20:00</td>
<td>O26</td>
<td>Delbert M Gatlin III</td>
<td>Review of use of plant proteins in aquafeeds</td>
</tr>
<tr>
<td>20:00-20:15</td>
<td>O27</td>
<td>MH Holme, V Crampton, K Ruohonén, R Taylor, K Østerhus and V Ohnstad</td>
<td>In replacing fishmeal, what are we replacing?</td>
</tr>
<tr>
<td>20:15-20:30</td>
<td>O28</td>
<td>Yan Wang</td>
<td>Potential and problems in the use of rendered animal protein ingredients as dietary protein sources for three marine fish species</td>
</tr>
<tr>
<td>20:30-20:45</td>
<td>O29</td>
<td>Crisantema Hernández, Ronald Hardy, Miguel A. Olivera-Novoa, Paul Varilla, Alan Santos, Denisse Marquez, Martin Valverde,</td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>Paper No</td>
<td>Title</td>
<td>Authors</td>
</tr>
<tr>
<td>--------------</td>
<td>----------</td>
<td>----------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------</td>
</tr>
<tr>
<td>20:45-21:00</td>
<td>O30</td>
<td>Effects of fish meal quality and fish meal substitution by animal protein blend on growth performance GH/IGF-I axis and flesh quality of Japanese seabass (Lateolabrax japonicus)</td>
<td>Liang Hu, M. Xue, L. Wang, X. Wu, H. Ge, Y. Zheng</td>
</tr>
<tr>
<td>21:00-21:15</td>
<td>O31</td>
<td>Effect of taurine supplement to graded fish meal feed on yellowtail Seriola quinqueradiata in net cage</td>
<td>Shuichi Satoh, Daisuke Kondoh, Yohsuke Futami, Yutaka Haga, Yoshihiro Yamamoto and Takaumi Oishi</td>
</tr>
<tr>
<td>21:15-21:30</td>
<td>O32</td>
<td>Effects of L-carnitine on growth, vitality and RNA/DNA ratio in first feeding larvae of grass carp, Ctenopharyngodon idella</td>
<td>Yuke Chen, Jing Xu, Lili Lin, Dan Song, Huaiquan Pei, Jing Dong, Lifang Wu, Chenxia Ge, Dongming Zhang</td>
</tr>
<tr>
<td>21:30-21:45</td>
<td>O33</td>
<td>Impacts of dietary hydroxyproline on growth, muscle firmness, collagen and PYD cross-links formation in Atlantic salmon (Salmo salar)</td>
<td>Sissel Albrekksen, Ellen Sirnes, Anders Aksnes and Ørjan Hagen</td>
</tr>
<tr>
<td>21:45-22:00</td>
<td>O34</td>
<td>Nucleotide nutrition in aquatic animals: current knowledge and future applications</td>
<td>Peng Li, Sergio Nates, Delbert M Gatlin III</td>
</tr>
</tbody>
</table>

June 2, 2010  
2nd F the Conference Centre

Session: Cellular and molecular nutrition
Chairpersons: Drs. Douglas Tocher & Sadasivam J. Kaushik

Invited Presentation

<table>
<thead>
<tr>
<th>Time</th>
<th>Paper No</th>
<th>Title</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>08:30-09:00</td>
<td>O36</td>
<td>Regulation of insulin-like growth factor (IGF) and IGF binding protein gene expression by nutrition and aquaculture related stressors: Implications in nutrient utilization and stress assessment</td>
<td>Cunming Duan, Qiang Feng, Yun Li, Ling Lu, Xianglei Wang, and Hiroyasu Kamei</td>
</tr>
<tr>
<td>09:00-09:15</td>
<td>O37</td>
<td>Gene expression pattern during European sea bass early stages and regulation by vitamins: consequences for skeletal development</td>
<td>Chantal L Cahu, M Darias, JL Zambonino-Infante and D Mazurais</td>
</tr>
<tr>
<td>09:15-09:30</td>
<td>O38</td>
<td>Preliminary results on the modulation of ACTH-induced expression of stress related genes by polyunsaturated fatty acids in head kidney from European sea bass, Dicentrarchus labrax</td>
<td>Montero, D. Negrín-Báez, D. Ganga, R. Navarro, A. Izquierdo, MS, Afonso, JM</td>
</tr>
<tr>
<td>09:30-09:45</td>
<td>O39</td>
<td>Zebrafish Elovl4 elongases: role in biosynthesis of very long-chain fatty acids and expression during embryogenesis</td>
<td>Óscar Monroig, Josep Rotllant, José M. Cerdá-Reverter, James R. Dick, Antonio Figueras, Douglas R Tocher</td>
</tr>
<tr>
<td>09:45-</td>
<td>O40</td>
<td>Stanko Skugor, Barbara Grisdale-Helland, Ståle Refstie, Sergey</td>
<td>Stanko Skugor, Barbara Grisdale-Helland, Ståle Refstie, Sergey</td>
</tr>
<tr>
<td>Time</td>
<td>Session</td>
<td>Title</td>
<td>Speaker(s)</td>
</tr>
<tr>
<td>--------------</td>
<td>---------------------------------------------------------------------------------------------</td>
<td>-----------------------------------------------------------------------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>10:00</td>
<td>Afanasyev, Jouni Vielma Aleksei Krasnov</td>
<td>Gene expression profiling reveals similar hepatic responses to restricted feeding and extracted soybean meal in diets for Atlantic salmon (<em>Salmo salar</em> L.)</td>
<td></td>
</tr>
<tr>
<td>10:00-10:30</td>
<td>Coffee break</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:30-10:45</td>
<td>O41 Lina Jin, Naisong Chen, Hengyong Zhou, Xiaojie Qiu</td>
<td>Arginine intake and carbohydrate to lipid ratios affect gene expression of anabolic hormones in largemouth bass, <em>Micropterus salmoides</em></td>
<td></td>
</tr>
<tr>
<td>10:45-11:00</td>
<td>O42 Chenglong Wu, Kangsen Mai, Wenbing Zhang, Qinghui Ai, Wei Xu, Xiaojie Wang, Hongming Ma, Zhiguo Liufu</td>
<td>Molecular cloning, characterization and mRNA expression of selenium-dependent glutathione peroxidase from abalone <em>Haliotis discus hannai</em> in response to dietary selenium, zinc and iron</td>
<td></td>
</tr>
<tr>
<td>11:00-11:15</td>
<td>O43 Chuanpeng Zhou, Bo Liu, Jun Xie, Xianping Ge, Pao Xu, Wenbin Liu</td>
<td>Effect of high carbohydrate dietary on the growth, blood immune parameter and gene expression of heat shock protein 70 of Wuchang bream (<em>Megalobrama amblycephala</em>)</td>
<td></td>
</tr>
<tr>
<td>11:30-11:45</td>
<td>O45 Giovanni M Turchini, David S Francis</td>
<td>The whole-body fatty acid balance method: advantages, limitations and opportunities</td>
<td></td>
</tr>
<tr>
<td>11:45-12:00</td>
<td>O46 Jocelyn A. Madrones-Ladja* and Milagros R. de la Peña</td>
<td>The effect of nutrients and light conditions on diatom propagation, and refinement of larval and postlarval culture techniques for the abalone <em>Haliotis asinina</em> Linne</td>
<td></td>
</tr>
<tr>
<td>12:00-13:30</td>
<td>Lunch</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Session: Broodstock and larval nutrition**

**Chairpersons: Drs. Marisol Izqueirdo & Liqiao Chen**

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Title</th>
<th>Speaker(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13:30-13:45</td>
<td>O47 Yutaka Haga, Saki Masui, Yuichiro Fujinami, Masato Aritaki, Shuichi Satoh</td>
<td>Early diagnosis of vertebral deformity induced by excessive vitamin A in live marine fish larvae by fluorescent calcein staining</td>
<td></td>
</tr>
<tr>
<td>13:45-14:00</td>
<td>O48 Trond M Kortner, Ingrid Overrein, Gunvor Øie, Per-Arvid Wold, Tora Bardal, Elin Kjørsvik and Augustine Arukwe</td>
<td>Molecular characterization of digestive ontogeny and endocrine functions in Atlantic cod (<em>Gadus morhua</em> L.) larvae as influenced by diet</td>
<td></td>
</tr>
<tr>
<td>14:00-14:15</td>
<td>O49 WingKeong Ng, Yan Wang, Yunyun Qian</td>
<td>Inclusion of crude palm oil in the broodstock diets of Nile tilapia, <em>Oreochromis niloticus</em>, affected tissue fatty acid and vitamin E composition resulting in enhanced reproductive performance</td>
<td></td>
</tr>
<tr>
<td>14:15-14:30</td>
<td>O50 Li Ji, Yutaka Haga, Toshihiro Nakamura, Shuzou Ishida, Toshio Takeuchi</td>
<td>Development of zebrafish model system to evaluate feed performance of microdiet for marine fish larvae</td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>Session: Crustacean nutrition/metabolism</td>
<td>Chairpersons: Drs. Elizabeth Cruz &amp; Chien Yew-hu</td>
<td>Invited Presentation</td>
</tr>
<tr>
<td>-----------</td>
<td>-----------------------------------------</td>
<td>-------------------------------------------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>14:30-15:00</td>
<td>O51</td>
<td>D Allen Davis, Daranee Sookying and Luke A Roy</td>
<td>Development and use of plant based diets for the production of the pacific white shrimp, <em>Litopenaeus vannamei</em></td>
</tr>
<tr>
<td>15:00-15:15</td>
<td>O52</td>
<td>Nick Wade, Christian de Santis, Simon Tabrett Simon Irvin, Melony Sellar, Brett Glencross, Nigel Preston</td>
<td>The effect of ration level, bioactive ingredients and genetic selection on muscle growth factor gene expression in the Black Tiger Prawn, <em>Penaeus monodon</em></td>
</tr>
<tr>
<td>15:30-15:45</td>
<td></td>
<td>Coffee break</td>
<td></td>
</tr>
<tr>
<td>15:45-16:00</td>
<td>O54</td>
<td>Ingrid Lupatsch, Lydia Cuthbertson and Robin J Shields</td>
<td>Assessing energy and protein requirements of juvenile European lobster <em>Homarus gammarus</em> designed for intensive culture</td>
</tr>
<tr>
<td>16:00-16:15</td>
<td>O55</td>
<td>Lenaïg Richard, Christiane Vachot, Anne Surget, Vincent Rigolet, Sadasivam Kaushik, Inge Geurden</td>
<td>Regulation of sulfur amino acid pathways by methionine, cysteine and choline in juveniles tiger prawn <em>Penaeus monodon</em></td>
</tr>
<tr>
<td>16:15-16:30</td>
<td>O56</td>
<td>Yanhua Huang, Guoxia Wang, Wenqing Huang, Ye Zhou, Shangzhi Dong</td>
<td>Effects of dietary probiotic lactic acid bacterium on growth, survival, digestive enzyme activities and vibriosis resistance of the shrimp <em>Litopenaeus vannamei</em></td>
</tr>
<tr>
<td>16:30-16:45</td>
<td>O57</td>
<td>Xugan Wu, Chaoshu Zeng, Greg Smith, Paul C Southgate, Mike Hall</td>
<td>The effects of different DHA/EPA ratios on the survival, growth and larval development of blue swimmer crab, <em>Portunus pelagicus</em> and ornate rock lobster, <em>Panulirus ornatus</em> based on live food enrichment</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time</th>
<th>Session: Feed processing and feeding</th>
<th>Chairpersons: Drs. Brett Glencross &amp; Stale Refstie</th>
<th>Detailed Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:45-17:00</td>
<td>O58</td>
<td>Y Gao, MM Nabulime, JF Hansen, V. Denstadli, HM Gjøen, T Storebakken</td>
<td>Solid-state fermentation with Aspergillus niger to improve the nutritional value of rapeseed meal as a feed ingredient for Nile tilapia (<em>Oreochromis niloticus</em> L.)</td>
</tr>
<tr>
<td>17:00-17:15</td>
<td>O59</td>
<td>Nan Bai, Kangsen Mai, Wenbing Zhang, Hongming Ma, Qinghui Ai, Xiaojie Wang</td>
<td>Effect of discontinuous administration of β-glucan and glycyrrhizin on the growth and immunity of Pacific white shrimp <em>Litopenaeus vannamei</em></td>
</tr>
<tr>
<td>17:15-18:15</td>
<td></td>
<td>Poster session</td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>Session</td>
<td>Presenters</td>
<td>Title</td>
</tr>
<tr>
<td>-----------</td>
<td>-------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>08:30-08:45</td>
<td><strong>Feed processing and feeding</strong></td>
<td>Brett Glencross, Wayne Hawkins, Peter Burridge, David Evans, Neil Rutherford, Peter McCafferty, Ken Dods, Rhys Hauler and Sofia Sipsas</td>
<td>Using Near-InfraRed Spectroscopy (NIRS) to predict the digestible protein and energy value of grain – The lupin case study</td>
</tr>
<tr>
<td>08:45-09:00</td>
<td><strong>Feed processing and feeding</strong></td>
<td>Basseer M Codabaccus, Wing-Keong Ng, Peter D Nichols, Chris G Carter</td>
<td>Effect of a finishing diet strategy on EPA and DHA restoration in rainbow trout, <em>Oncorhynchus mykiss</em>, previously fed dietary palm fatty acid distillate at two different water temperatures</td>
</tr>
<tr>
<td>09:00-09:15</td>
<td><strong>Feed processing and feeding</strong></td>
<td>Frieder J Schwarz, Andreas Lemme, LC Nwanna, Abdallah Metwally</td>
<td>Responses of common carp (<em>Cyprinus carpio</em> L.) to DL-methionine supplementation at different feeding strategies</td>
</tr>
<tr>
<td>09:15-09:30</td>
<td><strong>Feed processing and feeding</strong></td>
<td>Graeme S Mansfield, Stephanie A. Nilson, Atul R Desai, Janet E Hill, Murray D Drew, and Andrew G Van Kessel</td>
<td>Plant-based protein source and processing affect gastrointestinal inflammatory markers and growth rate in rainbow trout, <em>Oncorhynchus mykiss</em></td>
</tr>
<tr>
<td>09:30-09:45</td>
<td><strong>Feed processing and feeding</strong></td>
<td>Thea Morken, Olav F Kraugerud, Frederic T. Barrows, Mette Sørensen, Trond Storebakken, Margareth Øverland</td>
<td>Effect of sodium diformate and extruder temperature on nutrient digestibility in rainbow trout fed barley protein concentrate-based diets</td>
</tr>
<tr>
<td>09:45-10:00</td>
<td><strong>Feed processing and feeding</strong></td>
<td>Trond Storebakken, Gerd Marit Berge, Margareth Øverland, Karl D Shearer, Marie Hillestad, Åshild Krogdahl</td>
<td>Dietary potassium diformate protects against heat-induced reduction of protein digestibility in a mixture of full-fat soy and wheat when used in extruded diets for Atlantic salmon (<em>Salmo salar</em> L)</td>
</tr>
<tr>
<td>10:00-10:15</td>
<td><strong>Feed processing and feeding</strong></td>
<td>T Synnøve Aas, Bendik F Terjesen, Trygve Sigholt, Marie Hillestad, Jørgen Holm, Ståle Refstie, Grete Baeverfjord, Kjell-Arne Rorvik, Mette Sørensen, Maike Oehme, Gaojie He, Torbjørn Åsgård</td>
<td>The optimal pellet quality is a trade-off between durability and responses in the fish</td>
</tr>
<tr>
<td>10:15-10:45</td>
<td><strong>Feed processing and feeding</strong></td>
<td>T Synnøve Aas, Bendik F Terjesen, Trygve Sigholt, Marie Hillestad, Jørgen Holm, Ståle Refstie, Grete Baeverfjord, Kjell-Arne Rorvik, Mette Sørensen, Maike Oehme, Gaojie He, Torbjørn Åsgård</td>
<td>The optimal pellet quality is a trade-off between durability and responses in the fish</td>
</tr>
</tbody>
</table>

**Session: Nutrition and environment**

Chairpersons: Drs. Oyvind Lie & Shouqi Xie

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Presenters</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:45-11:15</td>
<td><strong>Invited Presentation</strong></td>
<td>Shouqi Xie</td>
<td>Feed, feeding and environment safety in aquaculture</td>
</tr>
<tr>
<td>11:15-11:30</td>
<td><strong>Invited Presentation</strong></td>
<td>Anders K. Kiessling, Eva Brännäs, Jana Pickova, Lars Edebo, Odd Lindahl, Anders Alanää, Kristina Sundell, Matilda Olstorpe,</td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>Session</td>
<td>Authors</td>
<td>Title</td>
</tr>
<tr>
<td>--------------</td>
<td>----------------------------------------------</td>
<td>------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td>11:30-11:45</td>
<td></td>
<td>Erik Sandblom, Afaf Kamal-Edin, Corine Sandström, Muhamed Muminovic, Leif Norrgren, Lars Ove Eriksson, Johan Schnürer, Jan Erik Lindberg, Torbjörn Lundh</td>
<td>A model for sustainable cage farming in semi closed water systems</td>
</tr>
<tr>
<td>11:45-12:00</td>
<td></td>
<td>Md. Al-Amin Sarker, Yutaka Haga, Md. Shah Alam Sarker, Misako Miwa, Yoji Yamamoto, Goro Yoshizaki, Shuichi Satoh1</td>
<td>Influences of low salinity and dietary fatty acids composition on biosynthesis potency of highly unsaturated fatty acids in red sea bream Pagrus major</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ashraf Suloma, Osama M El–Husseiny, Rania S Mabroke, TahounM</td>
<td>Effect of within-day feeding strategies on Nile tilapia fry performance: step towards deliver diets vary in nutrients proportion in terms of meals timing</td>
</tr>
<tr>
<td>12:00-13:30</td>
<td>Lunch</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13:30-14:00</td>
<td>Session: Nutrition and health</td>
<td>Delbert M Gatlin III</td>
<td>Invited Presentation Fish nutrition, health and welfare: An overview of their interrelationships</td>
</tr>
<tr>
<td></td>
<td></td>
<td>J Gu, AM Bakke, NH Sissener, Â Krogdahl</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Per Gunnar Fjelldal, Erik-Jan Lock, Tom Johnny Hansen, Rune Waagbo, Anna Wargelius, Laura Gil Martens, Adel El-Mowafi &amp; Robin Ørnsrud</td>
<td>A short and medium term study on healthy and sensitized Atlantic salmon, Salmo salar L., fed genetically modified maize</td>
</tr>
<tr>
<td>14:45-15:00</td>
<td>Session: Nutrient requirements and availability</td>
<td>S. Döll, G Baardsen, P Möller, W Koppe, I Stubhaug and S Dänicke</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>JW Schrama, I Geurden, LTN Heinsbroek, JAJ Verreth, S.J. Kaushik</td>
<td></td>
</tr>
<tr>
<td>15:00-15:15</td>
<td></td>
<td>Xueqin Jiang*, Erchao Li, Liqiao Chen</td>
<td>Effect of dietary lipid sources on growth performance and immune parameters of Darkbarbel catfish, Pelteobagrus vachelli</td>
</tr>
<tr>
<td>15:15-15:30</td>
<td></td>
<td>Basseer M Codabaccus, Andrew R Bridle, Peter D Nichols and Chris G Carter</td>
<td>Effect of feeding Atlantic salmon (Salmo salar L.) a diet enriched with stearidonic acid from parr to smolt on growth and n-3 LC-PUFA biosynthesis</td>
</tr>
</tbody>
</table>
15:30-15:45  Coffee break

15:45-16:00  O78  S. Wang, P.M. Encarnacao, R.L. Payne, and D.P. Bureau
Estimating dietary lysine requirements for live weight gain and protein deposition in juvenile rainbow trout (*Oncorhynchus mykiss*)

16:00-16:15  O79  Erik-Jan Lock, Pål A Olsvik, Bente E Torstensen, Anders Goksoyr, Johan Johansen, Trygve Sigholt, Nanne Joerum, Jan-Vidar Jakobsen, Rune Waagbo, Marit Bjørnevik, Per-Gunnar Fjelldal, Olav Breck, Marc H.G. Berntssen
Feeding Atlantic salmon a diet based on decontaminated fish oil during a full production cycle; effect on POP levels, long chain omega-3 fatty acids, vitamins, fillet quality and bone deformities

16:15-16:30  O80  Weidan Jiang, Lin Feng, Yang Liu, Jun Jiang, Kai Hu, Shuhong Li, Xiaojiu Zhou, Gangfu Chen*
Lipid peroxidation, protein oxidant and antioxidant status of muscle, intestine and hepatopancreas for juvenile Jian carp (*Cyprinus carpio var. Jian*) fed graded levels of myo-inositol

16:30-16:45  O81  Leon TN Heinsbroek, Cris Wijnen, Jan Cordewener, Twan America, Ingrid M van der Meer, Johan W Schrama and Johan AJ Verreth
How apparent is the apparent digestibility of plant proteins by a carnivorous marine fish: the use of protein gel analysis to characterize dietary and faecal proteins of turbot, *Psetta maxima*

16:45-17:00  O82  Mónica B Betancor, M José Caballero, Tíbiaín Benítez-Santana, Reda Saleh, Eyad Atalah, Javier Roo and Marisol Izquierdo
Histological and ultrastructural changes in sea bass (*Dicentrarchus labrax*) larvae muscle in response to high dietary content of DHA

17:00-18:00  Poster session

June 4, 2010  2nd F  the Conference Centre

Session: Nutrient requirements and availability  
Chairpersons: Drs. Ashild Krogdahl & Xufang Liang

08:30-08:45  O83  Wai Yee Ho, Andreas Lemme, Katheline Hua, Margaret Quinton, Dominique P. Bureau
Efficiency of Methionine Utilization of Rainbow Trout (*Oncorhynchus mykiss*)

08:45-09:00  O84  Wen Gao, Yongjian Liu, Lixia Tian, Kangsen Mai
Comparisons of glucose tolerance of herbivorous grass carp (*Ctenopharyngodon idella*) and omnivorous tilapia (*Oreochromis niloticus × O. aureus*)

09:00-09:15  O85  Qi Ye, Xiangjun Sun, Yongjun Liang, Liwei Song, Xiuting Qiao
Effects of dietary digestible carbohydrate on growth, blood biochemical indices, glycogens, digestive enzymes and carbohydrate metabolic enzymes of Jade Perch (*Scortum barcoo*)

09:15-09:30  O86  K. Ruohonen, D Nanton, MH Holme and A El-Mowafi
Modelling oil digestibility of Atlantic salmon feeds

09:30-09:45  O87  K M Randall, M J T Reaney, M D Drew
Strategies to increase highly unsaturated omega 3 fatty acids in rainbow trout fed canola, flax and camelina oils
<table>
<thead>
<tr>
<th>Time</th>
<th>Session Title</th>
<th>Speaker(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>09:45-10:00</td>
<td>Invited Presentation</td>
<td>Rune Waagbø, Bente E Torstensen, Lise Madsen &amp; Marc HG Berntssen</td>
</tr>
<tr>
<td></td>
<td>Feed and feeding affect quality and safety of aquaculture products, with</td>
<td></td>
</tr>
<tr>
<td></td>
<td>emphasis on salmonids</td>
<td></td>
</tr>
<tr>
<td>10:00-10:15</td>
<td>Effects of dietary copper sources and levels on performance, plasma</td>
<td>Xianping Shao, Wenbin Liu, Chao Liang, Kangle Lu, Wei Xia, Yangyang Jiang</td>
</tr>
<tr>
<td></td>
<td>antioxidant activities and relative copper bioavailability in <em>Carassius</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>auratus gibelio</em></td>
<td></td>
</tr>
<tr>
<td>10:15-10:45</td>
<td>Coffee break</td>
<td></td>
</tr>
<tr>
<td>10:45-11:15</td>
<td>Studies on the protease systems cathepsins and calpains/calpastatins and their</td>
<td>Magny S. Thomassen, Mari Gaarder, Turid Mørkøre, Kjell Rørvik, Eva Veiseth</td>
</tr>
<tr>
<td></td>
<td>impact on muscle quality in farmed Atlantic salmon (<em>Salmo salar</em>)</td>
<td>ent, Ragni Ofstad, Diane Bahauad</td>
</tr>
<tr>
<td></td>
<td>Influence of dietary lipid composition, addition of free amino acids and</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pre-slaughter stress</td>
<td></td>
</tr>
<tr>
<td>11:15-11:30</td>
<td>Evaluation of sensory quality indices and freshness assessment of Nile</td>
<td>Gabriel Gana Bake, Masato Endo, Atsushi Akimoto, Naoko Hamada-Sato,</td>
</tr>
<tr>
<td></td>
<td>tilapia <em>O. niloticus</em> fed recycled food waste material</td>
<td>Toshio Takeuchi</td>
</tr>
<tr>
<td>11:30-11:45</td>
<td>Effects on performance and product quality in Atlantic salmon fed diets</td>
<td>Jan Olli, Bente Ruyter, Turid Mørkøre, Harald Breivik, Aimo Oikari,</td>
</tr>
<tr>
<td></td>
<td>reduced in organic pollutants</td>
<td>Aleksei Krasnov, Olav Thorstad, Åshild Krogdahl, Gunnar Berge, Torbjørn</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Åsgård</td>
</tr>
<tr>
<td>12:00-13:30</td>
<td>Lunch</td>
<td></td>
</tr>
<tr>
<td>13:30-13:45</td>
<td>Toxicological, physiological responses and residue of aflatoxin B1 in</td>
<td>Shixi Deng, Lixia Tian, Fujia Liu, Shengjie Jin, Guiying Liang, Yongjian</td>
</tr>
<tr>
<td></td>
<td><em>Oreochromis niloticus × O. aureus</em></td>
<td>Liu</td>
</tr>
<tr>
<td>13:45-14:00</td>
<td>Dietary enzymes in aquaculture</td>
<td>Viviane Verlhac Trichet* &amp; Ester Santigosa i Culi</td>
</tr>
<tr>
<td>14:00-14:15</td>
<td>Applications of DVAQ® in aquaculture</td>
<td>Fanyi Meng</td>
</tr>
<tr>
<td>14:15-14:30</td>
<td>Effects of different supplementation levels of extracted soy peptide on</td>
<td>Janice A Ragaza, Saichiro Yokoyama, Manabu Ishikawa, Shunsuke Koshio</td>
</tr>
<tr>
<td></td>
<td>growth performance and tolerance to high temperature stress in juvenile</td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>Session</td>
<td>Title</td>
</tr>
<tr>
<td>--------------</td>
<td>---------</td>
<td>-------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>14:30-14:45</td>
<td>O98</td>
<td>Effects of dietary prebiotics on intestinal morphology and digestive enzyme activities of juvenile hybrid striped bass, <em>Morone chrysops</em> x <em>M. saxatilis</em> and red drum, <em>Sciaenops ocellatus</em></td>
</tr>
<tr>
<td>14:45-15:00</td>
<td>O99</td>
<td>Effects of supplemental enzymes on apparent nutrient digestibility in rainbow trout (<em>Oncorhynchus mykiss</em>) fed plant-based diets</td>
</tr>
<tr>
<td>15:00-15:15</td>
<td>O100</td>
<td>Supplementation of Methionine with 2-Hydroxy-4-Methylthio-butanoic Acid (HMTBA) in Low Fish Meal Diets for Fish and Shrimp</td>
</tr>
<tr>
<td>15:15-15:30</td>
<td>O101</td>
<td>Effect of increasing dietary doses of the lupin alkaloid sparteine on growth performance, body composition and histology of Rainbow Trout (<em>Oncorhynchus mykiss</em>)</td>
</tr>
<tr>
<td>15:30-15:45</td>
<td></td>
<td>Coffee break</td>
</tr>
<tr>
<td>15:45-16:00</td>
<td>O102</td>
<td>Use of probiotics in beluga (<em>Huso huso</em>) and Persian sturgeon (<em>Acipenser persicus</em>) aquaculture. Which bacteria <em>Lactobacillus curvatus</em> or <em>Leuconostoc mesenteroides</em> should we choose?</td>
</tr>
<tr>
<td>16:00-16:15</td>
<td>O103</td>
<td>Differing effects of saponin supplementation to plant meals in formulated feeds for Atlantic salmon</td>
</tr>
<tr>
<td>16:15-16:30</td>
<td>O104</td>
<td>Histidine supplementation to plant protein used diet improves growth and freshness of muscle in yellow tail <em>Seriola quinqueradiata</em></td>
</tr>
<tr>
<td>16:30-16:45</td>
<td>O105</td>
<td>Effect of dietary crystalline or microcapsuled amino acid supplementation on growth and plasma total free amino acids of common carp <em>Cyprinus carpio</em></td>
</tr>
<tr>
<td>16:45-17:45</td>
<td></td>
<td>Student travel award/Next symposium announcement</td>
</tr>
<tr>
<td>19:00-22:00</td>
<td></td>
<td>Farewell banquet and entertainment</td>
</tr>
</tbody>
</table>
### POSTERS

#### Alternative feed protein and lipid sources

<table>
<thead>
<tr>
<th>Posters</th>
<th>Authors</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>A. Mirza Aghazadeh*, M.S. Yousefi, R. Malekzadeh Viayeh</td>
<td>Replacement of wheat flour by macaroni wastes in diets of rainbow trout, <em>Oncorhynchus mykiss</em></td>
</tr>
<tr>
<td>P2</td>
<td>Feng Lu*, Shuichi Satoh, Yutaka Haga, Toshifumi Wakayama, Koichi Yamaguchi, Taro Akiyama</td>
<td>Use of maggot meal for substitution of fish meal in rainbow trout diets</td>
</tr>
<tr>
<td>P3</td>
<td>Youqing Miao, Qinghui Ai*, Kangsen Mai, Wei Xu, Wenbing Zhang, Xiaojie Wang, Zhiguo Liu</td>
<td>Isolation and Screening of Tannin or Phytic Acid Degrading Microbes</td>
</tr>
<tr>
<td>P4</td>
<td>Qingmei Zheng , Xiaobo Wen*, Chunyan Han</td>
<td>Effect of soybean meal replacement by cottonseed meal on growth, hematology, expression and activity of antioxidant enzymes of grass carp (<em>Ctenopharyngodon idellus</em>)</td>
</tr>
<tr>
<td>P5</td>
<td>Ye Jinyun, Pan Qian, Chen Jianming, Shen Binqian, Wang Youhui Zhang Xueshu</td>
<td>Effect of replacement of fish meal by soybean meal in the diet on growth of red-claw crayfish (<em>Cherax quadricarinatus</em>)</td>
</tr>
<tr>
<td>P6</td>
<td>Zonglin Zheng , Lizhi Jin , Kaiyu Wang, Xinghua Zhou, Xiao Xiang</td>
<td>Effect of oregano essential oil (<em>Origanum heracleoticum</em> L.) on intestinal bacterial flora of channel catfish (<em>Ictalurus punctatus</em>)</td>
</tr>
<tr>
<td>P7</td>
<td>Qiu Yan Ye Yuan-tu*  Cai Chun-fang Ma Hong Xiao shun-ying Dai Xiao-fang Gu Dong</td>
<td>Effects of Raffinose on Growth Performance and Physiological Effects of Grass Carp (<em>Ctenopharyngodon idellus</em>)</td>
</tr>
<tr>
<td>P8</td>
<td>Dai Xiao-fang Ye Yuan-tu*  Cai Chun-fang Jin Su-ya Xiao Shun-ying Xiang Chao-lin Hu Xian-qiong</td>
<td>Apple seed and pumpkin seed on growth performance and Part of the physiological, biochemical indexes in <em>Megalobrama amblycephala</em></td>
</tr>
<tr>
<td>P9</td>
<td>Xinxia Wang*, Yongjin Li, Chonglin Hou, Yang Gao, Yizhen Wang*</td>
<td>Utilization of different dietary lipid source in in yellow croaker (<em>Pseudosciaena crocea</em> R.): effects on growth performance, tissue fatty acid composition, histological changes and PPARγ expression</td>
</tr>
<tr>
<td>P10</td>
<td>Aliro Bórquez, Carolina Shenne, Adrián Hernández*, Mónica Rubilar, Patricio Dantagnan</td>
<td>Novel protein sources for aquaculture: potential use of solid state fermentation to improve nutritional value of plant feedstuffs</td>
</tr>
<tr>
<td>P11</td>
<td>Bergo Owari Ngandzali*, Fan Zhou, Qing Jun Shao, Wen Xiong, Yuan Jian Xu and Jun Zhuo Xu</td>
<td>Effects of partial replacement of fish meal with soybean protein concentrate on growth performance, body composition and nutrition digestibility of juvenile black sea bream, <em>Sparus macrocephalus</em></td>
</tr>
</tbody>
</table>
| P12 | Dadgar, sh., M. Alizadeh  
Application of some carbohydrate waste food in grower diet of rainbow trout, *Oncorhynchus mykiss* |
| P13 | Ana Paula O. Rodrigues, Maria do Carmo G. Rosa, Eduardo Cargnin-Ferreira, Alícia de Francisco, Débora M. Fracalossi  
Different utilization of plant sources by the omnivores jundiã catfish (*Rhamdia quelen*) and Nile tilapia (*Oreochromis niloticus*) |
| P14 | Felipe E. Reveco and Murray D. Drew  
Protein concentrates from wheat distillers’ grains as feed source for rainbow trout |
| P15 | Katerina Kousoulaki, Hanne Jorun Olsen*, Sissel Albrektsen, Eyolf Langmyhr, Paddy Campbell, Anders Aksnes  
Fractionation of stickwater (SW) by micro-, ultra- and nano- filtration. Effect of different SW fractions and supplemented hydroxyproline and taurine on Atlantic salmon (*Salmo salar L.*) performance fed very low fish meal diets |
| P16 | Murat Arslan, A. Necdet Sirkecioglu, Abdulkadir Bayir, Harun Arslan  
The influence of varying dietary lipid resources on growth, survival and fatty acid composition of brown trout, *Salmo trutta*. |
| P17 | Jun Yang*, Qi-Cun Zhou, Hua-lang Wang, Hai-Tao Zhang, Xian-Jun Cui  
Partial replacement of fish meal by wheat gluten meal in practical diets for juvenile cobia (*Rachycentron canadum*) |
| P18 | Jun Yang*, Qi-Cun Zhou, Hua-lang Wang, Hai-Tao Zhang, Xian-Jun Cui  
Partial replacement of fish meal by corn gluten meal in practical diets for juvenile cobia (*Rachycentron canadum*) |
| P19 | Tuo Wang*, Qi-Cun Zhou  
Partial replacement of fish meal with a mixture of canola meal and cottonseed meal in practical diets for *Litopenaeus vannamei* |
| P20 | M.H.S. Ariyaratne  
Cage culture of the GIFT strain of Nile tilapia (*Oreochromis niloticus*) fed with aquafeed prepared using local fishmeal and Ipil ipil leaves |
| P21 | Stephanie A. Nilson*, Andrew G. Van Kessel, Janet E. Hill, Murray D. Drew  
Effect of dietary inclusion rate of canola meal in the growth performance of rainbow trout, *Oncorhynchus mykiss* |
| P22 | Hafice A. Yilmaz, Tufan O. Ergoldogan*, Kenan Engin, Abdullatif Olgul, Oguz Tasbozan, Serhat Tirkmen  
Partial and total replacement of fish oil either canola or cotton seed oils in diets for European sea bass (*Dicentrarchus labrax*): effects on flesh and whole body fatty acid composition |
| P23 | Vidar Gundersen, Kjell A. Masoval and Eldar Asgard Bendiksen  
Feed sustainability of high-energy feeds with graded fishmeal inclusion for Atlantic salmon (*Salmo salar L.*) |
| P24 | Vivian O. Crampton*, Dominic A. Nanton, Kari Ruohonen, Adel El-Mowafi  
Demonstration of salmon farming as a net producer of fish protein and oil. |
Replacement of fish meal by skate meal and black cod viscera meal in diets for Pacific threadfin (*Polydactylus sexfilis*) |
| P26 | Lu Cheng*, Feng Huang  
Effect of fermented grain protein on growth and feed efficiency of *Carassius auratus gibelio* |
<p>| P27 | Rongbin Gao*, Longzhen Zhang, Ping Zhuang, Guangpeng Feng, Jianyi Liu |</p>
<table>
<thead>
<tr>
<th>NO.</th>
<th>Title</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>P28</td>
<td>Optimal replacement of fish meal by soybean protein in diets for <em>Siganus guttatus</em></td>
<td>Bin-Chong Qiu, Qing Pan*, Bi-wei Liu, Ying-Zuo Bi</td>
</tr>
<tr>
<td>P29</td>
<td>Effects of replacements of soybean meal with dried or fermented brewers’ grain on growth and body composition in juvenile tilapia <em>Oreochromis niloticus × O. aureus</em></td>
<td>Shengming Sun, Jinyun Ye *, Jianming Chen, Youhui Wang, Liqiao Chen *</td>
</tr>
<tr>
<td>P30</td>
<td>Effects of dietary cottonseed meal protein on growth, body composition and some blood indices of juvenile <em>Mylopharyngodon piceus</em></td>
<td>Xiao Taoyi *, Hu Yi, Huang Yun</td>
</tr>
<tr>
<td>P31</td>
<td>Effect of fish-meal replacement by fermented soybean meal on growth, flesh quality, nitrogenous and phosphorus metabolism of Japanese seabass, <em>Lateolabrax japonicus</em> under digestible amino acid profile</td>
<td>Liang HU, Min XUE*, Xiufeng WU, Yinghua ZHENG, Lanmei WANG, Hongyun GE</td>
</tr>
<tr>
<td>P32</td>
<td>Growth, nutrient utilization, gut and liver histology of rainbow trout (<em>Oncorhynchus mykiss</em>) fed extruded diets including medium and high levels of lupin and pea concentrate</td>
<td>Zhang, Yuexing, Øverland, Margareth, Shearer, Karl D., Penn, M., Sørensen, Mette, Denstadli, Vegard, Romarheim, Odd Helge, Storebakken, Trond. *</td>
</tr>
<tr>
<td>P33</td>
<td>Effects of Fish Meal Partly Replaced with Corn Gluten Meal on Digestive Enzyme Activities in <em>Fugu Obscurus</em></td>
<td>Zhong Guo-fang *, Hua Xue-ming, Han Bin, Zhou Hong-qi</td>
</tr>
<tr>
<td>P34</td>
<td>Growth performance and feed efficiency of rainbow trout (<em>Oncorhynchus mykiss</em>) juveniles fed on extruded diets with varying levels of Lupine (<em>Lupinus albus</em>), Peas (<em>Pisum sativum</em>) and Raps (<em>Brassica napus</em>)</td>
<td>Adrián Hernández *, David Román, Aliro Bórquez, Patricio Dantagnan, Javier Alcaino, Jamie Hooft</td>
</tr>
<tr>
<td>P35</td>
<td>Replacement of fishmeal by plant protein in turbot (<em>Psetta maxima</em>): performance, nitrogen budget and histological evaluation</td>
<td>Alessio Bonaldo *, Luca Parma, Luciana Mandrioli, Rubina Sirri Ramon Fontanillas, Wolfgang Koppe, Pier Paolo Gatta</td>
</tr>
<tr>
<td>P36</td>
<td>Organic vegetable proteins in feed for organic rainbow trout (<em>Oncorhynchus mykiss</em>)</td>
<td>Ivar Lund, Johanne Dalsgaard *, Alfred Jokumsen, Bodil K. Larsen</td>
</tr>
<tr>
<td>P37</td>
<td>Applications of apple snail meal as alternative protein source in white shrimp (<em>Penaeus vannamei</em>) feed.</td>
<td>Anut Kiriratnikom *, Suphada Kiriratnikom, Punthasit Chokswatdikorn and Kritsana Ruengklay</td>
</tr>
<tr>
<td>P38</td>
<td>The effects of feeding black or yellow seeded canola as a partial replacement for fishmeal in practical diets fed to rainbow trout</td>
<td>C. Zhang *, D. M. Anderson, M. D. Drew</td>
</tr>
<tr>
<td>P40</td>
<td>HaoKun LIU *, Xiaoming ZHU, Yunxia YANG, Dong. HAN, Shouqi. XIE</td>
<td></td>
</tr>
</tbody>
</table>
### Effect of replacement of dietary fish meal by soybean meal on growth performance and enzyme activities in gibel carp, *Carassius auratus gibelio*

**P41**

**Paulo Rema, Êlia Pedrosa, Giampaolo Grassi, Jorge Dias**

Dietary hempseed meal and hempseed oil enhanced growth performance in juvenile turbot (*Scophthalmus maximus*)

**P42**

**Ki-Min Bae, Sang-Min Lee, Suhee Hong, Kyoung-Duck Kim**

Effects of dietary Makgeolli by-products on growth performance and body composition of juvenile olive flounder, *Paralichthys olivaceus*

**P43**

**Kjell A. Måsøval, Patrick C. Campbell**

Lipid digestibility in feeds for Atlantic salmon (*Salmo salar L.*) containing different fish oil sources.

**P44**

**Daniel Sanchez A., Luis Héctor Hernández H.*, Mario Alfredo Fernández A., Teresa Ramírez Pérez, Omar Angeles López.**

Effects on growth of adding yeast (*Saccharomyces cerevisiae*) to diets with high contents of soybean meal to fed juvenile rainbow trout *Oncorhynchus mykiss*

**P45**

**Sandra Yesell Torres O., Luis Héctor Hernández H.*, Mario Alfredo Fernández A., Teresa Ramírez Pérez, Omar Angeles López.**

Use of earthworm meal as substitute of fish meal: effects on the growth of juveniles of rainbow trout *Oncorhynchus mykiss*

**P46**

**M. Saedi, M. M. Sajjadi, H. Hosseinzade, H. Emadi**

Effect of replacing soybean meal with fish meal on growth performance and body Composition at Red Pacu fish (*piaractus brachypomus*)

**P47**

**Alizadeh Morteza, R. Mahmoudi**

Study Replacement of soybean meal by canola meal on growth performance and body composition of rainbow trout (*Oncorhynchus mykiss*) in pre-growing stage

**P48**

**Mustafa Yıldız, O. Tufan Eroldoğan, Kenan Engin, Muhammed A. Baltaci**

Effects of dietary cottonseed and/or canola oil inclusion on the growth performance and fatty acid composition of the juvenile rainbow trout, *Oncorhynchus mykiss*

**P49**

**Sang-Min Lee, Dong-Gyu Kim, Hee-Guk Byun, Minh Anh Pham, Kyoung-Duck Kim**

Effects of dietary total replacement of fish oil by vegetable oils on flesh quality of juvenile and growing flounder, *Paralichthys olivaceus*: Recovery of fatty acid profiles by a fish oil finishing diet

**P50**

**Stefanie M. Colombo, Rolf E. Olsen and Santosh P. Lall**

Evaluation of *Calanus* Copepod and *Euphausia* Krill meal and Oil as the Dietary Protein and Lipid Supplements for Juvenile Atlantic Halibut (*Hippoglossus hippoglossus*)

**P51**

**Liao, W-L.*, Wu, L-C. and Ou, J-N.**

Effect of feed supplement with krill meal and vegetative ingredients replacing fish meal on the growth performance of red striped snapper, *Lutjanus erythropterus*.

**P52**

**Wing-Keong Ng*, Basseer M. Codabaccus, Chris G. Carter, Peter D. Nichols**

Replacing dietary fish oil with palm fatty acid distillate improves lipid utilization in rainbow trout, *Oncorhynchus mykiss*, raised at two different water temperatures

**P53**

**Jinghua Chen*, Kangsen Mai, Wei Xu, Qinghui Ai, Wenbing Zhang, Zhiguo Liufu, Hongming Ma, Xiaojie Wang, Beiping Tan**

Effects of partially replacing fish meal with fermented soybean meal and
<table>
<thead>
<tr>
<th>Session</th>
<th>Title</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>P54</td>
<td>Incorporation of a mixture of plant feedstuffs as substitute for soybean meal in diets of juvenile tilapia, <em>Oreochromis niloticus</em></td>
<td>Yunxue Guo, Xiaohui Dong, Beiping Tan, Qihui Yang, Shuyan Chi, Gang Chen, Lu Zhang</td>
</tr>
<tr>
<td>P55</td>
<td>The effect of Antarctic krill (<em>Euphausia superb</em>) meal on the growth and immunity of juvenile <em>Epinephelus coioides</em></td>
<td>Huang Yanqing*, Gao Lu jiao, Lu Jian xue</td>
</tr>
<tr>
<td>P56</td>
<td>Effects of dehulled soybean meal replaced fish meal on growth, body composition and biochemical parameters of clarias lazera</td>
<td>Lifang Wu*, Guixin Qin, Dongming Zhang, Honghe Wang, SUN Ze-wei, SUN Ling, ZHU Dan</td>
</tr>
<tr>
<td>P57</td>
<td>Studies on replacement of fish-origin meal and oil with those of vegetable-origin in diets for a marine teleost <em>Siganus canaliculatus</em></td>
<td>Shude Xu*, Liang Zhang, Shuqi Wang, Xuebing Liu, Yuanyou Li’</td>
</tr>
<tr>
<td>P58</td>
<td>Effects of replacement of fish meal by soy protein concentrate on growth performance and protein metabolism of <em>Pelteobagrus vachelli</em> juvenile</td>
<td>Kangsen Mai,Yinghao Yang*,Qinghui Ai</td>
</tr>
<tr>
<td>P59</td>
<td>Replacing dietary fish oil with vegetable oil affect oleic acid uptake and metabolism in Atlantic salmon (<em>Salmo salar</em> L.) hepatocytes</td>
<td>Jishu Zhou *, Ingunn Stubhaug, Anne V. Krøvel, Pål A. Olsvik, Bente E. Torstensen</td>
</tr>
</tbody>
</table>

### Physiological metabolism

<table>
<thead>
<tr>
<th>Session</th>
<th>Title</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>P60</td>
<td>Effect of Starvation on the Activities and mRNAs expression of Enzymes about Lipid Metabolism in Hybrid Tilapia (<em>Oreochromis niloticus</em> × <em>O.Areus.</em>)</td>
<td>Chunyan Han, Xiaobo Wen*, Qingmei Zheng, Haobo Li</td>
</tr>
<tr>
<td>P61</td>
<td>Dietary methylmercury and n-3/n-6 ratio affects lipid metabolism in Atlantic salmon</td>
<td>Bente E. Torstensen*, Lise Andreasen, Pål A. Olsvik &amp; Heidi Amlund</td>
</tr>
<tr>
<td>P62</td>
<td>Renal threshold for urinary lysine excretion by tilapia in response to orally administered and injected lysine</td>
<td>Chyng-Hwa Liou*, Yen-Yu Chen, Shuenn-Der Yang, Fu-Guang Liu</td>
</tr>
<tr>
<td>P63</td>
<td>Whole-body in vivo enzyme activity of fatty acid metabolism in barramundi, <em>Lates calcarifer</em></td>
<td>Ramez Alhazzaa , Andrew Bridle, Peter Nichols and Chris Carter</td>
</tr>
<tr>
<td>P64</td>
<td>Fatty acids absorption and lipid metabolism gene expression of Darkbarbel catfish, <em>Pelteobagrus fulvidraco</em>, fed different dietary lipid sources</td>
<td>Chuanjie Qin, Erchao Li, Liqiao Chen*</td>
</tr>
<tr>
<td>P65</td>
<td>Effect of dietary protein and starch levels on growth performance, feed utilization and hepatic amino acid metabolism of juvenile grass carp (<em>Ctenopharyngodon idella</em>) in practical extruded diets</td>
<td>Yong-Jun Chen*, Yong-Jian Liu, Li-Xia Tian, Hui-Jun Yang, Gui-Ying Liang, Lian Gan, Jian-Jun Liang</td>
</tr>
<tr>
<td>P66</td>
<td>Effects of dietary n-3 HUFA on growth performance and lipid metabolism in juvenile grass carp, <em>Ctenopharyngodon idellus</em></td>
<td>Hong Ji*, Jie Li, Pin Liu</td>
</tr>
<tr>
<td>Page</td>
<td>Authors</td>
<td>Title</td>
</tr>
<tr>
<td>------</td>
<td>---------</td>
<td>-------</td>
</tr>
<tr>
<td>P68</td>
<td>Lei Pan*, Gao D. Liang, Ping Xie, Wen S. Zhou</td>
<td>Hematological responses of southern catfish (Silurus meridionalis) to intraperitoneal injection of free gossypol with determination of LC50s</td>
</tr>
<tr>
<td>P69</td>
<td>Weifang Wang*, Dongfang Deng, Nicola De Riu, Silas S. O. Hung</td>
<td>Heat shock response (Hsp70) in white sturgeon and green sturgeon exposed to different stressors</td>
</tr>
<tr>
<td>P70</td>
<td>Ingunn Stubhaug, Geneviève Corraze, Catherine I. Kolditz, Sadasivam J. Kaushik, Gregory. Guernec2, Stéphane Panserat1, Françoise Médale1*</td>
<td>Changes in the liver transcriptome induced by a plant based diet compared to a marine based diet in two lines of rainbow trout selected for muscle fat content.</td>
</tr>
<tr>
<td>P73</td>
<td>Yuanhui DUAN*, Xiaoming ZHU, Yunxiao YANG, Dong. HAN, Shouqi. XIE</td>
<td>Effect of dietary choline on energy utilization of juvenile gibel carp (Carassius auratus gibelio)</td>
</tr>
<tr>
<td>P74</td>
<td>Guangpeng Feng*, Ping Zhuang, Longzhen Zhang, Jianyi Liu</td>
<td>Energy resource changes in blood of juvenile Chinese sturgeon Acipenser sinensis during starvation</td>
</tr>
<tr>
<td>P75</td>
<td>Hong Ji *, Haitao Sun, Wei Zhang</td>
<td>Partial characterization and activity distribution of proteases,α-amylase and lipase in paddlefish Polyodon spathula</td>
</tr>
<tr>
<td>P76</td>
<td>Shiliang Zhang, Hongming Ma*, Kangsen Mai, Qinghui Ai, Wenbing Zhang, Xiaojie Wang, Zhiguo Liufu, Wei Xu</td>
<td>Effects of dietary carbohydrate on growth performance and metabolism in juvenile yellow catfish, Pelteobagrus vachli</td>
</tr>
</tbody>
</table>

**Cellular and molecular nutrition**

<table>
<thead>
<tr>
<th>Page</th>
<th>Authors</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>P77</td>
<td>Indra Suharman, Shuichi Satoh*, Yutaka Haga, Masato Endo, Ikuo Hirono, and Takashi Aoki</td>
<td>Tissues distribution of foreign DNA from genetically modified soybean meal in practical diets for Nile tilapia, Oreochromis niloticus.</td>
</tr>
<tr>
<td>P78</td>
<td>Peiqiang Yu*</td>
<td>Inherent Structural Characteristics of Feed at a Cellular Level in Relation to Nutrition Availability: A Novel Approach</td>
</tr>
<tr>
<td>P79</td>
<td>Xiaozhong Zheng, Zhaokun Ding, Youqing Xu, Oscar Monroig, Sofia Morais, Douglas R. Tocher</td>
<td>Characterisation of cDNAs of fatty acyl Δ6 desaturase and elovl5 elongase of cobia (Rachycentron canadum)</td>
</tr>
<tr>
<td>P80</td>
<td>Xu-Fang Liang</td>
<td>Molecular and functional characterization of leptin genes in major Chinese cultivated freshwater fishes</td>
</tr>
<tr>
<td>P81</td>
<td>Jun Jiang, Lin Feng, Yang Liu, Wei-Dan Jiang, Kai Hu, Shu-Hong Li,</td>
<td></td>
</tr>
<tr>
<td>Paper No.</td>
<td>Title and Authors</td>
<td></td>
</tr>
<tr>
<td>-----------</td>
<td>------------------</td>
<td></td>
</tr>
</tbody>
</table>
| P82 | Real-time PCR quantification of the in vitro effects of glutamine on glutaminase and target of rapamycin gene expression in intestinal epithelial cells of Jian carp (*Cyprinus carpio* var. Jian)  
Xiao-Qiu Zhou* |
| P83 | Effects of threonine on growth, protein synthesis and TOR gene expression in intestinal epithelial cells of Jian carp (*Cyprinus carpio* var. Jian)  
Lin Feng, Weidan Jiang, Jun Jiang, Kai Hu, Yang Liu, Shuhong Li, Xiaoqiu Zhou* |
| P84 | Effects of emodin, Vitamin E on the growth performance, gene expression of heat shock protein 70 (HSP70) and disease resistance of Wuchang bream (*Megalobrama amblycephala*)  
Liu Bo*, Ming Jianhua, Xie Jun, Ge Xianping, Xu Pao, Liu Wenbin |
| P85 | Molecular cloning, characterization and mRNA expression of selenium-binding protein in abalone (*Haliotis discus hannai* Ino) in response to dietary selenium, iron and zinc  
Chenglong Wu, Wenbing Zhang, Kangsen Mai, Xufang Liang, Wei Xu, Jia Wang, Hongming Ma |
| P86 | Changes in gene expression in gut tissue from rainbow trout (*Oncorhynchus mykiss*) fed mycelium biomass from fungi (*Rhizopus oryzae*)  
Liv Torunn Mydland*, Stanko Skugor, Thor Landsverk, Tone S. Martinsen, Anders Kiessling, Trond Storebakken, Lars Edebo and Aleksei Krasnov |
| P87 | Time response expression profiles of genes, possibly associated with effects of altered intestinal microbiota in Atlantic salmon (*Salmo salar L.*) fed soybean meal.  
Elin C Valen*, Inderjit Singh Marjara, Trond M Kortner, Åshild Krogdahl and Anne Marie Bakke |
| P88 | In vitro study of fish adipocyte differentiation by using a primary culture system  
Hiromi Oku* |
| P89 | Transcriptomics (oligo microarray) to assess the effects of different diets on gene expression in heart skeletal muscle inflammation virus infection in Atlantic salmon  
Laura Martínez*, Sofia J. Morais, Gordon Bell, José L. González Vecino, Simon Wadsworth, Douglas R. Tocher |
| P90 | Growth and muscle proteome response to fish protein hydrolysates in the diet of zebrafish  
Mahaut de Vareilles*, Pedro Gomêz-Requeni, Katerina Kousoulaki, Odete Cordeiro, Tomé S. Silva, Nadège Richard, Luis E.C. Conceição, Pedro M. Rodrigues and Ivar Rønnestad |
| P91 | Expression of genes of long-chain polyunsaturated fatty acid (LC-PUFA) biosynthesis during early embryogenesis in cobia (*Rachycentron canadum*)  
| P92 | Evaluation of candidate reference genes for quantitative real-time PCR (qPCR) assays of distal intestinal tissues after partial soybean meal replacement in diets for Atlantic salmon (*Salmo salar L.*)  
Trond M Kortner*, Elin C Valen, Elvis Chikwati and Åshild Krogdahl |
| P93 | Construction of the hepatopancreas and kidney cDNA subtractive library of abalone (*Haliotis discus hannai* Ino. fed with vitamin C-deficiency diet  
Jia Wang*, Hongming Ma, Kangsen Mai, Wenbing Zhang, Zhiguo Liufo |
| P93 | Brett Glencross*, Simon Tabrett, Simon Irvin, Nick Wade, David Smith, Greg Coman, Nigel Preston  
Genotype by diet interactions in feed utilisation by Black tiger shrimp, *Penaeus monodon* |
| P94 | Xavier Rollin*, Christophe Manssens, Yvan Larondelle  
The effect of protein growth rate on the optimum dietary indispensable amino acid pattern in growing rainbow trout (*Oncorhynchus mykiss*) fry including requirements for maintenance and for tissue accretion |
| P95 | Xavier Rollin*, Quentin Watthez, Frédéric Dumonceau, Yvan Larondelle  
The influence of adiposity of brown trout (*Salmo trutta fario*) fry on their feeding motivation, agonistic behavior and territorial ability at low density |
| P96 | Reza Malekzadeh Vialyeh and Habib Mohammadi  
Taking steps toward mass culture of two native *Brachionus* rotifers from Iran: estimation of temperature preference |
| P97 | E. Gisbert*, A. Skalli, Y. Kotzamanis, Zambonino-Infante, J.L.  
Effects of inclusion of yeast and pig blood protein hydrolysates in microdiets on larval quality of gilthead sea bream *Sparus aurata* |
| P98 | G. Rotllant*, E. Gisbert and M. Solé  
Effect of captivity in digestive and metabolic enzymes in the spider crab *Maja brachydactyla* broodstock |
| P99 | Qiyou XU, Changan WANG, Hong XU, Jiasheng Yin, Jianzhang MA  
Effects of Glutamine Dipeptide on the Growth Performance and Antioxidant of *Hucho Taimen* larvae |
| P100 | Franco Daprá, Inge Geurden, Geneviève Corraze*, José-Luis Zambonino-Infante, Stéphanie Fontagné-Dicharry  
Comparison of the effects of dietary phospholipids on intestinal lipid absorption between rainbow trout fry and juveniles |
Effects of different dietary vitamin A levels on the spawning performance and offspring quality of gilthead sea bream *Sparus aurata* broodstock |
| P102 | I. Fernández, F. Hontoria and E. Gisbert  
Vitamin A and Senegal sole *Solea senegalensis* larvae: the effect of timing and dietary content on larval performance. |
| P103 | Nadège Richard*, Mahaut de Vareilles, Paulo J. Gavaia, Tomé S. Silva, Odete Cordeiro, Manuel Yúfera, Pedro M. Rodrigues and Luis E.C. Conceição  
Inclusion of protein hydrolysates in the diet of white seabream (*Diplodus sargus*) larvae: impacts on skeleton quality and larvae proteome expression |
| P104 | Valeria S. Scabini*, H. Fernández-Palacios, L. Robaina, T. Kalinowski and Marisol Izquierdo  
Inclusion of natural carotenoids in diets for gilthead sea bream broodstock (*Sparus aurata* L.) and it’s effect on spawning |
| P105 | Reda Saleh Mohamed Ibrahim, M.B. Betancor, E. Atalah, J. Roo, T. Benítez-Santana and M.S. Izquierdo  
Effects of different dietary phospholipids levels on development of gilthead sea bream (*Sparus aurata*) larvae |
<p>| P106 | Noélie Bodin, Tran Thi Nang Thu, Eric Le Boulengé, Yvan Larondelle, |</p>
<table>
<thead>
<tr>
<th>ISFNF 2010</th>
<th>Qingdao • China</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>P107</strong></td>
<td>Xavier Rollin*, Frédéric Dumonceau, Yvan Larondelle</td>
</tr>
<tr>
<td></td>
<td>The effect of adiposity of brown trout (Salmo trutta fario) fry on survival, body composition and energy utilization during long-term fasting at three different temperatures</td>
</tr>
<tr>
<td><strong>P108</strong></td>
<td>Xavier Rollin*, Adrien Nemry, Yvan Larondelle</td>
</tr>
<tr>
<td></td>
<td>Adiposity depress voluntary feed intake and lipid deposition, but not growth and protein retention efficiency in brown trout (Salmo trutta fario) fry</td>
</tr>
<tr>
<td><strong>P109</strong></td>
<td>Tran Thi Nang Thu, Julien Henrotte, Yvan Larondelle, Xavier Rollin*</td>
</tr>
<tr>
<td></td>
<td>Partial substitution of fish meal by sesame oil cake (Sesamum indicum) in the diet of brown trout (Salmo trutta fario) and rainbow trout (Oncorhynchus mykiss) fry: an interspecific comparison</td>
</tr>
<tr>
<td><strong>P110</strong></td>
<td>Shou Wang*, Torstein Harboe, Ivar Ronnestad</td>
</tr>
<tr>
<td></td>
<td>Growth and gut transit time of Atlantic halibut larvae reared under shifting light regime</td>
</tr>
</tbody>
</table>

**Crustacean nutrition/metabolism**

<p>| <strong>P111</strong>    | Yu wei, Junming Cao*, Hongxia Zhao, Yanhua Huang, Xiaohua Liu, Bing Chen, Hanbing Lan  |
|             | Effect of different selenium sources on growth performance and oxidation resistance of Litopenaeus vannamei |
| <strong>P112</strong>    | Dan-dan Xu*, Jun-ming Cao, Han-bing Lan, Yan-hua Huang, Guo-li Li, Hong-xia Zhao, Bing Chen  |
|             | Effects of dietary nucleotides level on growth performance and immune activities for juvenile Litopenaeus vannamei |
| <strong>P113</strong>    | Jie Chang, Wen-Bing Zhang*, Kang-Sen Mai  |
|             | Effects of dietary β-glucan and glycyrrhizin on non-specific immunity and disease resistance of white shrimp, Litopenaeus vannamei challenged with Vibrio alginolyticus |
| <strong>P114</strong>    | Ai Chunxiang, Chen Li-qiao*, Liu Xiao-ling, Gao Lu-jiao  |
|             | Effect of Dietary Vitamin E on the non-specific immunity of Chinese Mitten-Handed Crab, Eriocheir sinensis |
| <strong>P115</strong>    | Ai Chunxiang*, Xu Hua, Chen Yufeng, Li Shaojing  |
|             | Effects of Cr⁶⁺ Stress on the non-specific immunity of Scylla paramamosain |
| <strong>P116</strong>    | Hong-Xia Zhao, Jun-Ming Cao*, An-Li Wang, Chao Xia Yea, Han-Bing Lan, Xiao-Hua Liu, Guo-Li Li, Shui-Hua Liu  |
|             | Effect of long-term administration of dietary β-1,3-glucan on growth, physiological and immune responses in Litopenaeus vannamei (Boone, 1931) |
| <strong>P117</strong>    | Xiaoying Chen*, Junming Cao, Yanhua Huang, Guoli Li, Bing Chen, Hongxia Zhao, Hanbing Lan  |
|             | Effects of two types of vitamin B1 on growth Performance, Body Composition and Biochemical Indices of Litopenaeus vannamei |
| <strong>P118</strong>    | Sui-hua Liu, Jun-ming Cao*, Yan-hua Huang, Hong-xia Zhao, Guo-li Li, Han-bing Lan, Li Liu  |
|             | Influence of different dietary 18:3n-3/18:2n-6 ratio on growth performance, body composition and fatty acid composition in Pacific white shrimp, Litopenaeus |</p>
<table>
<thead>
<tr>
<th>Page</th>
<th>Title</th>
<th>Authors</th>
<th>Abstract</th>
</tr>
</thead>
<tbody>
<tr>
<td>P119</td>
<td>Effects of two types of vitamin B&lt;sub&gt;6&lt;/sub&gt; on growth performance, body composition and serum biochemical indices of <em>Litopenaeus vannamei</em></td>
<td>Wei-liang Jiang*, Jun-ming Cao, Yan-hua Huang, Jun-ru Hu, Hong-xia Zhao, Bing Chen, Han-bing Lan</td>
<td></td>
</tr>
<tr>
<td>P120</td>
<td>Apparent digestibility coefficients of selected feed ingredients for white shrimp <em>Litopenaeus vannamei</em>, Boone</td>
<td>Yi-Rong Yue*, Yong-Jian Liu, Li-Xia Tian, Hui-Jun Yang, Gui-Ying Liang, Lian Gan, Yu-Jie Gao, Wen-Jia Luo</td>
<td></td>
</tr>
<tr>
<td>P121</td>
<td>Expression of fusionprotein of fortilin, TAT and GFP in <em>Pichia pastoris</em> and effects on immune activities of haemocyte of white shrimp <em>Litopenaeus vannamei</em></td>
<td>Yi Zhou, Wenbing Zhang, Kangsen Mai, Xiaojie Wang, Qinghui Ai, Hongming MA, Wei Xu, Zhiguo Liu</td>
<td></td>
</tr>
<tr>
<td>P122</td>
<td>Influence of salinity on growth and survival of <em>Litopenaeus vannamei</em> and <em>L. setiferus</em> reared at 24°C</td>
<td>Binh Thanh Nguyen*, Saichiro Yokoyama, Manabu Ishikawa, Shunsuke Koshio</td>
<td></td>
</tr>
<tr>
<td>P124</td>
<td>Glutamate dehydrogenase and Na&lt;sup&gt;+&lt;/sup&gt;-K&lt;sup&gt;+&lt;/sup&gt; ATPase mRNA expression and growth of pacific white shrimp, <em>Litopenaeus vannamei</em>, fed on different dietary protein</td>
<td>Erchao Li, Leticia Arena, Gabriel Lizama, Gabriela Gaxiola, Gerard Cuzon, Carlos Rosas, Liqiao Chen, Alain Van Wormhoudt</td>
<td></td>
</tr>
<tr>
<td>P126</td>
<td>The effects of dietary Chinese herbs on growth, digestive enzyme and immunity of <em>Litopenaeus vannamei</em></td>
<td>Li Zhuo-Jia*, Lu Xin, Lin Hei-Zhao, Yuan Feng-Hua</td>
<td></td>
</tr>
<tr>
<td>P127</td>
<td>Effects of feeding timing of dietary Chinese herbs on digestive and immune enzymes of <em>Litopenaeus vannamei</em></td>
<td>Lin Hei-Zhao*, Lu Xin, Li Zhuo-Jia, Yuan Feng-Hua</td>
<td></td>
</tr>
<tr>
<td>P128</td>
<td>Effect of seven different carbohydrates on the growth, survival, nutrients composition and enzymes activities of liver 6-phosphogluconate Dehydrogenase and glucokinase</td>
<td>Niu Jin*, Lin Heizhao, Liu Yongjian, Tian Lixia, Chen Xu, Huang Zhong</td>
<td></td>
</tr>
<tr>
<td>P129</td>
<td>Effects of stocking density on growth and optimal dietary protein requirement of Pacific white shrimp, <em>Litopenaeus vannamei</em></td>
<td>Li-Ping Shang*, Ni-Na Gou, Li-Rong Bai, Yu-Tao Miao, An-Li Wang</td>
<td></td>
</tr>
<tr>
<td>P130</td>
<td>The dietary effect of xylooligosaccharides and immune response of <em>Litopenaeus vannamei</em> on low temperature stress</td>
<td>Yang Yang*, Yu-Tao Miao, Jun-Wa Huang, Xiao-Chun Wu, Dan Jiang, An-Li Wang</td>
<td></td>
</tr>
<tr>
<td>P131</td>
<td>The dietary effect of xylooligosaccharides and immune response of <em>Litopenaeus vannamei</em> on low temperature stress</td>
<td>Chaoxia Ye, Anli Wang, Jian’an Xian, Weina Wang, Yutao Miao, Shaoan Liao</td>
<td></td>
</tr>
<tr>
<td>Paper Number</td>
<td>Title</td>
<td>Authors</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>----------------------------------------------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>P132</td>
<td>Flow cytometry as a tool to study the effects of dietary zinc on <em>Litopenaeus vannamei</em> under nitrite stress</td>
<td>Fanny Yasumaru, Daniel Lemos*</td>
<td></td>
</tr>
<tr>
<td>P133</td>
<td>Quality control of raw materials for juvenile <em>Litopenaeus vannamei</em> diets: <em>in vitro</em> prediction of protein digestibility</td>
<td>Chyng-Hwa Liou, Carina Miranda Tayag, Yong-Chin Lin, Jiann-Chu Chen*</td>
<td></td>
</tr>
<tr>
<td>P134</td>
<td>Dietary administration of <em>Spirulina platensis</em> increased the immune response of white shrimp <em>Litopenaeus vannamei</em></td>
<td>Shogo Harakawa*, Shunsuke Koshio, Manabu Ishikawa, Saichiro Yokoyama</td>
<td></td>
</tr>
<tr>
<td>P135</td>
<td>Effect of the polychaetes extracts on growth, stress tolerance and immune responses of kuruma shrimp (<em>Marsupenaeus japonicus</em>) juveniles</td>
<td>Wutiporn Phromkunthong*, Manthana Jaiyen, Amonrat Sermwatanakul, Mali Boonyaratpalin, Paiboon Bunlipatanon and Patcharee Soonson</td>
<td></td>
</tr>
<tr>
<td>P136</td>
<td>Dietary administration of <em>Spirulina platensis</em> increased the immune response of white shrimp <em>Litopenaeus vannamei</em></td>
<td>Shogo Harakawa*, Shunsuke Koshio, Manabu Ishikawa, Saichiro Yokoyama</td>
<td></td>
</tr>
<tr>
<td>P137</td>
<td>Dietary administration of <em>Spirulina platensis</em> increased the immune response of white shrimp <em>Litopenaeus vannamei</em></td>
<td>Wutiporn Phromkunthong*, Manthana Jaiyen, Amonrat Sermwatanakul, Mali Boonyaratpalin, Paiboon Bunlipatanon and Patcharee Soonson</td>
<td></td>
</tr>
<tr>
<td>P138</td>
<td>Dietary Vitamin B1 Requirement of Prawn, <em>Litopenaeus vannamei</em></td>
<td>He Zhi-jiao, Cao Jun-ming, Chen Bing, Lan Han-bing, Huang Yan-hua, Chen Xiao-ying, Pan Qing</td>
<td></td>
</tr>
<tr>
<td>P139</td>
<td>Dietary Vitamin B6 Requirement of Prawn, <em>Litopenaeus vannamei</em></td>
<td>He Zhi-jiao, Cao Jun-ming, Chen Bing, Lan Han-bing, Huang Yan-hua, Jiang Wei-liang, Pan Qing</td>
<td></td>
</tr>
<tr>
<td>P140</td>
<td>Dietary Vitamin B1 Requirement of Prawn, <em>Litopenaeus vannamei</em></td>
<td>Chen Bing, Cao Jun- ming, CHEN Ping-jie, Zhao Hong-xia, Lan Han-bing, Zhu Xuan</td>
<td></td>
</tr>
<tr>
<td>P141</td>
<td>Dietary Vitamin B6 Requirement of Prawn, <em>Litopenaeus vannamei</em></td>
<td>Chen Cheng-xun*, Bai Dong-qing, Xing Ke-zhi, Guo Yong-jun, Zheng Yan</td>
<td></td>
</tr>
<tr>
<td>P142</td>
<td>Dietary Vitamin B1 Requirement of Prawn, <em>Litopenaeus vannamei</em></td>
<td>Shengming Sun, Ming Chen, Liqiao Chen*, Haibo Jiang, Erchao Li</td>
<td></td>
</tr>
<tr>
<td>P143</td>
<td>Dietary Vitamin B1 Requirement of Prawn, <em>Litopenaeus vannamei</em></td>
<td>Hongbo Jiang, Liqiao Chen*, Erchao Li</td>
<td></td>
</tr>
<tr>
<td>P144</td>
<td>Dietary Vitamin B1 Requirement of Prawn, <em>Litopenaeus vannamei</em></td>
<td>Hongbo Jiang, Liqiao Chen*, Erchao Li</td>
<td></td>
</tr>
<tr>
<td>P145</td>
<td>Dietary Vitamin B1 Requirement of Prawn, <em>Litopenaeus vannamei</em></td>
<td>Yue Wang, Liqiao Chen*, Erchao Li</td>
<td></td>
</tr>
</tbody>
</table>

* indicates corresponding author
<table>
<thead>
<tr>
<th>ISFNF 2010</th>
<th>Qingdao • China</th>
</tr>
</thead>
</table>
| **P146** | WANG Xing-qiang*, CAO Mei  
Effects of dimethylhydantoin and dietary carbohydrate levels on growth and immune factors of *Litopenaeus vannamei* |
| **P147** | Qihui Yang*, Qicun Zhou, Beiping Tan, Xiaqiu Zhou, Xiaohui Dong, Shiyuan Chi  
Effect of partial replacement of dietary fish meal by corn gluten meal on growth, feed utilization and apparent nutrient digestibility of white shrimp *Litopenaeus vannamei*, Boone |
| **P148** | Weijing Zhong, Shengpeng Zhang, Weipei Huang, Jinfeng Li, Anli Wang*  
Effect of conjugated linoleic acid on immune-related genes changes in the Pacific white shrimp *Litopenaeus vannamei* |
| **P149** | Yong Li*, Su D. Xia, Wen Q. Wang, Shi Q. Tang, Hua Wang, Guo X. Sun  
Effect of dietary protein level on growth, water quality, immunity and eco-nutrition requirement for *Litopenaeus vannamei* in high density culture system |
| **Feed processing and feeding** |
| **P150** | Nan Bai*, Wenbing Zhang, Kangsen Mai, Hongming Ma, Qinghui Ai, Xiaojie Wang, Min Gu  
Effects of continuous and alternate administration of β-glucan and mannan-oligosaccharide on the growth, immunity and resistance against *Vibrio splendidus* of sea cucumber *Apostichopus japonicus* |
| **P151** | Jin Xing Xiao*, Qing Jun Shao, Fan Zhou, Bergo Owari Ngandzali, Yuan Jian Xu and Jun Zhuo Xu  
Effects of short-term feed deprivation and refeeding on growth performance, body composition and serum parameters of juvenile black sea bream, *Sparus macrocephalus* |
| **P152** | Keke Zheng*, Mengqing Liang, Qing Chang, Wei Fang  
Feeding strategies for turbot (*Scophthalmus maximus*) juvenile |
| **P153** | Andreas Lemme and Mark S. Redshaw  
Variation of amino acid levels in fishmeal – a review on amino acid analysis |
| **P154** | Ashraf Suloma*, Rania S Mabroke, Osama M. El–Husseiny  
Effect of mixed feeding schedules versus regular feeding diet on Nile tilapia *Oreochromis niloticus* fry performance: Reevaluation using constant ingredient composition |
| **P155** | Namyong Hwang, Jun-Ho Lee, Seunghyung Lee, Young Chul Kim, Jun-Young Bae, Okorie E. Okorie, Mahmoud Mohseni, Gun Hyun Park and Sungchul C. Bai*  
The effects of feeding rate in juvenile olive flounder, *Paralichthys olivaceus*, fed commercial diet at the low temperature season |
| **P156** | Hua Xueming*, Han Jiafeng, Wang Jun, Yu Ning, Zhou Hongqi  
Effects of feeding modes of chitosan on growth and natural killer cells activity of *Ctenopharyngodon idella* |
| **P157** | Yong Li*, Mei Q. Wang, Ting T. Gao, Hua Wang, Su D. Xia, Guo X. Sun  
Effects of protein and satiation degrees on growth and immunity of *Cynoglossus Semilaevis Günther* in industrial culture |
| **P158** | SHUAI Ke, GAO Qi-ping  
Effects of feeding diets of different quality within one day on survival, growth |
<table>
<thead>
<tr>
<th>Page</th>
<th>Title</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>P159</td>
<td>The effect of different feeding rate on the grow performances of Jian carp (Cyprinus carpio Var.Jian)</td>
<td>SHUAI Ke, LI Yun-lan, GAO Qi-ping</td>
</tr>
<tr>
<td>P160</td>
<td>Comparison of the activity of gut enzymes of wild and aquacultured groper (Polyprion oxygeneios) using fluorgenic methylumbelliferyl substrates</td>
<td>Anna Yu. Kilimnik*, Sally A. Anderson, Vicky L. Webb</td>
</tr>
<tr>
<td>P161</td>
<td>Combined effect of vitamin c and vitamin e microdiets for gilthead sea bream Sparus aurata</td>
<td>E. Atalah*, C. M. Hernández Cruz, E. Ganausa, T. Benítez-Santana, R. Ganga, J. Roo, H. Fernández-Palacios, and M.S. Izquierdo</td>
</tr>
<tr>
<td>P162</td>
<td>Feeding behaviour of the burbot (Lota lota L.), a new candidate for freshwater aquaculture</td>
<td>Hendrik Wocher, Frieder J. Schwarz</td>
</tr>
<tr>
<td>P163</td>
<td>Centre for Feed Technology, Fôrtek: Research opportunities</td>
<td>Zimonja, O.</td>
</tr>
<tr>
<td>P164</td>
<td>Development of a pH-Stat method to measure in vitro degree of protein hydrolysis using enzymes from pyloric caeca of Atlantic cod (Gadus morhua L.)</td>
<td>Sean M. Tibbetts, Joyce E. Milley, Neil W. Ross, Santosh P. Lali</td>
</tr>
<tr>
<td>P165</td>
<td>Enzyme pretreatment of high fibre plant ingredients and effects on feed utilisation in rainbow trout (Onchorhynchus mykiss)</td>
<td>Vegard Denstadli, Marie Hillestad, Viviane Verlhac, Mikkel Klausen, Margareth Øverland</td>
</tr>
<tr>
<td>P166</td>
<td>Effect of multi-cycled feeding with low dietary protein and optimal dietary protein on growth and feed utilization of hybrid sturgeon (Acipenser ruthenus Linnaeus ♂ × A. baeri Brandt ♀)</td>
<td>Ying Huang*, Xiaoming Zhu, Wu Lei, Yunxia Yang, Dong Han, Shouqi Xie</td>
</tr>
<tr>
<td>P167</td>
<td>Effect of protein restriction with subsequent realimentation on compensatory growth of juvenile Chinese soft-shelled turtles (Pelodiscus sinensis)</td>
<td>Quansen Xie*, Junwei Li, Yujuan Li, Zhencai Yang</td>
</tr>
<tr>
<td>P168</td>
<td>Effects of different binders on physical properties of microdiets and on growth status of Larval turbot, Scophthalmus maximus</td>
<td>Xiaobing Chen, Kangsen Mai*, Xiaojie Wang, Qinghui Ai, Wenbing Zhang, Hongming Ma, Hui Xu, Zhiguo Liu</td>
</tr>
<tr>
<td>P169</td>
<td>Sodium diformate and extruder temperature affects physical quality of barley protein concentrate-based fish feed</td>
<td>Thea Morken*, Olav F. Kraugerud, Frederic T. Barrows, Mette Sørensen, Trond Storebakken, Margareth Øverland</td>
</tr>
</tbody>
</table>

**Nutrition and environment**

<table>
<thead>
<tr>
<th>Page</th>
<th>Title</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>P170</td>
<td>Response to various culture salinities in two native Brachionus rotifers from Iran</td>
<td>Reza Malekzadeh Viyah and Habib Mohammadi</td>
</tr>
<tr>
<td>P172</td>
<td>Stress response in seabream (Sparus aurata) held under crowded conditions and fed diets with different levels of inclusion of linseed and/or soybean oil</td>
<td>Ganga, R., Bell, J.G., Montero, D., Atalah, E., Acerete, L., Tort, L., Benitez Santana, T., Fernández-Vaquero, A. and Izquierdo, M.S.</td>
</tr>
<tr>
<td>Page</td>
<td>Title</td>
<td>Authors</td>
</tr>
<tr>
<td>------</td>
<td>----------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>P173</td>
<td>Oxygenated nanobubbles as a means to improve nutrition and the environment of finfish and shellfish in order to increase growth and population density</td>
<td>Phil Hamilton* and Peter Appleton</td>
</tr>
<tr>
<td>P174</td>
<td>Effects of crowding stress on environment, growth performance, body composition and some immune factors of <em>Litopenaeus vannamei</em></td>
<td>Ran Guo*, Ya-kun Chen, Hui Xia</td>
</tr>
<tr>
<td>P175</td>
<td>Diversity, abundance and spatial distribution of sediment ammonia-oxidizing Beta-proteobacteria in mariculture environment</td>
<td>Li Jing*, Dang Hongyue, Geng chunxiang, Sun xiuhi</td>
</tr>
<tr>
<td>P176</td>
<td>Accumulation and distribution of heavy metals and total phosphorus in intensive aquatic farm sediments</td>
<td>Wu Xiao-Yi, Yang Yu-Feng*</td>
</tr>
<tr>
<td>P177</td>
<td>The effect of supplementary feeding on water quality during cage culture practice of Oreochromis niloticus in Lake Kuriftu, Ethiopia</td>
<td>Ashagrie Gibtan, Abebe Getahun &amp; Seyoum Mengistou</td>
</tr>
<tr>
<td>P178</td>
<td>Elevated sea temperature push farmed Atlantic salmon to anorexia: Local effects of global warming</td>
<td>Ernst M. Hevrøy, Rune Waagbø, Olav Breck, Harald Takle, Sven M. Jørgensen, Thomas Torgersen, Sissel Susort, Leiv Tvenning, Tom Hansen</td>
</tr>
</tbody>
</table>

**Nutrition and health**

<table>
<thead>
<tr>
<th>Page</th>
<th>Title</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>P181</td>
<td>The Application of Quorum-quenching Enzyme in Anti-infection immunity of Zebrafish (<em>Danio rerio</em>)</td>
<td>Yanan Cao**, Zhigang Zhou*</td>
</tr>
<tr>
<td>P182</td>
<td>Effect of dietary glucan extraction from <em>Ganoderma lucidum</em> on growth performance, immune response and survival in gibel carp (<em>Carassius auratus gibelio</em>) challenged with <em>Edwardsiella tarda</em></td>
<td>Yuhang Chen*, Xiaoming ZHU, Yunxia YANG, Dong. HAN, Shouqi. XIE</td>
</tr>
<tr>
<td>P183</td>
<td>Dietary antibiotics induced changes in the intestinal microbiota alter tilapia physiological responses to infection with <em>Aeromonas hydrophila</em></td>
<td>Zhigang Zhou*, Suzx He, Yuchun Liu, Yanan Cao, Kun Meng, Bin Yao, Einar Ringø, Ilkyu Yoon</td>
</tr>
<tr>
<td>P184</td>
<td>Effects of dietary β-glucan and glycyrrhizin on non-specific immunity and disease resistance of sea cucumber (<em>Apostichopus japonicus</em> Selenka) challenged with <em>Vibrio splendidus</em></td>
<td>Jie Chang, Wen-Bing Zhang*, Kang-Sen Mai</td>
</tr>
<tr>
<td>ID</td>
<td>Title</td>
<td>Authors</td>
</tr>
<tr>
<td>-----</td>
<td>----------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>P187</td>
<td>Assessment of dietary supplementation with a multi-strain probiotic on performance, intestinal microflora, non-specific immune response and antioxidant status of rainbow trout (<em>Oncorhyncus mykiss</em>)</td>
<td>Ilias A. Giannenas, Efstathios Chronis, Sophia Stavrakakis, Maritsa Margaroni, Pedro Encarnacao, Elisabeth Mayer and Evdokia E. Karagouni</td>
</tr>
<tr>
<td>P188</td>
<td>ER stress and ROS play important regulatory roles during development of white adipocytes in Atlantic salmon.</td>
<td>Todorčević M, Škugor S, Krasnov A, Ruyter B</td>
</tr>
<tr>
<td>P189</td>
<td>Aflatoxin B₁ reduces growth performance, physiological response and disease resistance in Tra catfish (<em>Pangasius hypophthalmus</em>).</td>
<td>Tu Do Cam, Pedro Encarnação, Le Thanh Hung</td>
</tr>
<tr>
<td>P190</td>
<td>Effects of dietary yeast culture (DVÁQUA®) on growth performance, intestinal autochthonous bacterial community and non-specific immunity of hybrid tilapia (<em>Oreochromis niloticus ♀ × O. aureus ♂</em>) cultured in cages.</td>
<td>Shuxu He, Zhigang Zhou, Yuchun Liu, Pengjun Shi, Bin Yao, Einar Ringø, Ilkyu Yoon</td>
</tr>
<tr>
<td>P191</td>
<td>Improved disease resistance in sea bass (<em>Dicentrarchus labrax</em>) fed mannan oligosaccharides (MOS)</td>
<td>Silvia Torrecillas, Alex Makol, María José Caballero, Tibiábin Benítez-Santana, Daniel Montero, John Sweetman, Viswanath Kiron and Marisol Izquierdo</td>
</tr>
<tr>
<td>P194</td>
<td>Effects of β-glucan, mannan oligosaccharide and their combinations on growth, immunity and resistance against <em>Vibrio splendidus</em> of sea cucumber, <em>Apostichopus japonicus in vitro</em> and <em>in vivo</em>.</td>
<td>Min Gu, Hongming Ma, Kangsen Mai, Wenbing Zhang, Qinghui Ai, Xiaojie Wang</td>
</tr>
<tr>
<td>Session</td>
<td>Title</td>
<td>Authors</td>
</tr>
<tr>
<td>----------</td>
<td>----------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>P198</td>
<td>Effects of dietary vitamin C and β-glucan on the growth performance, immunity and disease resistance of sea cucumber (Apostichopus japonicus)</td>
<td>Qin Zhang, Kangsen Mai*, Hongming Ma, Wenbing Zhang, Zhiguo Liu, Wei Xu</td>
</tr>
<tr>
<td>P199</td>
<td>Dietary administration of yeast culture enhances the non-specific immunity of sea cucumber Apostichopus japonicus and its resistance against Vibrio splendidus</td>
<td>Qin Zhang, Kangsen Mai*, Hongming Ma, Wenbing Zhang, Zhiguo Liu, Wei Xu</td>
</tr>
<tr>
<td>P200</td>
<td>Immune response of sea cucumber Apostichopus japonicus coelomocytes to several immunostimulants in vitro</td>
<td>Min Gu, Hongming Ma*, Kangsen Mai, Wenbing Zhang, Qinghui Ai, Xiaojie Wang</td>
</tr>
<tr>
<td>P201</td>
<td>The effects of dietary Vitamin C on the oxidative stress of juvenile olive flounder, Paralichthys olivaceus, fed cadmium</td>
<td>Jun-Ho Lee, Seunghyung Lee, Young Chul Kim, Jun-Young Bae, Okorie E. Okorie, Mahmoud Mohseni, Gun Hyun Park, Nam young Hwang and Sungchul C. Bai*</td>
</tr>
<tr>
<td>P202</td>
<td>The potential role of proteinase-activated receptor 2 in soybean meal induced inflammation of the intestine of Atlantic salmon (Salmo salar)</td>
<td>Christian Sahlmann*, Jim Thorsen, Elin C Valen, Trond M Kortner, Åshild Krogdahl</td>
</tr>
<tr>
<td>P203</td>
<td>Effect of zinc on growth, non-specific immunological responses and resistance to Streptococcus agalactiae of sex-reversed red tilapia, Oreochromis niloticus x O. mossambicus</td>
<td>Kidchakan Supamattaya, Boonkob Viriyapongsutee, Jirawat Tadkaew, Chutima Tantikitti*, Wutiporn Phromkunthong</td>
</tr>
<tr>
<td>P204</td>
<td>The combined used of nucleotides and immune-stimulant (Sanictum®) in feed against salmon rickettsia syndrome</td>
<td>José L. Gonzalez Vecino*, Simon Wadsworth, Javier Gonzalez, Kari Ruohonen</td>
</tr>
<tr>
<td>P205</td>
<td>Interaction of arachidonic acid and vitamin E on the immune response to pathogen challenge in juvenile Atlantic salmon (Salmo salar)</td>
<td>Patricio Dantagnan*, Katerina Gonzáles, Martín Hevia, Takahiro Ogura, Adrián Hernández, Aliro Bóquez, Javier Alcaimo</td>
</tr>
<tr>
<td>P206</td>
<td>Fucoidan derived from seaweed give effectiveness on enhancement of non-specific immune response in Japanese flounder Paralichthys olivaceus</td>
<td>Takuma Sakurai*, Saichiro Yokoyama, Manabu Ishikawa, Shunsuke Koshio</td>
</tr>
<tr>
<td>P207</td>
<td>In vivo and in vitro reduced gut bacterial translocation in relation to mucus production and immune activity and disease resistance in sea bass (Dicentrarchus labrax) fed mannan oligosaccharides (MOS)</td>
<td>Silvia Torrecillas*, Alex Makol, Maria José Caballero, Daniel Montero, John Sweetman and Marisol Izquierdo.</td>
</tr>
<tr>
<td>P208</td>
<td>Effects of dietary selenium and vitamin E on glutathione peroxidase activities in soft-shelled turtle, Pelodiscus sinensis</td>
<td>Ying-Jhih Lin*, Chen-Huei Huang</td>
</tr>
<tr>
<td>P209</td>
<td>Effects of iron and zinc levels on total antioxidant capacity and nitric oxide</td>
<td>Li-Rong Bai*, Yu-Tao Miao, Hong-Yan Kou, Li-Ping Shang, Hai-Lan Lian, Zhao-Hui Zhu, Yong-Xiang He, Jian-An Xian, Wei-Jing Zhong, An-Li Wang</td>
</tr>
<tr>
<td>ISBN: F 2010</td>
<td>Qingdao • China</td>
<td></td>
</tr>
<tr>
<td>---------------------------------------------</td>
<td>------------------------------------------</td>
<td></td>
</tr>
<tr>
<td><strong>P210</strong></td>
<td><strong>Qing Chang</strong>, Meng-Qing Liang, Jin Zhou</td>
<td></td>
</tr>
<tr>
<td>Synthase of Tilapia (Oreochromis niloticus × O. aureus)</td>
<td>Effect of dietary vitamin E and selenium on growth, nonspecific immune responses and disease resistance in Japanese flounder (Paralichthys olivaceus)</td>
<td></td>
</tr>
<tr>
<td><strong>P211</strong></td>
<td><strong>Wei-na Xu</strong>, Wen-bin Liu, Xing-xing Fang, Xian-ping Shao, Guang-zhen Jiang</td>
<td></td>
</tr>
<tr>
<td>The protection of vitamin C on trichlorfon-stress in Carassius auratus gibelio</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>P212</strong></td>
<td><strong>Yancui Zhao</strong>*, Hongming Ma, Kangsen Mai, Wenbing Zhang, Qinghui Ai, Wei Xu, Xiaojie Wang, Zhiguo Liu Fu</td>
<td></td>
</tr>
<tr>
<td>Influence of dietary probiotic Bacillus TC22 and fructooligosaccharide on growth, immune capacity, microflora and disease resistance in sea cucumber Apostichopus japonicus</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>P213</strong></td>
<td><strong>Houguo Xu</strong>, Qinghui Ai*, Kangsen Mai, Wenbing Zhang, Wei Xu, Xiaojie Wang, Hongming Ma, Zhiguo Liu Fu, Jun Wang, Rantao Zuo</td>
<td></td>
</tr>
<tr>
<td>The effects of dietary glycyrrhizic acid on growth, survival and immune response of large yellow croaker, Pseudoscaena crocea</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>P214</strong></td>
<td><strong>Boon kob Viriyapongsutee</strong>, Kiron Viswanath, Naraid Suanyuk, Kidchakan Supamattaya, Wutiporn Phromkunthong and Chutima Tantikitti</td>
<td></td>
</tr>
<tr>
<td>Effects of inulin and Lactobacillus plantarum on growth, bacterial quantity in hepatopancreas and intestine and resistance to Vibrio harveyi of Pacific white shrimp (Litopenaeus vannamei)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Nutrient Requirements and Availability</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>P215</strong></td>
<td>Xavier Rollin*, Jonathan Brel, Junio Dort, Mathieu Haerinck, Tarik Abboudi, Yvon Carpentier, Yvan Larondelle</td>
<td></td>
</tr>
<tr>
<td>Nutrient Requirements and Availability</td>
<td>Long-term effect of a severe n-3 polyunsaturated fatty acid-restricted diet during the juvenile period on the efficacy of a linseed oil finishing strategy and on apparent in vivo D-6 desaturase activity in rainbow trout (Oncorhynchus mykiss)</td>
<td></td>
</tr>
<tr>
<td><strong>P216</strong></td>
<td>Ying Hua*, Guan Yun Zhong, Qing Jun Shao, Fan Zhou, Berge Owari Ngandzali, Yuan Jian Xu and Jun Zhuo Xu</td>
<td></td>
</tr>
<tr>
<td>Effects of different phosphorus sources on the growth performance of juvenile black sea bream, Sparus macrocephalus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Effects of dietary copper levels on growth, hematological parameters and tissue copper accumulation in juvenile olive flounder (Paralichthys olivaceus)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>P218</strong></td>
<td>Rossita Shapawi*, Saleem Mustafa, Ng Wing-Keong</td>
<td></td>
</tr>
<tr>
<td>Effects of dietary carbohydrate source and level on growth, feed utilization and body composition of the humpback grouper, Cromileptes altivelis</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>P219</strong></td>
<td>JunMing Cao*, Yan Chen, XuanZhu, Yan-huaHuang, Hong-xia Zhao, Guo-li Li, Han-bing Lan, Bing Chen, Qing Pan</td>
<td></td>
</tr>
</tbody>
</table>
| Study on Dietary L-Lysine Requirements for Juvenile Pelteobagrus fulvidraco | *
<p>| <strong>P220</strong>                                   | Li Luo, Shi Chen, Yun Li, Shi-mei Lin, Fu-bao Wang, Ya-ge Wang, Hua Wen |
| Dietary leucine requirement of juvenile Grass carp Ctenopharyngodon idella | |
| <strong>P221</strong>                                   | Ya-ge Wang, Li Luo, Hua Wen, Fu-bao Wang, Shi Chen |
| Dietary valine requirement of juvenile grass carp Ctenopharyngodon idella | |
| <strong>P222</strong>                                   | Chao-ming Wang, Li Luo, Shi-mei Lin, Gui-zhong Zhang, Wei-min Shang, Zhong Yan, Ben-xiang Liu |</p>
<table>
<thead>
<tr>
<th>Paper No.</th>
<th>Title</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>P224</td>
<td>Effects of variation in dietary protein content on growth performance and feed utilization of mature Chinese softshell turtle, Pelodiscus sinensis</td>
<td>Mingchun Ren*, Qinghui Ai, Kangsen Mei, Wei Xu, Wenbing Zhang, Xiaojie Wang, Zhiqiu Liufu</td>
</tr>
<tr>
<td>P226</td>
<td>Effects of dietary lipid levels on growth performance, nutrient digestibility and blood biochemical indices of gift tilapia (Oreochromis niloticus)</td>
<td>WANG Ai-Min, HAN Guang-Ming, LV Fu, FENG Gong-Neng, YU Ye-Bing, YANG Wen-Ping, GUO Jia-Hao, WANG Tian, XU Pao*</td>
</tr>
<tr>
<td>P227</td>
<td>Effect of dietary iron levels on growth, body composition and intestinal enzyme activities of juvenile Jian carp (Cyprinus carpio var. Jian)</td>
<td>Yurong Zou, Kangsen Mai*, Qinghui Ai</td>
</tr>
<tr>
<td>P228</td>
<td>Study on the specific growth rate and changes of digestive enzyme activities of the juvenile soft-shelled turtle, Trionyx sinensis</td>
<td>Zhang Jun, Ye Yuan-tu*, Cai Chun-fang, Yin Xiao-jing, Jin Su-ya, Yin Yong-feng</td>
</tr>
<tr>
<td>P229</td>
<td>Effect of wheat on the growth performance of grass carp (Ctenopharyngodon idellus)</td>
<td>Ying Xiao-jing, Ye Yuan-tu*, Jin Su-ya, Xiang Chao-lin, Qiu Yan Zang Jun</td>
</tr>
<tr>
<td>P231</td>
<td>Effect of Different Protein Level on Growth and Survival of the Catla catla (Hamilton) Reared in glass aquaria</td>
<td>Thanongsak Thanuthong*, David S. Francis, Paul L. Jones, Shyamalie D Senadheera, Giovanni M. Turchini</td>
</tr>
<tr>
<td>P232</td>
<td>Canceled</td>
<td></td>
</tr>
<tr>
<td>P233</td>
<td>In vitro pH-stat determination of protein digestibility in fish species: recovery and standardization of enzyme extracts for acid and alkaline hydrolysis assays in juvenile rainbow trout and Nile tilapia</td>
<td>Daniel Lemos*, Fanny Yasumaru</td>
</tr>
<tr>
<td>P234</td>
<td>The effects of total dietary C18 PUFA on growth performance and fillet fatty acid composition of rainbow trout (Oncorhynchus mykiss) and subsequent fatty acid wash-out using a finishing period</td>
<td>Thanongsak Thanuthong*, David S. Francis, Paul L. Jones, Shyamalie D Senadheera, Giovanni M. Turchini</td>
</tr>
<tr>
<td>P235</td>
<td>Efficacy of ALA/LA ratio on growth performance and fillet fatty acid profile of Murray cod and subsequent fatty acid restoration by a finishing diet</td>
<td>Shyamalie D. Senadheera*, David S. Francis, Thanongsak Thanuthong, Giovanni M. Turchini</td>
</tr>
<tr>
<td>P236</td>
<td>Dhanapong Sangsue*, Orapint Jintasataporn, Oratai Trivutanon, and</td>
<td></td>
</tr>
<tr>
<td>ID</td>
<td>Title</td>
<td>Authors</td>
</tr>
<tr>
<td>------</td>
<td>----------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------</td>
</tr>
<tr>
<td>P237</td>
<td>Reduction of protein in diets for hybrid catfish (Clarias macrocephalus x Clarias gariepinus) with special focus on amino acid supplementation</td>
<td>Andreas Lemme</td>
</tr>
<tr>
<td>P238</td>
<td>Fishmeal reduction in diets for sex-reversed red hybrid tilapia (Oreochromis spp.) with focus on amino acid supply</td>
<td>Orapint Jintasataporn, Prathak Tabthipwon, Dhanapong Sangsue and Andreas Lemme</td>
</tr>
<tr>
<td>P239</td>
<td>Dietary lysine requirement of juvenile Pacific threadfin (Polydactylus sexfilis)</td>
<td>Dong-Fang Deng, Warren Dominy, Zhi Yong Ju, Shunsuke Koshio, Ryan Murashige</td>
</tr>
<tr>
<td>P240</td>
<td>Dietary L-methionine requirement of juvenile black sea bream Sparus macrocephalus at a constant dietary cystine level</td>
<td>Fan Zhou, Qing Jun Shao, Jin Xing Xiao, Bergo Owari Ngandzali, Yuan Jian Xu and Jun Zhuo Xu</td>
</tr>
<tr>
<td>P241</td>
<td>Dynamic and integrated production biology of farmed salmon in sea. Effects of dietary supplementation of the bioactive fatty acid TTA.</td>
<td>Magny S. Thomassen, Henriette Alne, Maike Oehme, Rørvik K-A</td>
</tr>
<tr>
<td>P242</td>
<td>Effects of feeding non-marine raw materials on performance, gut microbiota and intestinal histology in Atlantic salmon (Salmo salar L.) – New Microbial Insights</td>
<td>Mali Bjerkhaug Hartviksen, Jose L. González Vecino, Simon Wadsworth, Åshild Krogdahl, Anu Kettunen, Einar Ringø</td>
</tr>
<tr>
<td>P243</td>
<td>Supplemental effects of some crude ingredients in improving feed intake and performances of red sea bream, Pagrus major fed high soy protein concentrate diet</td>
<td>Md. Abdul Kader, Saichiro Yokoyama, Manabu Ishikawa, Shunsuke Koshio</td>
</tr>
<tr>
<td>P244</td>
<td>Study of dietary protein and energy requirements of rainbow trout (Oncorhynchus mykiss) in inland brackish water condition</td>
<td>Alizadeh, M.</td>
</tr>
<tr>
<td>P245</td>
<td>Protein requirement of Arctic charr (Salvelinus alpinus).</td>
<td>Olafur Ingi Sigurgeirsson, Jón Arnason, and Aðalheiður Ólafsdóttir</td>
</tr>
<tr>
<td>P246</td>
<td>Effects of dietary carbohydrate sources on growth performance and hepatic carbohydrate metabolic enzyme activities of juvenile cobia (Rachycentron canadum)</td>
<td>Xian-Jun Cui, Qi-Cun Zhou, Hai-Ou Liang, Jun Yang, Li-Mei Zhao</td>
</tr>
<tr>
<td>P247</td>
<td>Vitamin and mineral status in young rainbow trout fed extruded feeds with graded inclusions of a vitamin and mineral premix</td>
<td>Rune Waagbo, Eldar A. Bendiksen, Kim S. Ekmann, Arne Guttvik, Gro-Ingunn Hemre</td>
</tr>
<tr>
<td>P248</td>
<td>Use of low-phosphorus feed for Biwa salmon aquaculture</td>
<td>Shozo Sugiura, Takashi Taguchi, Akira Yamaoka, Hidetomo Tanaka, Kunihiko Kuwamura</td>
</tr>
<tr>
<td>P249</td>
<td>Gastrointestinal evacuation time in European sea bass (Dicentrarchus labrax) fed diets containing mixture of vegetable oils at high temperature</td>
<td>Eroldoğan O. Tufan, Çiçek C. İşıl, Yılmaz H. Asuman, Dedeler H., Türkmen, S.</td>
</tr>
<tr>
<td>P250</td>
<td>Gastrointestinal evacuation time in European sea bass (Dicentrarchus labrax) fed diets containing mixture of vegetable oils at high temperature</td>
<td>Anaïs Boglino, Maria J. Darias, Filiz Ozcan, Karl B. Andree, Alicia Estévez, Enric Gisbert</td>
</tr>
<tr>
<td>Presentation ID</td>
<td>Title</td>
<td>Authors</td>
</tr>
<tr>
<td>----------------</td>
<td>----------------------------------------------------------------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>P251</td>
<td>Dietary ratio of (n-3)/(n-6) Polyunsaturated Fatty Acids (PUFAs) during the Artemia feeding period affects the growth of Senegal sole (Solea senegalensis) larvae but not larval morphogenesis</td>
<td>Naisong Chen*, Hengyong Zhou, Jianzhong Ma, Jie Zhou</td>
</tr>
<tr>
<td>P252</td>
<td>Effects of dietary different carbohydrate to lipid ratios on the growth performance, body composition and non-specific immunity of largemouth bass</td>
<td>Xiaojie Qiu*, Naisong Chen, Hengyong Zhou, Jianzhong Ma</td>
</tr>
<tr>
<td>P253</td>
<td>Effect of dietary carbohydrate-to-lipid ratios on growth performance, body composition, nutrient utilization and hepatic enzymes activities of omnivorous tilapia (Oreochromis niloticus × O. aureus)</td>
<td>Wen Gao*, Yong-Jian Liu, Li-Xia Tian, Kang-Sen Mai</td>
</tr>
<tr>
<td>P254</td>
<td>Comparisons of carbohydrate utilization of herbivorous grass carp (Ctenopharyngodon idella) and omnivorous tilapia (Oreochromis niloticus × O. aureus)</td>
<td>Wen Gao*, Yong-Jian Liu, Li-Xia Tian, Kang-Sen Mai</td>
</tr>
<tr>
<td>P255</td>
<td>Effects of dietary lysine requirement in largemouth bass, Micropterus salmoides</td>
<td>Hu Yi, He Lanbo, Xiao Tiaoyi, Zhou Dinggang</td>
</tr>
<tr>
<td>P256</td>
<td>Effects of dietary different carbohydrate to lipid ratios on the growth performance, body composition, nutrient utilization and hepatic enzymes activities of omnivorous tilapia (Oreochromis niloticus × O. aureus)</td>
<td>Guang-zhen Jiang*, Wen-bin Liu*, Xing-xing Fang, Xue-jiao Jiang, Dan-ni Liang, Su Zhuang</td>
</tr>
<tr>
<td>P257</td>
<td>Dietary manganese requirement for Juvenile cobia, Rachycentron canadum</td>
<td>Kang Liu*, Xiaojie Wang, Qinghui Ai, Kangsen Mai, Wenbing Zhang</td>
</tr>
<tr>
<td>P258</td>
<td>Dietary magnesium requirements of juvenile grass carp, Ctenopharyngodon idella</td>
<td>Fu-bao Wang, Li Luo, Shi-mei. Lin, Yun Li, Shi Chen, Yage. Wang, Hua Wen, Chongjiang Hu</td>
</tr>
<tr>
<td>P259</td>
<td>Growth and postprandial plasma profiles as affected by dietary lipid level and source in rainbow trout (Oncorhynchus mykiss)</td>
<td>Lin Luo*, M Xue, C. Vachot S. Kaushik, I. Geurden</td>
</tr>
<tr>
<td>P260</td>
<td>Effect of different diets on weight gain, hepatic lipase and antioxidant enzyme of juvenile silver pomfret (Pampus argenteus)</td>
<td>Peng Shi-ming*, Yin Fei, Sun Peng, Shi Zhao-hong, Wang Jian-gang</td>
</tr>
<tr>
<td>P261</td>
<td>Optimum dietary level of protein for sea cucumber, Apostichopus japonicus</td>
<td>T. Ren*, L. He, Zh-Q. Jiang, Y. Han, B. Jiang</td>
</tr>
<tr>
<td>P262</td>
<td>Effects of different protein levels on blood biochemical parameters of carp (Cyprinus carpio L. minor) at different temperatures</td>
<td>Tang Ling, Xu Qi-You, Wang Chang-An, Yin Jia-sheng</td>
</tr>
<tr>
<td>P263</td>
<td>Estimating the Requirements of dietary essential amino acid pattern for Young Amur Sturgeon Acipenser schrenckii</td>
<td>QiYou Xu, JunLing Yang, ChangAn Wang, Hong Xu, DaJiang Sun</td>
</tr>
<tr>
<td>P264</td>
<td>Effects of dietary pantothenic acid on growth, body composition and hepatic pantothenate content for Japanese seabass</td>
<td>zhang Chun-xiao, Mai Kang-hui, Zhang Lu</td>
</tr>
<tr>
<td>Page</td>
<td>Title</td>
<td>Authors</td>
</tr>
<tr>
<td>-------</td>
<td>----------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------</td>
</tr>
<tr>
<td>P265</td>
<td>Assessment of arginine requirement for largemouth bass, <em>Micropterus salmoides</em></td>
<td>Hengyong Zhou*, Naisong Chen, Xiaojie Qiu, Jianzhong Ma</td>
</tr>
<tr>
<td>P266</td>
<td>Effect of pre-digestion on absorption, retention and metabolism of lipids in Atlantic cod (<em>Gadus morhua</em>) larvae</td>
<td>Kristin Hamre*, Ingochouba M. Lukram, I.E., Ivar Ronnestad, Andreas Nordgreen, Øystein Sæle</td>
</tr>
<tr>
<td>P267</td>
<td>The optimal lysine level of large yellow croaker (<em>Pseudosciaena crocea</em>, Richardson) larvae</td>
<td>Fengjun Xie, Qinghui Ai*, Kangsen Mai, Xiaojie Wang, Wei Xu, Wenbin Zhang, Hongming Ma</td>
</tr>
<tr>
<td>P268</td>
<td>Apparent digestibility of common and alternative ingredients in youth of silver arowana <em>Osteoglossum bicerrihus</em></td>
<td>Jenny M. Moreno Poveda, Yolanda Ojeda Torres, Adriana P. Muñoz-Ramírez*</td>
</tr>
<tr>
<td>P269</td>
<td>Effect of dietary protein and lipid levels on growth, survival and feed utilization of Mexican silverside (<em>Menidia estor</em>) juveniles</td>
<td>Jesús López-Garcia, Carlos A. Martínez-Palacios*, M. Gisela Ríos-Durán</td>
</tr>
<tr>
<td>P270</td>
<td>Effects of dietary lysine level on growth of soft-shelled turtle, <em>Pelodiscus sinensis</em></td>
<td>Chih-Sheng Lu*, Chen-Huei Huang</td>
</tr>
<tr>
<td>P271</td>
<td>Dietary carbohydrate sources on growth, feed utilization and intermediary enzymes in Asian seabass (<em>Lates calcarifer</em>)</td>
<td>Chutima Tantikitti*, Soykaew Iang-ubon, Manee Srichanun</td>
</tr>
<tr>
<td>P272</td>
<td>Canceled</td>
<td></td>
</tr>
<tr>
<td>P273</td>
<td>Digestibility of alternative protein-rich feedstuffs for channel catfish, <em>Ictalurus punctatus</em></td>
<td>Renato E. Kitagima and Dèbora M. Fracalossi*</td>
</tr>
<tr>
<td>P274</td>
<td>Lipid storage and liver size in Atlantic cod fed energy diluted diet</td>
<td>Gro-Ingunn Hemre, Ørjan Karlsen, Anette Lekva, Ann-Cecilie Hansen and Grethe Rosenlund</td>
</tr>
<tr>
<td>P275</td>
<td>Effects of different diets on the digestive physiology of Norway lobster <em>Nephrops norvegicus</em></td>
<td>G. Rotllant*, E. Gisbert, I.T. Karapanagiotidis, E. Gkolomazou and E. Mente</td>
</tr>
<tr>
<td>P276</td>
<td>Rainbow trout regulates lean body growth but does not adjust feed intake of diets non-limiting in protein with varying lipid levels</td>
<td>I. Geurden*, J.W. Schrama, F. Terrier, F. Médaile, S.J. Kaushik</td>
</tr>
<tr>
<td>P277</td>
<td>Effect of different diets on growth performances and composition body at Red Pacu fish (<em>Piaractus brachypomus</em>)</td>
<td>M. Saedi, B. Tizkar, M.M. Sajjadi</td>
</tr>
<tr>
<td>P278</td>
<td>Effects of different levels of dietary vitamin C (L-ascorbyl-2-polyposphate) on liver total ascorbic acid concentrations and its changes under acute hypoxia in juvenile Mexican silverside (<em>Menidia estor</em>)</td>
<td>M. Gisela Ríos-Durán*, Carlos A. Martínez-Palacios</td>
</tr>
<tr>
<td>P279</td>
<td>Is there a digestive maturation model in agastric fish species?</td>
<td>E. Mayra Toledo-Cuevas*, Francisco J. Moyano López, Carlos A. Strüssmann, Manuel Diaz, Carlos A. Martínez Palacios</td>
</tr>
<tr>
<td>P280</td>
<td>Comparative study of digestive enzymes in rainbow and brown trout</td>
<td>Renata Baric-Rafaj*, Josipa Kules, Emil Gjursevic, Zvonimir Kozaric</td>
</tr>
<tr>
<td>P281</td>
<td>Comparative study of digestive enzymes in rainbow and brown trout</td>
<td>Jonathan Brel, Junio Dort, Mathieu Haerinck, Tarik Abboudi, Yvon,</td>
</tr>
<tr>
<td>P282</td>
<td>Chen Cheng-xun, Wang Qing-kui, Xing Ke-zhi, Bai Dong-qing, Yu Wen-wen, Lv Zhi-min</td>
<td></td>
</tr>
<tr>
<td>------</td>
<td>--------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Effects of dietary zinc supplementation on growth, digestive enzyme activity and antioxidant ability in grouper, <em>Epinephelus malabaricus</em></td>
<td></td>
</tr>
<tr>
<td>P283</td>
<td>Hong-Yan Kou*, Yu-Tao Miao, An-Li Wang, Shu-Ying Xu</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Estimation of optimal dietary protein and lipid requirements for <em>Trachinotus ovatus</em></td>
<td></td>
</tr>
<tr>
<td>P284</td>
<td>Xiaoqing Li, Kangsen Mai†, Qinghui Ai, Wei Xu, Wenbing Zhang, Xiaojie Wang</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Effects of dietary carbohydrate levels on growth performance and body composition of Turbot (<em>Scophthalmus maximus</em> Linnaeus)</td>
<td></td>
</tr>
<tr>
<td>P285</td>
<td>Yan Li, André Moreira Bordinhon, D. Allen Davis*, Xuezhi Zhu</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Protein : energy ratio in practical diets for Nile tilapia</td>
<td></td>
</tr>
<tr>
<td>P286</td>
<td>Xingwang Liu, Kangsen Mai†, Qinghui Ai, Zhiqiu Liu, Wei Xu, Wenbing Zhang, Xiaojie Wang</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Effects of protein to energy ratios in practical diets on growth and body composition of Half-Smooth Tongue-Sole, <em>Cynoglossus semilaevis</em> Gunther</td>
<td></td>
</tr>
<tr>
<td>P287</td>
<td>Kangsen Mai†, Xingwang Liu, Qinghui Ai, Zhiqiu Liu, Wei Xu, Wenbing Zhang, Xiaojie Wang</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Effects of dietary carbohydrate levels on growth and body composition of Turbot (<em>Scophthalmus maximus</em> Linnaeus)</td>
<td></td>
</tr>
<tr>
<td>P288</td>
<td>Wang Qing-kui†, Chen Cheng-xun, Xing Ke-zhi, Bai Dong-qing, Yu Wen-wen, Yu Xue-quan</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Effects of dietary copper supplementation on growth, digestive enzyme activity and antioxidant ability in grouper, <em>Epinephelus malabaricus</em></td>
<td></td>
</tr>
<tr>
<td>P289</td>
<td>Yuting Wei*, Xiaojie Wang, Kangsen Mai, Qinghui Ai, Wei Xu</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Effect of dietary vitamin E level on growth, tissue lipid peroxidation, and tissue antioxidant capacity of juvenile turbot (<em>Scophthalmus maximus</em>)</td>
<td></td>
</tr>
<tr>
<td>P290</td>
<td>QiYou Xu, QiuShan Zheng, Hong Xu, ChanAn Wang, Dajiang Sun</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Studies on Dietary Phosphorus Requirement of Young Siberian Sturgeon <em>Acipenser baeri</em></td>
<td></td>
</tr>
<tr>
<td>P291</td>
<td>Rantao Zuo, Qinghui Ai†, Kangsen Mai, Wei Xu, Wenbing Zhang, Jun Wang, Houguo Xu, Hiskia Asino</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Effects of dietary n-3 HUFA on growth, fatty acid metabolism and immunity of large yellow croaker (<em>Pseudosciaena crocea</em>)</td>
<td></td>
</tr>
<tr>
<td>P292</td>
<td>Yan Li, Qinghui Ai†, Kangsen Mai, Wei Xu, Zhenyan Cheng, Zhigang He, Xiaojie Wang</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dietary leucine requirement of juvenile Japanese seabass, <em>Lateolabrax japonicus</em> and large yellow croaker, <em>Pseudosciaena crocea</em> R</td>
<td></td>
</tr>
<tr>
<td>P293</td>
<td>Chen Qing-Hua, Liu Wei, Tang Fu-Jiang</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Comparison of nutritive component among four culters of Shinkai River</td>
<td></td>
</tr>
<tr>
<td>P294</td>
<td>Susana Muñoz, José Caquilpan, Lilia Masson, Cesar Martinez and Paulo Palacios</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Effect of dietary lipid on the content of ω-3 fatty acids EPA and DHA in the fillet of rainbow trout (<em>Oncorhynchus mykiss</em>)</td>
<td></td>
</tr>
<tr>
<td>Page</td>
<td>Title</td>
<td></td>
</tr>
<tr>
<td>------</td>
<td>-------</td>
<td></td>
</tr>
<tr>
<td>P295</td>
<td>Eryalçın, K.M., Roo, J., Betancour, M., Atalah, E., Benítez, T. and Izquierdo, M.S. Microphotographic observation of gut contents and ingestion rates of fed different microalgae included diets by Gilthead Sea Bream larvae (<em>Sparus aurata</em> L. 1758)</td>
<td></td>
</tr>
<tr>
<td>P296</td>
<td>Liu Yi, Jun-Ho Lee, Seunghyung Lee, Young Chul Kim, Okorie E. Okorie, Mahmoud Mohseni, Gun Hyun Park, Namyoung Hwang and Sungchul C. Bai* Evaluation of dietary cadmium threshold level of toxicity in juvenile olive flounder (<em>Paralichthys olivaceus</em>)</td>
<td></td>
</tr>
<tr>
<td>P298</td>
<td>Agus Kurnia, Shuichi Satoh, Hiroaki Kudo, Makoko Nakada, Hiroaki Matsumura, Yutaka Watanabe and Syougo Adachi Effect of marine bacteria (<em>Paracoccus</em> sp.) as an astaxanthin source in practical red sea bream culture</td>
<td></td>
</tr>
<tr>
<td>P299</td>
<td>Suphada Kiriratnikom*, Anut Kiriratnikom, Punthasit Choksawatdikorn and Kritsana Ruengklay Studies on effects of carotenoid sources and its levels on growth performance, body color and total carotenoids contents in the Slender walking catfish (<em>Clarias nieuhofii</em>).</td>
<td></td>
</tr>
<tr>
<td>P300</td>
<td>Jian Gao*, Saichiro Yokoyama, Manabu Ishikawa, Shunsuke Koshio Influences of oxidized fish oil with vitamin C supplementation on growth performance and reduction of oxidative stress in Red Sea Bream (<em>Pagrus major</em>)</td>
<td></td>
</tr>
<tr>
<td>P301</td>
<td>Haiyan Liu**, Peng Jia, Min Xue, Xiufeng Wu, Zhencai Yang, Huaixia Mu, Peipei Zhang Effects of dietary supplementation of lutein on growth performance, skin colour and tissue accumulation of the Chinese soft-shelled turtle, <em>Pelodiscus sinensis</em></td>
<td></td>
</tr>
<tr>
<td>P302</td>
<td>Chang-an Wang*, Qi-you Xu, Hong Xu, Jia-sheng Yin, Tong Zhang, Yang Wang, Ling Tang Influence of dietary protein and water temperature on growth and flesh quality of <em>Cyprinus carpio specularis</em></td>
<td></td>
</tr>
<tr>
<td>P303</td>
<td>Dongqing Bai Shanshan Yan* Primary Study β-carotene on the Growth, Colouring and Deposition of Red-white koi carp (<em>Cyprinus carpio var. koi</em> L.)</td>
<td></td>
</tr>
<tr>
<td>P304</td>
<td>Jón Arnason*, Olafur Ingi Sigurgeirsson and Áðalheiður Ölfusdóttir Effect of dietary protein level on protein content and quality of Arctic charr</td>
<td></td>
</tr>
<tr>
<td>P305</td>
<td>Kanjana Payooha, Jitra Simawan, Chamnan Kawmanee and Chutima Thongkaew Effect of dietary carbohydrate on the growth performance, fillet composition and flesh quality of <em>Pangasius bocourti</em></td>
<td></td>
</tr>
<tr>
<td>P306</td>
<td>Geneviève. Corraze, Sadasivam J. Kaushik, Georges Choubert, Françoise Médale Changes in nutritional and sensory properties induced by a plant based diet in two lines of rainbow trout selected for muscle fat content.</td>
<td></td>
</tr>
<tr>
<td>Paper No.</td>
<td>Authors</td>
<td>Title</td>
</tr>
<tr>
<td>-----------</td>
<td>---------</td>
<td>-------</td>
</tr>
<tr>
<td>P307</td>
<td>GAO Lu-jiao, Huang Yan-qing, Xia Lian-jun, Lu Jian-xue, Liu Sheng-cong</td>
<td>Comparison of nutritional components and physical characteristics of muscle of <em>Fugu rubripes</em> from different zones</td>
</tr>
<tr>
<td>P308</td>
<td>Yuzhe Han, Zhiqiang Jiang, Tongjun Ren, Shunsuke Koshio, Jian Gao, Hongyue Shi</td>
<td>Effect of oxidized fish oil blended with palm oil on antioxidant capacity and histology of Japanese sea bass (<em>Lateolabrax maculatus</em>) juvenile</td>
</tr>
<tr>
<td>P309</td>
<td>Wang Qiu-rong*, Lin Li-min, Wang Zhi-yong, Xi Feng</td>
<td>Comparison of biochemical compositions of muscle of wild large yellow croaker (<em>Pseudosciaena crocea</em> R.) from different parts of China Sea</td>
</tr>
</tbody>
</table>

**Feed additives**

<table>
<thead>
<tr>
<th>Paper No.</th>
<th>Authors</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>P310</td>
<td>Fuxian Feng, Hongming Ma, Kangsen Mai, Qinghui Ai, Wenbing Zhang, Yi Hu, Qingfei Li, Yun Huang</td>
<td>Effects of dietary arginine on survival, growth, nonspecific immune responses and disease resistance in juvenile darkbarbel catfish (<em>Pelteobagrus vachelli</em>)</td>
</tr>
<tr>
<td>P311</td>
<td>Gan Lian*, Yong-Jian Liu, Li-Xia Tian, Hui-Jun Yang, Yi-Rong Yue, Yong-Jun Chen, Jian-Jun Liang, Gui-Yin Liang</td>
<td>Effects of Lysine and methionnine supplementation to different protein level practical diet for grass carp <em>Ctenopharyngodon idella</em></td>
</tr>
<tr>
<td>P312</td>
<td>Yu-Jie Gao*, Yong-Jian Liu, Li-Xia Tian, Gui-Ying Liang, Yi-Rong Yue, Wen-Jia Luo, Shu-Yun Zhou</td>
<td>Effect of ghrelin on the food intake and growth of grouper (<em>Epinephelus coioides</em>)</td>
</tr>
<tr>
<td>P313</td>
<td>Li-wei Liu*, Yu-liang Luo, Hong-li Hou</td>
<td>Effect of partial replacement of calcium dihydrogen phosphate with neutral phytase on growth performance, Body compositions and haemato-biochemical parameters of <em>Ctenopharyngodon idella</em></td>
</tr>
<tr>
<td>P314</td>
<td>Wei-min Shang, Li Luo, Si-chao Jia, Chaoming Wang, Guizhong Zhang</td>
<td>Effects of different acidifiers on the growth performance, metabolism and non-specific immunity of <em>Ctenopharyngodon idella</em></td>
</tr>
<tr>
<td>P315</td>
<td>Song li ping, Wang ai ying, Hu bin, Mao shu quan, Han bo</td>
<td>Effects of dietary phytase on growth and feed utilization of jade perch, <em>Scortum bacoo</em></td>
</tr>
<tr>
<td>P316</td>
<td>WANG Ji-ting XIAO Xin-wu SUN Meng-ming WAN Wen-ju*</td>
<td>Effect of acidifier and feed nutrient digestibility of common carp (<em>Cyprinus carpio</em>)</td>
</tr>
<tr>
<td>P318</td>
<td>Tan Fangfang, Ye Yuantu*, Xiao Shunying, Ma Hong, Li Bing</td>
<td>Effects of Microcapsule Lysine and Methionine on the Growth Performance of Grass Crap(<em>Ctenopharyngodon idella</em>)</td>
</tr>
<tr>
<td>P319</td>
<td>Amal Biswas, Biswajit K. Biswas, Yang S. Kim, Norishige Yagi, Kenji Takii</td>
<td>Does amino acids, glycosidase and phytase supplementation help to increase replacement level of fish meal by soybean meal in the diet of Pacific bluefin tuna juveniles?</td>
</tr>
<tr>
<td>P320</td>
<td>Chhorn Lim*, Phillip H. Klesius and Christian Lückstädt</td>
<td></td>
</tr>
<tr>
<td>ID</td>
<td>Title</td>
<td>Authors</td>
</tr>
<tr>
<td>------</td>
<td>----------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>P321</td>
<td>Effects of dietary levels of potassium diformate on growth, feed utilization and resistance to <em>Streptococcus iniae</em> of Nile tilapia, <em>Oreochromis niloticus</em></td>
<td>F. Liebert*</td>
</tr>
<tr>
<td>P322</td>
<td>Efficiency of microbial phytase sources in low phosphorus plant based diets for Tilapia (<em>Oreochromis niloticus</em>)</td>
<td>F. Liebert, Wen Gao, C. Kobler and A. Lemme</td>
</tr>
<tr>
<td>P324</td>
<td>Evaluation of probiotics and prebiotics to improve the growth of juvenile leopard grouper <em>Mycteroperca rosacea</em></td>
<td>Reyes-Becerril, M.*, Macias, R. M. E., Ascencio, F., Meseguer, J., Esteban Abad, M. A.</td>
</tr>
<tr>
<td>P325</td>
<td>The Study of Effect of dietary Mannan oligosaccharides as prebiotic on growth performance and some blood metabolites in rainbow trout (<em>Oncorhynchus mykiss</em>)</td>
<td>M. Norouzi*, H. Meftah, S. Karimzadeh</td>
</tr>
<tr>
<td>P327</td>
<td>Effects of dietary α-lipoic acid on growth, antioxidative responses of juvenile abalone <em>Haliotis discus hannai</em></td>
<td>Wenbing Zhang*, Qiyong Chen, Kangsen Mai, Wei Xu, Xiaojie Wang, Zhiguo Liu Fu</td>
</tr>
<tr>
<td>P328</td>
<td>Effects of Dietary Phytase (Cibenza Phos) on The Growth of Juvenile Tilapia (<em>Oreochromis niloticus</em>)</td>
<td>Yin Long *, Yong-Jian Liu, Hong Cao, Hui-jun Yang, Fu-Cun Guo, Gui-Ying Liang, Craig Browdy, Li-Xia Tian</td>
</tr>
<tr>
<td>P330</td>
<td>Effect of Aquanin PlusTM on Growth and Feed Utilization of Nile Tilapia, <em>Oreochromis niloticus</em></td>
<td>Nontawith Areechon, Qingsong Tan*</td>
</tr>
<tr>
<td>P331</td>
<td>Dietary L-alanyl-L-glutamine supplementation improves growth performance and physiological function of hybrid sturgeon <em>A. schrenckii♀×A. baerii♂</em></td>
<td>Chang-an Wang*, Qi-you Xu, Hong Xu, Qing Zhu, Jun-ling Yang, Da-jiang Sun</td>
</tr>
<tr>
<td>P332</td>
<td>Effect of two kinds of Chinese herbs polysaccharide on respiratory burst and proliferation of head kidney macrophages in <em>Peleobagrus fulvidraco</em></td>
<td>Dongqing Bai Xuan Wu*</td>
</tr>
<tr>
<td>P333</td>
<td>Responses of grass carp (<em>Ctenopharyngodon idellus</em>) to supplemental methionine and lysine</td>
<td>Tianyi Liu, Hong Qin Li*, Xuan Zhu* and Andreas Lemme</td>
</tr>
<tr>
<td></td>
<td>Authors</td>
<td>Title</td>
</tr>
<tr>
<td>---</td>
<td>-----------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>P334</td>
<td>Xuan Zhu*, Wenhua, Ming Jiang and Andreas Lemme</td>
<td>Responses of juvenile Nile tilapia (<em>Oreochromis niloticus</em>) to supplemental methionine</td>
</tr>
<tr>
<td>P335</td>
<td>Huihui Zhou*, Hongming Ma, Wenbing Zhang, Wei Xu, Zhiguo Liu, Kangsen Mai</td>
<td>Effects of potential probiotics on growth performance and immune response of the juvenile sea cucumber (<em>Apostichopus japonicus</em>)</td>
</tr>
<tr>
<td>P336</td>
<td>Gui Qin-Wang*, Hong Mei-Lu, Xiao Tian-Niu, Zi Ping-Li</td>
<td>Effects of Ala-Gln dietary supplementation on growth and some stress parameters of Jian Carp (<em>Cyprinus carpio</em>) juveniles, reared in different stocking density</td>
</tr>
<tr>
<td>P337</td>
<td>Maria L. Cuvin-Aralar, K.-J. Kühlmann, K. Schroeder and C. Lückstädt*</td>
<td>Effect of potassium diformate (KDF) on growth performance of male Nile Tilapia (<em>Oreochromis niloticus</em>)</td>
</tr>
<tr>
<td>P339</td>
<td>Dong-Hoon Lee*, Jeong-Dae Kim</td>
<td>Effects of dietary garlic extracts on growth, feed utilization, whole body fatty acid profile, muscle free amino acids composition and blood plasma changes of Juvenile sterlet sturgeon (<em>Acipenser ruthenus</em>)</td>
</tr>
<tr>
<td>P340</td>
<td>F. Liebert, K. Mohamed and C. Lückstädt</td>
<td>Effects of diformates on growth and feed utilization of all male Nile Tilapia fingerlings (<em>Oreochromis niloticus</em>) reared in tank culture</td>
</tr>
<tr>
<td>P341</td>
<td>K. Mohamed and F. Liebert</td>
<td>Effects of a probiotic feed additive (<em>Enterococcus faecium</em>) in diets for Tilapia (<em>Oreochromis niloticus</em>) on growth, feed efficiency and microbiological parameters of the gut</td>
</tr>
<tr>
<td>P342</td>
<td>Fredrik Venold, Elvis Chikwati, Michael Penn*, Junior Molatelo Madibana, Anne Marie Bakke, Jens Rohloff, Arne Guttvik, Marie Hillestad and Ashild Krogdahl</td>
<td>Supplementation to plant feeds on the growth performance, digestibility, and retention in Atlantic salmon.</td>
</tr>
<tr>
<td>P343</td>
<td>Jorge Dias*, Paulo Rema, Viviane Verlhac</td>
<td>Protease supplementation enhances the apparent digestibility of nutrients and energy in Nile tilapia fed plant-protein rich diets with variable crude protein content</td>
</tr>
<tr>
<td>P344</td>
<td>Lourens F de Wet, Willem Visagie</td>
<td>Evaluating CHD-FA Carbohydrate-derived Fulvic Acid for use in diets of Mozambique Tilapia (<em>Oreochromis mossambicus</em>).</td>
</tr>
<tr>
<td>P345</td>
<td>Alizadeh Morteza, A. Farzanfar, G. R. Lashtuaghai, M. Bayati, R. Ghorbani</td>
<td>Effect of dietary different levels of probiotic on growth performance of rainbow trout Oncorhynchus mykiss in larvae stage</td>
</tr>
<tr>
<td>P346</td>
<td>Maike Oehme, Fabian Grammes, Harald Takle, José-Luis Zambonino-Infante, Ståle Refstie, Thomassen M.S, Kjell-Arne Rorvik and Bendik Fyhun Terjesen</td>
<td>Dietary supplementation of glutamate and arginine to Atlantic salmon (<em>Salmo salar L.</em>) increases growth during the first autumn in sea cages</td>
</tr>
<tr>
<td>P347</td>
<td>Wei-Hsiang Lin*, Chen-Huei Huang</td>
<td>Dietary attractants improve feed consumption and growth of soft-shelled turtles</td>
</tr>
<tr>
<td>Paper ID</td>
<td>Authors</td>
<td>Title</td>
</tr>
<tr>
<td>----------</td>
<td>---------</td>
<td>-------</td>
</tr>
<tr>
<td>P348</td>
<td>Chen Cheng-xun, Wang Qing-kui, Xu Da-wei, Ji Yan-bin, Zhao Jing-quan, Yang Shu-yuan</td>
<td>Effects of dietary supplementation of compound Chinese herbal medicine on antioxidant ability of <em>Claris lareza</em></td>
</tr>
<tr>
<td>P349</td>
<td>Shuyan Chi, Beiping Tan, Kangsen Mai, Qinghui Ai, Xiaohui Dong, Qihui Yang, Qicun Zhou</td>
<td>Effect of supplementation of microcapsule or crystalline methionine in diets with low fish meal on growth performance of juvenile cobia <em>Rachycentron canadum</em> (Linnaeus)</td>
</tr>
<tr>
<td>P350</td>
<td>Jin Gao, Kangsen Mai, Qinghui Ai, Hongming Ma, Xiaojie Wang, Wenbing Zhang, Wei Xu, Zhiguo Liu</td>
<td>Effects of dietary compound probiotics on growth, survival, activities of digestive enzymes and stress resistance of large yellow croaker (<em>Pseudosciaenid crocea</em>) larvae</td>
</tr>
<tr>
<td>P351</td>
<td>Qingfei Li, Qinghui Ai, Kangsen Mai, Wenbing Zhang, Wei Xu, Hongming Ma, Xiaojie Wang, Yuefu Zheng</td>
<td>In vitro effects of arachidonic acid on leucocytes immune functions of large yellow croaker (<em>Pseudosciaena crocea</em>) head-kidney</td>
</tr>
<tr>
<td>P352</td>
<td>Lin Jian-bin, Zhu Qing-huo, Ling Ping, Chen Du-huan, Qin Zhi-qing, Ai Chun-xian</td>
<td>Effects of different feed additives and combination on growth and non-specific immunity of European eel (<em>Anguilla anguilla</em>)</td>
</tr>
<tr>
<td>P353</td>
<td>Zi-Jiao He, Qing Pan, Yan Chen, Bin-Chong Qiu, Ying-Zuo Bi</td>
<td>Effects of phosphorus and phytase on growth performance and body composition in juvenile tilapia fed fishmeal-free diet</td>
</tr>
<tr>
<td>P354</td>
<td>Tang Ling, Xu Qi-You</td>
<td>Effects of natural plant additives garlic stem powder and oregano leaf powder on growth performance, digestive enzyme activity and serum biochemical indexes of the carp (<em>Cyprinus carpio L. minor</em>)</td>
</tr>
<tr>
<td>P355</td>
<td>XiFeng Wang, Qiurong Lin, Linlimin</td>
<td>A study on the reducing-drug-residue additives for <em>Anguilla japonica</em></td>
</tr>
<tr>
<td>P356</td>
<td>Fa Lan Zhao, Wu Ying Chu, Rui Xue Zhou, Tao Meng, Jia Cheng, Jian She Zhang</td>
<td>Effects of dietary lactosucrose on growth performance and serum biochemical indices in <em>ctenopharyngodon idellus</em></td>
</tr>
<tr>
<td>P357</td>
<td>Wangming Zhu, Meng Zhou</td>
<td>Effects of different dietary levels of bile acid product on growth performance and serum biochemical indexes of hybrid Snakehead, <em>Channa maculate ♀ × C. argus ♂</em></td>
</tr>
<tr>
<td>P358</td>
<td>Kathryn A. Schuller, Melissa K. Gregory, Valene H.L. See and Robert A. Gibson</td>
<td>Cloning and functional characterization of fatty acyl desaturase and elongase genes from southern bluefin tuna <em>Thunnus maccocyii</em></td>
</tr>
<tr>
<td>P359</td>
<td>Kathryn A. Schuller, Drew L. Sutton, Grace H. Loo and R. Ian Menz</td>
<td>Cloning and functional characterization of a 2-Cys peroxiredoxin from southern bluefin tuna <em>Thunnus maccocyii</em></td>
</tr>
</tbody>
</table>
ORAL PAPERS

PRESENTED AT THE

14th INTERNATIONAL SYMPOSIUM ON
FISH NUTRITION & FEEDING

MAY 31--JUNE 4, 2010

QINGDAO, CHINA
Achievements and prospects of aquaculture and aquafeed industry in China

Kangsen Mai
Fisheries College, Ocean University of China

Abstract: This article presents a comprehensive review on the achievements obtained in the past a few decades and the prospects in aquaculture and aquafeed industry in China. The total production of aquaculture in China increased from 2.38 million tons in 1978 to 36 million tons in 2008, growing 15 times in 30 years. As aquaculture, China has also become the world champion in the annual output of aquafeed, from only 0.75 million tons in 1991 to 13 million tons in 2008, increasing 17 times in 17 years. China’s aquafeed industry is the indispensable basis to support the fastest growth and the largest scale aquaculture in the world.

This review analyzes the major reasons for the great success of China aquafeed industry in such short period. China has the highest diversity in aquacultured species (more than 100 species), farming patterns and geographical distributions. It is impossible to establish a comprehensive database of nutrient requirements for so many species in such a short duration and with limited research funding. Hence, Chinese aquaculture nutritionists decide to select representative species for systematic nutritional studies, on the basis of their taxonomy, feeding habits, geographical distributions, farming patterns and culture scales. These research findings subsequently are applied to the feed formulations of all other relative cultured species. This R & D strategy has been demonstrated to be very successful. Additionally, China is deficient in raw feedstuff, particularly the high quality protein sources, such as fishmeal and soybean, 70% of which depends on importation at present. However, China has succeeded in diversifying its feed ingredients and improving their bioavailability by the developments of technology and commercial products, such as neutral phytase, compound fermentation, feeding attractants, micro-comminution, essential amino acid (EAA) encapsulation and formulation EAA balance.

The progress and present status of the feed additives and aquafeed processing machinery are also presented and discussed. The research frontier in nutritional manipulation and its applications in aquaculture are discussed in more details. The principles of nutritional (dietary) manipulations for animal reproduction, larval culture, decreasing nutrient requirements, animal health, behavior, environmental adaptation, product quality and safety, and culture environmental sustainability are introduced, and the application examples of nutritional manipulation technology in aquaculture practices are given.

The main problems and challenges for China aquaculture nutritional studies and aquafeed industry are scrutinized. The most important challenge for China aquafeed industry is lastingly the shortage feed protein sources, particularly fishmeal and high quality plant proteins. The quality, safety and sustainability of China aquaculture will closely depend on this issue. The R & D of China aquaculture nutrition and aquafeed industry should focus on exploring new non-fishmeal protein sources and improving their utilization efficiency. Hence, a comprehensive database of nutrient requirements and bioavailability of feed ingredients for more and more cultured species, and even for different life stages of a same species should be gradually established to provide a more reliable basis for precise feed formulations to save the limited protein sources. It is proposed that direct use of raw feed ingredients and trash fishes in aquaculture should be stopped by legislation because it is an important way to save feed ingredient sources, and to protect food and environment safety. The modern molecular biotechnology, such as nutrigenomics, should be applied to aquaculture nutrition studies to fully understand the molecular nutritional mechanisms for developing more reliable and precise technology of nutritional manipulation. The industries of feed additives and feed processing machinery need to be gradually consummated according to the special needs of aquaculture animals and the real situation of China.

Key words: China; Aquaculture; Aquafeed industry; Achievements; Prospects
Microbes – a potential sustainable aquafeed resource

M. Øverland1*, L. T. Mydland1, O. H. Romarheim1, T. Landsverk2, A. Skrede1
1Aquaculture Protein Centre, CoE, Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, Aas, Norway. 2Department of Basal Sciences and Aquatic Medicine, Norwegian School of Veterinary Science, Oslo, Norway.

Abstract

Microorganisms, i.e. bacteria, yeast and algae, represent a potential future nutrient source because they can be grown rapidly on substrates with minimum dependence on soil, water, and climate conditions and may relieve pressure on limited protein sources like fish meal. Microorganisms as sources of protein and fat represent an exciting research area, where further knowledge will be required to develop new sustainable feed ingredients for farmed fish. Bacteria grown by fermentation of natural gas, ammonia and mineral salts (mainly Methylococcus capsulatus) is a promising protein source. The spray-dried meal (BM) contains about 70% crude protein and 10% lipids and has a favourable amino acid composition compared with the requirements of salmonids. Experiments have shown that BM can partially replace high-quality fish meal in diets for Atlantic salmon and rainbow trout without impairing growth performance. The nucleic acids in BM (about 10%) may be considered semi-essential nutrients that have a N-sparing effect and increase feed efficiency and N-retention in rapidly growing salmon. Yeast produced from second-generation biofuel also represents a potential protein source in aquafeeds. Yeasts like Rhizopus oryzae have been shown to be efficient in fermenting co-products from wood processing and agricultural industries into a biomass rich in proteins, lipids, and nucleic acids. Rainbow trout fed diets containing Rhizopus oryzae obtained similar growth rate and feed efficiency as those fed a fish meal-based diet. Other yeast species (i.e. Pichia spp. and Kluyveromyces spp.) have shown a crude protein content of about 50-60%, with favorable amino acid profile and a high digestibility. Microalgae have the potential to serve as a valuable source of protein and lipid. To be commercially interesting, they must have acceptable contents of essential fatty acids and protein of a high quality. Evaluation of the algae Nannochloropsis oceanica, Phaeodactylum tricornutum, and Isochrysis galbana showed a crude protein content ranging from 23 to 50% of DM, and a lipid content ranging from 8 to 18%. In general, the amino acid profile was favorable, but there was a large difference in the apparent digestibility of crude protein among the algae, ranging from 19% in I. galbana to 80% in P. tricornutum.

To conclude, microorganisms have a great potential as a feed ingredient for farmed fish. They have a rapid growth rate and can convert non-food substrates into valuable protein and lipid. Production and down-stream processing conditions need optimizing to obtain products with a high nutritional value and a competitive prize.

Keywords: Bacteria; Yeast; Algae; Protein Sources; Sustainability

*E-mail address: margareth.overland@umb.no (M. Øverland).
O-003

Effects of feeding a northern, southern fish oil or DHA-rich algal meal replacement feed on the persistent organic pollutant (POP) levels in diets and flesh of Atlantic salmon

M. Sprague1*, J. Walton2, J. Dick1, F. Strachan1, D. R. Tocher1, J. G. Bell1
1 Institute of Aquaculture, University of Stirling, Stirling, FK9 4LA Scotland.
2 BioMar Ltd. North Shore Road, Grangemouth Docks, Grangemouth FK3 8UL Scotland

Abstract

Sourcing sustainable alternative oils and meals is required in order to address the decreasing availability and increasing price of fish oils. However, whilst plant based feeds often result in an advantageous reduction in the levels of persistent organic pollutants (POPs) such as dioxins (PCDD/Fs), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), their use is also related to a reduction in the beneficial effects (n-3 LC-PUFA) typically associated with fish oils. The current study investigated replacing fish oils with a DHA-rich algal meal and comparing growth and flesh composition together with levels of POPs (29 WHO-TEQ PCDD/Fs and PCBs and 9 PBDE congeners) in Atlantic salmon (Salmo salar L.) flesh and feeds. Following a conditioning period with a vegetable oil feed, triplicate groups of Atlantic salmon post-smolts were fed one of four commercial-type feeds consisting of either a Northern hemisphere fish oil (NFO; control) with a DHA/EPA of 10/8, a South American anchovy fish oil (SFO) with DHA/EPA of 2/3, or a rapeseed oil based feed with a low (5.5%) or high (11%) inclusion of DHA-rich algal meal (AM5 and AM11, DHA/EPA of 6/1 & 2/1 respectively). Fish were reared in 125 m³ net pens for 135 days with growth performance (SGR, TGC) and feed conversion (FCR) monitored. At the end of the trial, flesh samples were removed for nutritional and POP analysis. Dietary PBDE levels were reduced by 53% in SFO as compared to NFO with a similar reduction (43%) in PCDD/Fs and PCBs. Moreover, POP levels in both AM diets were reduced by around 90% and 95% when compared to NFO and SFO diets respectively. Similar trends were reflected in flesh POP levels with the order being NFO > SFO > AM. There was no difference in growth or flesh pigment levels amongst the dietary treatments. However, flesh fatty acid compositions varied according to the different diets fed with DHA levels significantly different between all treatments and EPA differing between all except the AM fed fish. Conversely, total n-3 PUFA levels were similar between NFO and SFO treatments but significantly higher than AM fed fish. Additionally, total n-3 LC-PUFA showed a small but significant difference between AM5 and AM11 fed fish. These results indicate that AM feeds can be used to minimize POP exposure without affecting growth performance and fulfill n-3 LC-PUFA levels when incorporated at appropriate inclusion rates.

Keywords: Atlantic salmon, Persistent organic pollutants (POPs), Fish oil replacement

* E-mail address: matthew.sprague@stir.ac.uk
Effects of replacing soybean meal with *Moringa oleifera* leaf meal on growth performance, protein digestibility, haematology and antioxidant action against lipid peroxidation in juvenile tilapia *Oreochromis niloticus*

**Bundit, J.***, Taweesin, A.

Fisheries Science Program, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, 10520 THAILAND

**Abstract**

Substitution of *Moringa oleifera* air-dried leaf meal with soybean meal diets in juvenile tilapia, *Oreochromis niloticus*, was conducted by Complete Randomized Design (CRD). The five treatments, consisting four replicates, were fed a diet substituted with moringa leaf meal at 0 (as control), 25, 50, 75 and 100%. The test diets contained isonitrogenous crude protein of 38.1 ±0.1% and isocaloric gross energy of 3889.2 ±120 kcal Kg^{-1} diet. The study was divided into two trials. The first trial was conducted to evaluate the in vivo dry matter and protein digestibility of fish fed different substituted moringa leaf meal levels. The results indicated dry matter and protein digestibility of fish decreased when the amount of dietary moringa leaf meal was increased up to 100%. Protein digestibility was 80, 83, 79, 79 and 75% (p<0.05) and the dry matter digestibility was 71, 77, 73, 75 and 72% (p>0.05) for 0, 25, 50, 75 and 100% substitution, respectively. The second trial was carried out for 60 days to evaluate growth performance, feed efficiency, haematology and antioxidant action against lipid peroxidation of fish fed diets containing the same levels of moringa substitution as described above. The results indicated that there were significant differences (p<0.05) for final weight gain among treatments which were highest in groups of fish fed 0, 25 and 50% substitution but were not significant (p>0.05). Feed conversion ratio was poorest in fish fed 100% substitution, the same trend was observed in protein efficiency ratio values. However, the net protein utilization was lowest in fish fed on the control diet (p<0.05).

Even though there were no significant differences in survival rate in all treatments (p>0.05), fish fed 50% substitution showed a 100% survival rate. Besides, fish fed diets containing moringa leaf meal showed an improvement in health and haematological parameters compared with the control (p<0.05). The analyzed muscle TBAR were 29.54, 23.60, 22.88, 22.64 and 21.04 µmol MDA g^{-1}, liver TBAR were 136.11, 128.40, 123.47, 106.93 and 84.72 µmol MDA g^{-1} and plasma TBAR were 0.25, 0.20, 0.19, 0.18, 0.15 µmol MDA g^{-1} in fish fed 0, 25, 50, 75 and 100% substitution, respectively. The hepatosomatic indices (HSI) were 2.46, 2.16, 2.09, 1.75 and 1.51% in fish fed 0, 25, 50, 75 and 100% substitution, respectively. The percentages of haematocrit, haemoglobin contents increased substantially with increasing levels of moringa (p<0.05).

**Keywords:** Moringa oleifera; Moringa leaf meal; Growth performance; Feed efficiency; haematology; Health responses; Lipid peroxidation; Tilapia; Oreochromis niloticus

*E-mail address: bundit_jatuporn@kmitl.ac.th*
Bacterial meal counteracts intestinal inflammation caused by soybean meal in Atlantic salmon

Odd H. Romarheim¹*, Margareth Øverland¹, Liv T. Mydland¹, Anders Skrede¹, Thor Landsverk²

¹ Aquaculture Protein Centre, CoE, Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, Aas, Norway.
² Department of Basic Sciences and Aquatic Medicine, Norwegian School of Veterinary Science, Oslo, Norway.

Abstract

A main limitation for the use of solvent extracted soybean meal (SBM) in diets for salmonids is the onset of an inflammation in the distal intestine. The inflammation is commonly referred to as “soybean meal-induced enteritis” and is caused by one or more alcohol soluble components. Removal of alcohol soluble components enables higher dietary inclusion of soy, but it is a costly process. A bacterial meal (BM), containing mainly the methanotrophic bacteria Methylococcus capsulatus, has shown to be a high-quality protein source for fish. Bacterial meal is also rich in nucleic acids, phospholipids, lipopolysaccharides and small peptides that might be immune defense modulators. The objective of the present experiment was to study if BM could prevent development of SBM-induced enteritis in salmon, and to study growth and feed utilization in diets with BM and SBM. Four extruded diets were made; a control diet based on fish meal (FM), a diet with 30% BM, a diet with 20% SBM, and a diet with both 30% BM and 20% SBM. Each diet was fed to three groups of Atlantic salmon (Salmo salar) for 80 days (start weight: 134g). Fish fed the SBM diet developed typical SBM-induced enteritis with atrophy of intestinal folds and signs of inflammation in the lamina propria, whereas the intestine of fish fed the fish meal control, the BM-based diet or the diet containing both SBM and BM appeared normal. Fish fed diets with inclusion of BM had higher intestinal weight than fish fed the two other diets. Fish fed the SBM and the BM/SBM diet had poorer growth and feed conversion ratio (FCR) than fish fed the FM diet. No significant (p>0.05) difference in growth rate was found between fish fed the BM and the FM diet, but fish fed the BM diet obtained the best FCR. Digestibility of crude protein and lipid was slightly lower in fish fed the BM diet than in fish fed the FM diet, whereas the uptake of phosphorus was 44 and 35% higher per kg eaten feed in fish fed the BM and BM/SBM diet, respectively, compared to fish fed the FM diet. To conclude, BM prevents development of SBM-induced enteritis, and dietary inclusion of BM allows a greater use of SBM in diets for Atlantic salmon without adverse gut health effects.

Keywords: bacterial protein meal, soybean meal, enteritis, Atlantic salmon, protein sources.

* E-mail address: odd-helge.romarheim@umb.no (O.H. Romarheim).
Sustainable feed for Atlantic salmon; the fish in feed to fish produced

Ole Torrissen*1 and Reidar Toresen†
1 Institute of Marine Research, P.O. Box 1870 Nordnes, 5817 Bergen, Norway

Abstract

There has been a strong focus on sustainability of feeds for aquaculture, and in particular feed for carnivorous fish. This discussion has mainly been along two axis: 1) The ecological aspect where focus has been the effect of harvesting small pelagic fishes for feed on the marine ecosystem (Naylor, Goldburg et al. 2000) and 2) The ethical aspect by actually consuming “more” edible fish than the salmon farming industry is producing (Tacon and Metian 2009). In this discussion it is assumed that vegetable protein and lipid sources are more sustainable than protein from marine resources.

The Brundtland commission defines sustainable development as the development that meets the needs of the present without compromising the ability of future generations to meet their own needs (Brundtland 1987). Based on this definition aquaculture sustainability depends both on the long term impact of the activity on the ecosystems and the amount of non renewable resources used, and where permanent impact is of particular concern. Harvest of small pelagic fishes will create impact both up and down the marine food web, however harvest within safe biological limits of renewable resources will have low or none permanent effects on the marine ecosystem. The challenge is unregulated or inadequate managed stocks. On contrary, industrialized agricultural production of grains will definitely have lasting impacts on landscape, biodiversity and give effluents to waters and finally to the ocean.

Fish in feed in relation to fish produced is complicated as raw material for fish oil and meal production varies with respect to lipid level with specie and season. Total annual use of whole fish for fishmeal production is approximately 17 mill tons per year in addition to 6 million tons byproducts. Hardly any of these calculations take into account the difference in edible yield between salmon and the alternative species used for fish oil and meal. Some species do not have an alternative use as food for humans while other have a relative low yield in production of food for humans.

The present presentation will discuss principles of sustainable harvest of marine feed resources for aquaculture salmon production both in relation to effects on marine ecosystems and in a global seafood supply.

*E-mail address: olet@imr.no

Reference:
O-007

The effects of size-fractionated fish hydrolysate in the diet on growth, and feed utilization of turbot (Scophthalmus maximus)

Liang Mengqing*, Yao Hongbo, Zheng Keke, Changqingm, Wang Jialin
Yellow Sea Fisheries Research Institute, 106 Nanjing Road Qingdao 266071 P. R China

Abstract

4 experimental diets were fed to turbot to examine the effect of fish hydrolysate and ultra filtered fish hydrolysate on growth performance, feed utilization and growth regulation using diets low in dietary fish meal inclusion. Diets A,B contained 3.7%,1.2% fish hydrolysate to replace fish meal respectively and the fish hydrolysate in two diets was ultra filtered to keep low molecular weight compounds. The diets A, C were identical in composition except that the fish hydrolysate in the diet C was not ultra filtered to have higher molecule weight. A diet containing only intact protein (fish meal and wheat meal) was used as a control (diet D). All diets were made equal in protein, lipid, energy. Each experimental diet was fed to juvenile turbot (27.8±0.04) g in triplicate for 56 days.

Weight gain, specific growth rate and feed efficiency were highest in fish fed diet A, followed by diet B, control and C, did not differ in the control and C diet. Protein and dry matter digestibilities, NPU of fish fed with diet A were higher than that of in the other groups, it was similar between fish fed the diet B and C and lowest in the group fed diet control. Nitrogen retention of fish fed diet A was significantly higher than that of other groups, Nitrogen retention of fish fed diet B was higher significantly than that of fed diet C, Nitrogen retention of fish fed diet Control was not different significantly than that of fed diet C and diet B respectively. Acid phosphatase (ACP), alkaline protease (AKP), lysozyme (LZM) and superoxide dismutase (SOD) activity were not affected by diet. Some of these small compounds in fish hydrolysate thus seem to be essential for biological performance. Results of this study indicate that the best overall growth and feed utilization of turbot juveniles were obtained with a dietary contained small compounds in fish hydrolysate.

Key words: Tutbot; Fish meal; Fish hydrolysate; Growth performance; Feed utilization

* E-mail address: liangmq@ysfri.ac.cn
O-008

The effect of soybean meal (SBM) and its four chemical factors on growth performance and intestinal morphology of juvenile allogynogenetic silver crucian carp (Carassius auratus gibelio♀×Cyprinus carpio♂)

Chunfang Cai1**, Wenjuang Wang1, Wei Zhang1, A. Krogdahl2, Yuantu Ye1*

1 School of Basic Medicine and Biological Science, Soochow University, Suzhou 215123, PR China
2 Aquaculture Protein Centre, CoE, Norwegian School of Veterinary Science, P.O. Box 8146 Dep, NO-0033 Oslo, Norway

Abstract

A series of experiments were conducted to investigate the effect of soybean meal (SBM) and its chemical factors, i.e. raffinose, stachyose, isoflavones and saponins, on growth performance and intestinal morphology of juvenile allogynogenetic silver crucian carp (Carassius auratus gibelio♀×Cyprinus carpio♂). (1) Fish fed diets with fish meal (FM) and 300 g kg⁻¹ SBM respectively during an 8-week feeding trial, while gut was sampled at 0, 3, and 8 weekend for histology analysis. The results showed 300 g kg⁻¹ SBM decreased the growth performance significantly (P < 0.05); a serious symptom of non-infectious enteritis of foregut, midgut and hindgut were noticed in fish fed SBM diet after 3-week feeding, however, the symptom alleviated after 8-week feeding. (2) Refer to the concentration of raffinose and stachyose in soybean, 6.67 g kg⁻¹ raffinose, 26.06 g kg⁻¹ stachyose and 6.67 g kg⁻¹ raffinose + 26.06 g kg⁻¹ stachyose were added to the FM based diets respectively. Fish fed the test diets for 8 weeks and no significant change were noticed on growth performance and intestinal morphology. (3) 0.5 - 8.0 g kg⁻¹ isoflavones were added to the FM based diets and fed fish for 17 weeks. Significant negative effect on growth performance were noticed only when the concentration of isoflavones was 8.0 g kg⁻¹. However, compare with the control group, symptom of non-infectious enteritis as well as low density of microvilli were noticed at each concentration. (4) 1.0 - 6.0 g kg⁻¹ saponins were added to the fishmeal based diets and fed fish for 10 weeks. Significant negative effect on growth performance were noticed only when the saponins concentration was 6.0 g kg⁻¹. Widening of the central lamina propria within the intestinal folds, with increased amounts of connective tissue, and infiltration of a mixed leucocyte population in the lamina propria were observed in fish fed diets with 6.0 g kg⁻¹ isoflavones. (5) The mixture of saponins and isoflavones seems aggravated the symptom of non-infectious enteritis introduced by isoflavones or saponins. However, no change in intestinal morphology were observed when fish fed mixture of saponins, raffinose and stachyose.

Keywords: Soybean meal; Raffinose; Stachyose; Isoflavones; Saponins; Growth performance; Intestinal morphology

* E-mail address: szcfcai@yahoo.com.cn.
The water soluble fraction in fish meal (stickwater) stimulates growth in Atlantic salmon (*Salmo salar* L.) given high plant protein diets

Katerina Kousoulaki*1, Sissel Albrektsen1, Eyolf Langmyhr1, Hanne Jorun Olsen2, Paddy Campbell2, Anders Aksnes1

1 Nofima Ingrediens, Kjerreidviken 16, N-5141 Fyllingsdalen, Norway
2 BioMar S.A.

Abstract

Fish solubles have gained attention due to their potential use as fish meal replacement in feeds but also due to the experimental evidence on the positive effect they exert on farmed fish performance, especially when incorporated in diets with high plant content. The mechanism behind this effect is not yet fully understood. In this study, increasing levels of stickwater (SW) separated during the fish meal production process were added to produce 5 experimental fish meals. Five experimental diets were prepared which consisted of 50 g kg⁻¹ diet commercial fish meal and 50 g kg⁻¹ diet of the different experimental fish meals with graded inclusion levels of SW. The remaining dietary protein sources consisted of a mixture of plant protein sources (soy protein concentrate; 215 g kg⁻¹, corn gluten; 215 g kg⁻¹; field beans 140 g kg⁻¹ diet; wheat gluten 55 g kg⁻¹) giving 17% of total dietary protein as protein from marine sources. All the experimental diets were isoproteic, isolipidic and isoenergetic. Two more low fish meal diets were prepared containing the low (permeate) or high (retentate) molecular weight fraction of fractionated and ultra filtrated SW. A control diet containing 300 g kg⁻¹ diet commercial fishmeal was prepared. The diets were given to 8 triplicate populations of salmon (initial body weight 137 g) for 74 days. Fish growth was significantly positively affected by the increasing inclusion level of SW in the low fish meal diets. The low fish meal diets performed the same, both in terms of growth rate and feed utilization, as the control diet. The growth parameters were also positively correlated to the dietary levels of taurine and hydroxyproline. No significant differences were found in feed intake or feed conversion ratios, nor was there any difference in ADC of protein, lipid, gross energy or phosphorus (P) among any of the diets containing the experimental fish meals. Whole body proximate composition and total and soluble P content of fish were not affected by the inclusion level of SW in the diets. Significant effects were identified in the free amino acid profile in the fillet of fish fed the different experimental diets. The present work gives further evidence for the importance of marine water soluble fractions and small marine water soluble components in high plant protein diets for salmon. By the addition of SW to the diets higher flexibility in the choice of protein sources may be obtained.

Keywords: Salmon; Plant protein; Stickwater; Marine water solubles; Taurine; Hydroxyproline

*E-mail address: katerina.kousoulaki@nofima.no (Katerina Kousoulaki).
Effects of fish oil replacement by increasing levels of cotton seed oil (CSO) on ammonia and urea excretion rates in the juvenile European sea bass, *Dicentrarchus labrax*

Kenan Engin1*, O. Tufan Eroldoğan2, İlgin Özşahinoğlu2, O. Taşbozan2, H. Asuman Yılmaz2, Mustafa Yıldız3, Pınar Mumoğullarında2

1Faculty of Fisheries and Aquaculture, Mersin University, Yenişehir kampüsü C Blok Kat:2 33169, Mersin, Turkey
2Faculty of Fisheries, University of Çukurova, 01330, Adana, Turkey
3Faculty of Fisheries, University of Istanbul, Ordu cad. No: 200, 34130 Laleli, Istanbul, Turkey

Abstract

Measurement of total ammonia and urea-nitrogen excretion can give an insight into nitrogen metabolism in fish under the effects of various environmental and nutritional factors. Alternative oil sources are increasingly being investigated to replace fish oil in aquafeeds but there is little known of their effects on nitrogenous excretion. Therefore, this study aimed at demonstrating the effects of fish oil replacement by increasing levels of cotton seed oil (CSO) on daily total ammonia and urea-nitrogen excretion rates in the juvenile European sea bass (*Dicentrarchus labrax*). Five iso-nitrogenous and iso-lipidic diets (48 % CP and 22 % CL on a dry matter basis) were formulated to contain cotton seed oil to replace fish oil by 0, 40, 60, 80 and 100 %. A total of 300 fish (67.8±3.2 AMBW) were randomly assigned to 15 500 l (350 l rearing volume) and fed 3 % BW/d two times to apparent satiation. Diurnal total ammonia (TAN) and urea nitrogen excretion rates were measured using phenol-hypochlorite and urease methods respectively. There was no significant difference in feed intake among dietary treatments. Increasing levels of cotton seed oil inclusion in diets did not change daily TAN excretion rates in the juvenile European sea bass compared to fish oil diet but significantly lower (P<0.05) excretions in fish fed diets with 40 and 100 % cotton seed oil following 8 h of afternoon feeding were noteworthy. However, the urea-nitrogen excretions in fish fed diets containing increasing levels of cotton seed oil were significantly higher (P<0.05) than the fish oil diet 8 h following the afternoon feed. It appeared that higher urea-nitrogen excretion might have been the consequences of a strategy adopted by fish to deal with influx of plasma TAN caused most probably by the distorted cell membrane permeability and fluidity. These results also indicate a higher energetic cost associated with increasing cotton seed oil inclusion in diets since urea-nitrogen excretion requires at least two additional molecules of ATP in fish.

Keywords: European sea bass; Total ammonia-nitrogen; Urea-nitrogen, Excretion, Cotton seed oil

* E-mail Address: kengin@mersin.edu.tr (K. Engin).
Effects of partial substitution of dietary Fish Oil (FO) with Crude Palm Oil (CPO) and Palm Fatty Acid Distillate (PFAD) on growth, feed efficiency, Muscle Fatty Acid Composition, biological Indices and the Activities of Hepatic Lipogenic Enzymes in Snakehead (Channa striatus, Bloch 1793) Fingerling.

Mohammed Aliyu-Paiko1*, Roshada Hashim1, Alexander A. S. CHONG1

1Laboratory of Feeds and Feed Development, Aquaculture Research Group, School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia.

Abstract

The effects of partial substitution of dietary fish oil (FO) with crude palm oil (CPO) and palm fatty acid distillate (PFAD) on growth performance, feed efficiency, muscle fatty acid composition, biological indices and activities of hepatic lipogenic enzymes were studied in Snakehead (Channa striatus, Bloch 1793) fingerling. Three diets were formulated to be iso-nitrogenous (45 g/Kg), iso-lipidic (6.5 g/Kg) and iso-energetic (18.5 KJ/g), varying only in their lipid sources (as designated); 100% FO (100FO), 50%FO50%CPO (50CPO) and 50%FO50%PFAD (50PFAD). Feed were hand fed to homogenous groups of 12 C. striatus fingerlings (mean weight 3.5 ± 0.3 g) per tank in triplicate for 12 wk, in a recirculation system. Growth performance and feed intake in the 50CPO and 50PFAD treatments were significantly higher than in the fish fed the reference diet (100FO) respectively, whereas FCR was similar and lower among the 100FO and 50CPO treatments compared with the 50PFAD. The biological indices monitored (HSI, VSI and CY) showed no significant variations among all the treatments respectively. The muscle FA profile of fish was substantially influenced by the FA composition of the diets fed, whereas no noticeable differences were recorded in the activities of the hepatic lipogenic enzymes (FAS, CCE and ME) monitored. Whole body proximate composition analysis revealed that 50PFAD treatment contained significantly higher protein and ash, but lower lipid contents, although the muscle contents were similar among all the treatments. It is concluded based on the results of this trial that CPO and PFAD can be used to partially substitute FO in diets for C. striatus fingerling for high survival and good performance without adverse effects or compromising the muscle FA profile.

Keywords: Channa striatus, lipogenic enzymes, fatty acid, CPO, PFAD, VO, FO

* E-mail address: moleepaiko@yahoo.com (M. Aliyu-Paiko).
O-012

Reduced reliance on marine wild fishery resources: progress in combined replacement of fish meal and fish oil in fish feeds under the EU-Aquamax project

Kaushik, S.J., 1 Corraze, G., 1 Bell, J.G., 2 Tocher, D.R., 2 Perez-Sanchez, J., 3 Csengeri, I., 4 Giri, S.S., 5 Mohanty, S., 5 Torstensen, B., 6 Berntssen, M., 6 Lie, O., 6

1 INRA Nutrition Aquaculture and Genomics Research Unit, UMR 1067- NuAGe, INRA Pôle d’Hydrobiologie, 64310 St-Pée-sur-Nivelle, France
2 Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland, UK
3 Institute of Aquaculture Torre de la Sal, CSIC, 12595 Ribera de Cabanes, Castellon, Spain
4 Research Institute for Fisheries, Aquaculture and Irrigation (HAKI), 5541 Szarvas, Hungary
5 Central Inst of Freshwater Aquaculture (CIFA), ICAR, Bhuvaneswar 751 002, India
6 National Inst of Nutrition & Seaood Research (NIFES), P.O. Box 176 Sentrum, 5804 Bergen, Norway

Abstract

The production of safe and healthy seafood from aquaculture starts with the feed and continuing expansion of fish farming requires the development of sustainable feeds. Most intensively farmed species require feeds relying rather heavily on fish meal and fish oil as sources of essential macro and micronutrients. It is recognised that besides the shortage and/or high costs of such marine resources, the levels of potential contaminants in such ingredients require an integrative approach to find alternatives to make the entire aquaculture production chain sustainable in the long term. Under “Aquamax”, an integrated EU project, (http://www.aquamaxip.eu), one of the objectives was hence “to develop feeds based largely on sustainable alternatives to fish meal (FM) and fish oil (FO) to produce healthy, minimally contaminated fish that are highly nutritious and acceptable to consumers”. Under this project, studies were undertaken with Atlantic salmon, rainbow trout, gilthead seabream and cyprinids to develop feeds with targeted low levels of both FM and FO over long growth periods (FM: 12-16% in salmon, 5 % in trout, 15% in seabream; FO: 8-12% in salmon, 5% in trout and 5% in seabream).

Besides growth, feed and nutrient utilisation, a number of criteria (biochemical, molecular, sensory analysis) were used to evaluate the consequences in terms of overall performance, flesh quality, fish health and welfare etc., As was already shown in a number of species, the possibility of tailoring flesh fatty acid composition was also confirmed. Completing the studies under laboratory conditions, feeds developed with low FM-FO levels have also been tested and validated under field conditions involving feed manufacturers and fish farmers. The results demonstrate that considerable reductions in FM and FO levels are possible even for intensively reared totally feed-based fish, significantly reducing the pressure on wild-marine fishery resources, thus reducing the “fish-in fish-out (FIFO)” ratios. These data are also complemented with life cycle analyses of feeds and fish production.

Keywords: Fishmeal, Fish oil, replacement, sustainability

* E-mail address: kaushik@st-pee.inra.fr (S.J. Kaushik).
Thermal regulation of lipid metabolism in barramundi, *Lates calcarifer*

Ramez Alhazzaa\(^1,2\*\), Andrew Bridle\(^1\), Peter Nichols\(^2\) and Chris Carter\(^3\)

\(^1\) NCMCRS, University of Tasmania, Locked Bag 1370, Launceston, TAS 7250, Australia
\(^2\) CSIRO Food Futures Flagship, Division of Marine and Atmospheric Research, TAS 7001, Australia
\(^3\) TAFI, University of Tasmania, Private Bag 49, Hobart, TAS 7001, Australia

**Abstract**

Aquaculture of barramundi, *Lates calcarifer*, is spread over wide ranges of latitudes through tropical and temperate Asia and Australia and usually occurs outdoor in ponds and cages where seasonal differences lead to changes in water temperature. At low temperatures, barramundi feed intake is significantly reduced and growth efficiency decreases. Lipid is involved in energy storage and generation, along with other physiological functions. Lowering temperature may lead to changes in absolute or relative amounts of total lipid and the proportion of lipid classes and fatty acid groups in fish body. Biochemical functions related to these lipid classes and their metabolites will be affected as well as flesh quality. We investigated the influence of different dietary lipid sources fed to barramundi in a controlled environment and the changes in lipid profile following dropping water temperature as can occur in farms. Fingerlings (50 ± 2 g body weight) were kept in 15 ppt salinity, 24 h light photoperiod and fed on three dietary treatments differing only in their lipid source: fish oil (FO), canola oil (CO) and oil from *Echium plantagineum* seeds (EO). Following 5 weeks at 30°C, the water temperature was dropped to 20°C for one week. At the end of week 5, FO and EO treatments had comparable growth performance parameters which were higher than for the CO treatment. At 20°C, fish almost stopped eating and daily feed ratio were readjusted in control temperature treatments to match, but fish could sustain their weights in all treatments through this one-week period compared with control. Whole body content of total lipid was not different between treatments at the end of the 30°C period and dropped at similar rates in different dietary treatments after one week at 20°C. Relative levels of different lipid classes were not significantly affected by different diets before and after the temperature drop except for the polar lipid percentage which were lowest for FO-fed fish and highest for the EO treatment. These findings indicate efficient growth and lipid metabolism of barramundi fed on different dietary lipid sources and demonstrates them as a competent eurythermal aquaculture species. Feeding barramundi on plant oils can achieve comparable growth rates to FO at a wide range of temperature. It can be practical to formulate a diet with FO and plant oil to reduce the dependency on FO using sustainable alternative sources for aquafeeds.

**Keywords:** barramundi, lipid, temperature

\(^*\) E-mail address: alhazzaa@utas.edu.au (R. Alhazzaa).
Distribution of melamine related crystal in walking catfish (Clarias batrachus) organs

Nopadon Pirarat¹, Takayuki Katagiri², Kunihiko Futami², Makoto Endo², Masashi Maita²
¹ Department of Pathology, faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand
² Laboratory of Fish Health Management, Tokyo University of Marine Science and Technology, Tokyo, Japan

Abstract
The evidence of melamine and its related compound toxicity has been increasingly reported in various animals. The metabolism, bioaccumulation and pathological study revealed that melamine and its related compound were accumulated only in kidney suggesting a specific nephro-toxic compound. However, in fish, the toxicological data of melamine and its related compound was very limited. Here we examined the histopathological changes and the distribution of melamine related crystal induced in walking catfish by oral administration of melamine and cyanuric acid. Fish were divided into 7 groups (control, 0.5%M, 0.5%Cy, 2%M, 2%Cy, 0.25%M+0.25%Cy and 1%M+1%Cy). There were no fish died during two weeks experimental period. Melamine related crystals were evidenced in fish fed with melamine and cyanuric acid. Melamine related crystals were diffusely precipitated in trunk kidney, liver, spleen, heart and corpuscles of stannius. Foreign body granulomas were also seen in all affected tissues. In kidneys, melamine related crystals were distributed in both glomerular and nephron units especially in distal convoluted tubules. Melamine and cyanuric acid was approximately detected in muscular tissues as 402.33 and 800.55 ppm respectively. In contrast with the previous reports of melamine and cyanuric acid toxicity in various animals and human, our results showed that toxicity of melamine and its related compound in fish were not limited only in kidney. Various tissues were also affected. The result suggested that the bioaccumulation, the clearance metabolism and the pathology of melamine and its related compound in fish should be further focused.

Keywords: melamine, cyanuric acid, walking catfish

* E-mail address: nopadonpirarat@hotmail.com.
Why do fish have limited capacity for carbohydrate utilization?

Åshild Krogdahl* and Anne Sundby
Aquaculture Protein Centre (a CoE), Department of Aquatic Medicine and Nutrition, Norwegian School of Veterinary Science, P.O. Box 8146 Dep, NO-0033 Oslo, Norway

Abstract
Fish are often claimed to have limited capacity for carbohydrate metabolism. Rates of glucose turnover and glucose oxidation in fishes are in most cases 1 - 2 orders of magnitude below those of mammals of comparative size. Lower body temperature and more sluggish metabolic rate may account for some of the difference. Hepatic gluconeogenesis in fish shows modest rate and absence of an alanine-glucose cycle may indicate that glucose is not a metabolic focus point in fish in contrast to terrestrial animals. More than 60 years ago, it was suggested that the optimum level of starch was 12% in diets for rainbow trout. Subsequent publications have supported this suggestion, but also shown that omnivore and herbivores can tolerate more. At low levels of inclusion carbohydrates can replace protein and spare amino acids also in carnivores. Inclusion above 10 – 12% of dietary carbohydrates reduces feed utilization in carnivores whereas omnivores have the ability to metabolize more carbohydrates. Omnivores and herbivores are to a greater extent able to increase feed intake to compensate for the lower energy density of diets high in carbohydrate and thereby to keep up growth rate. Carbohydrate tolerance in fish seems to increase with increasing water temperature. Likewise, protein sparing effect of carbohydrates has been found to increase with water temperature. Starch promotes better growth and higher liver lipogenic enzyme activities than glucose or other mono-saccharides. Studies to quantify conversion of glucose to glycogen and lipids are few. The results indicate however, that only minor amounts of ingested carbohydrates are converted to glycogen in the liver and muscle, and an even smaller fraction is converted to lipids in the liver. It therefore seems that fish, as many mammals have limited capacity to store surplus carbohydrate energy as glycogen or fat. If so, maintenance energy requirement of a fish species limits the level of carbohydrates that can be efficiently utilized by fish. A quantitative consideration will be given regarding carbohydrate metabolism and utilization based on biochemical and physiological aspects of carbohydrate metabolism and recent knowledge on energy requirements for maintenance in fish.

Key words: Carbohydrate utilization; Metabolism, Regulation, Glycogen, Lipid

* E-mail address: ashild.krogdahl@nvh.no

Pirozzi, I.*1 & Booth, M.A.2

1 School of Marine and Tropical Biology, James Cook University, Townsville, QLD, Australia.
2 NSW Department of Primary Industries and Aquafin Cooperative Research Centre, Port Stephens Fisheries Institute, Taylors Beach, NSW, Australia

Abstract

The partitioning and quantification of dietary energy is important in the study of nutritional energetics because it provides a convenient platform to predict the energy balance of individuals based on a number of biotic and abiotic factors. Specific dynamic action (SDA) is the energy expended on the physiological processes associated with meal digestion and is strongly influenced by the characteristics of the meal and the body weight (BW) and temperature of the organism. The effects of temperature (14, 20 and 26 °C) and body weight on the routine metabolic rate (RMR; 60 g to 1.14 kg) and postprandial metabolic response (60 g to 240 g) were assessed in mulloway. The effect of BW on the mass-specific RMR (mg O₂ kg⁻¹ h⁻¹) varied significantly depending on the temperature with a greater relative increase for smaller mulloway with increasing temperature. No statistical differences were found between the mass exponent (b) values at each temperature when tested against $H_0$: b = 0.8. Both SDA duration and time to peak SDA were influenced by temperature and BW; SDA duration occurred within 41-89 h and peak time occurred within 17 - 38 h of feeding. The effect of BW on peak metabolic rate varied significantly depending on temperature, generally increasing with temperature and decreasing with increasing BW. Peak gross oxygen consumption ($M_{\text{O}_2}$: mg O₂ fish⁻¹ h⁻¹) scaled allometrically with BW. Temperature, but not BW, significantly affected SDA scope, although the difference was numerically small. There was a trend for $M_{\text{O}_2}$ above RMR over the SDA period to increase with temperature; however, this was not statistically significant. The average proportion of energy expended over the SDA period (SDA coefficient) ranged from approximately 7 - 13% of the total DE intake while the proportion of total energy expended on SDA above RMR ranged from approximately 16 to 27%. The highly variable nature of parameter values dependent on the individual or interactive effects of fish size, temperature, diet and other factors has important implications on the accuracy of any system attempting to model rates of nutrient deposition and subsequent feed requirements in animals; predictions can only be valid within the physiological and environmental contexts from which the parameter values were derived. This variability indicates that the inappropriate use of fixed parameter values in bioenergetic models may lead to spurious predictions of energy requirements.

Keywords: Nutritional energetics; Specific dynamic action; Metabolic scaling; Postprandial oxygen consumption; Mulloway

* E-mail: Igor.Pirozzi@jcu.edu.au
O-017

Family-specific responses to dietary fish oil replacement by vegetable oil in Atlantic salmon: microarray analysis of liver transcriptome

Sofia Morais\(^1\)*, Jarunan Pratoomyot\(^1\), John B. Taggart\(^1\), James E. Bron\(^1\), J. Gordon Bell\(^1\), Douglas R. Tocher\(^1\)

\(^1\)Institute of Aquaculture, University of Stirling, FK9 4LA Stirling, Scotland, UK

Abstract

Vegetable oils (VO) have the potential to replace, at least partly, fish oil (FO) from wild stocks for the production of more sustainable fish feeds for aquaculture. Nonetheless, growth of fish on VO results in lower levels of n-3 LC-PUFA in their flesh, compromising their nutritional value. Given it is unavoidable that major changes in current commercial feeding practices will occur, one potential strategy would be to select and use strains of fish with higher ability to retain and/or biosynthesise n-3 LC-PUFA. However, this requires that the trait “n-3 LC-PUFA flesh content” is under genetic influence and, in order to investigate the viability of this approach, large-scale in depth studies exploring genotype-nutrient interactions are essential. In this study we have performed a feeding trial in which smolts, drawn from families predicted as either “fat” or “lean”, based on muscle adiposity (Landcatch Natural Selection), were grown from 90g to 3kg over 55 weeks using feeds with 44% plant meals and 25% fish meal and either 100% FO or 100% VO blend. We then analysed the relationship between the phenotype “fat deposition in muscle” (“fat” vs. “lean”) and gene expression in liver, using the TRAITS/SGP salmon 17k cDNA microarray. The results show that “lean” and “fat” families reacted differently, in terms of expression of lipid metabolism-related genes in liver. In general, the diet change affected mostly metabolism-related genes, particularly those related to lipid metabolism and also some for glucose and intermediary metabolism. A few genes involved in oxidative stress were also affected, indicating a potentially lower stress in fish fed VO. Finally, immune response-related genes were also quite highly altered, which might be related to a hypothesized anti-inflammatory role of FO. On the other hand, when comparing both families independently of their diet, much lower differences were noticed in metabolism genes and the majority of altered genes are involved in signaling and gene transcription. However, some important interactions were found between family and diet for several inter-related genes that participate in lipid and lipoprotein metabolism, cholesterol transport and biosynthesis. In addition, although the expression of HUFA-biosynthesis genes was affected in both families (increased expression in fish fed the VO diet), the magnitude of change was considerably higher in the “lean” family. This indicates that, for some metabolic pathways, the effects on gene expression when dietary FO was replaced by VO depended on which family the fish belong to, i.e., there was also an effect of the genetic origin.

Keywords: Atlantic salmon; Fish oil replacement; Sustainable diets; Nutrigenomics; Microarrays

*E-mail address: sofia.morais@stir.ac.uk (Sofia Morais).
O-018

Evidences of the action of essential fatty acids on sea bream neuronal activity

Tibiábin Benítez-Santana¹, Mónica Betancor¹, Mª José Caballero¹, Eyad Atalah¹, Eduardo Juárez-Carrillo¹, Silvia Torrecillas¹, Carmen M. Hernández-Cruz¹ and Marisol Izquierdo¹

¹Grupo de Investigación en Acuicultura (GIA), Instituto Canario de Ciencias Marinas and Universidad de Las Palmas de Gran Canaria. P.O. Box 35200 Telde, Canary Islands, Spain.

Abstract

Highly unsaturated fatty acids (HUFA), particularly docosahexaenoic acid, are known to be essential for normal development of fish neural system and, subsequently, fish behaviour. However, there is a limited information on the type of effect of these fatty acids on neuron development and functioning. Deficiency on those essential fatty acids impairs larval response to different stressors, reducing the escaping behaviour. Mauthner cells are certain neurons particularly responsible for the escaping behaviour in fish. The objective of the present study was to evaluate the effect of EFA on larval behaviour and Mauthner cells development and functioning. Two experiments were conducted feeding 18 dah larvae for 2 weeks on different dietary sources of HUFA to evaluate their effects on larval behaviour and brain development, particularly in relation to Mauthner cells. In trial 1, larvae were fed with microdiets containing soybean, linseed, rapeseed or fish oil, while in trial 2 microdiets contained different ratios of EPA/DHA (4/1; 4/3; 1.5/9; 0.3/0.6). Behaviour was determined by measuring swimming speed of larvae from all groups was measured to obtain burst-swimming speed to sonorous stimulus. Mauthner cells activity was studied by ChAT immuno-fluorescence since acetylcholine is a major neurotransmitter in the enteric nervous system. Behaviour showed a higher response in fish fed either fish oil and or 1.5/9 microdiets suggesting the importance of DHA for the tonicorous stimulus. Besides, a marked immunoreactivity was found in Mauthner neurons from those fish denoting the requirement of DHA for the correct activity of those neurons and their implications on burst swimming behaviour. Adequate intake of n-3 PUFA can leads to increased amounts of DHA in brain membranes and to associated learning and behavioural actions. These results show the first evidences of the action of EFA on fish neuronal activity and their implications in fish behaviour.

Keywords: Sea bream; Mauthner cells; Behaviour; Essential fatty acids

* E-mail address: tibi.benitez.santana@gmail.com (T. Benítez-Santana).
Studies on HUFA biosynthesis in a marine teleost Siganus canaliculatus

Yuan-You Li*, Liang Zhang, Su-Qi Wang, Shu-De Xu, Chang-bo Hu, Yi-Jun Zheng
Guangdong Provincial Key Laboratory of Marine Biology in Shantou University, Shantou, Guangdong, China

Abstract

Marine fish are incapable or have a deficient capacity to synthesize highly unsaturated fatty acids (HUFA) from C18 precursors, and thus fish meal and fish oil (FO) are needed in their formulated diets. With the growth of aquaculture, the illogicality between the demand and deficiency of FO will become more and more serious, and so sustainable alternatives to FO are urgently required. Vegetable oils (VOs), which are devoid of HUFA, are the obvious choice. In these years, we chose the rabbitfish Siganus canaliculatus, an herbivorous marine species, which can also live in brackish water with enormous commercial interest in Southeast China, as the experimental animals to study the characteristics of HUFA biosynthesis. The results based on growth performance and liver fatty acids composition showed that S. canaliculatus may have the ability to convert linoleic and α-linolenic acids into HUFA in both 10 ppt and 32 ppt, and such ability in low salinity was higher than that in high salinity. The replacing ratio of soybean oil (SO) to FO may be up to 67%, whereas the best replacing ratio was 45%, on the basis of comparison of growth performance, feed conversion ratio and protein efficiency rate between the FO- and SO-replacement dietary groups. For obtaining the molecular evidences of HUFA biosynthetic ability and studying the regulatory mechanism, the genes encoding three key enzymes for HUFA biosynthesis were cloned from this fish. The full lengths of cDNAs for delta-5 and delta-6 desaturase, and elongase are 1854 bp (GenBank accession no. GU594278), 1868 bp (EF424276) and 1277 bp (GU597350), respectively. They encode peptides with 445, 443 and 291 amino acids, respectively, which show 67-83% similarities with those of other fishes. Their mRNA expression were mainly detected in liver, brain and intestine. Dietary HUFA inhibited, while linoleic and α-linolenic acids increased their mRNA expression. The mRNA levels of delta-6 desaturase were higher in low salinity than those in high salinity. To our knowledge, this is the first report on the relationship between HUFA biosynthetic ability and ambient salinity in fish, and also the first clone of delta-5 desaturase cDNA from a marine fish. The results increase our understanding on the characteristics of fatty acid metabolism in fish, and are of important significance for directing the development of rabbitfish formulated diets and for clarifying the molecular regulation of HUFA biosynthesis, which will allow us to manipulate and optimize the activity of the pathway to enable efficient and effective use of VOs in future aquaculture.

Keywords: Marine fish; Biosynthesis of poly-unsaturated fatty acids; Nutritional regulation; Salinity regulation

* E-mail address: yyli@stu.edu.cn (Y.-Y. Li).
Effect of different dietary vitamin D levels on growth and calcium and phosphorus metabolism in Japanese seabass, *Lateolabrax japonicus*

Lu Zhang¹, Kangsen Mai², Qinghui Ai², Chunxiao Zhang², Huitao Li², Junli Wan², Jiaming Zhang², Shixuan Zheng¹

¹Guangdong Yuehai Feed Group, 22 Airport Road, Xiashan District, Zhanjiang 524017, P.R. China
²The Key Laboratory of Mariculture (Education Ministry of China), Ocean University of China, 5 Yushan Road, Qingdao 266003, P.R. China

Abstract

A 9-week feeding experiment was conducted to estimate the effect of dietary vitamin D levels on growth and calcium and phosphorus metabolism of Japanese seabass, *Lateolabrax japonicus*. Six isonitrogenous (43% crude protein) and isoenergetic (20KJ g⁻¹ dry matter) purified diets (Diets 1 - 6) containing 34.2, 219.4, 393.8, 775.9, 1534.1 and 3091.2 IU vitamin D (supplied as cholecalciferol, D₃) per kg diet were fed twice daily to triplicate groups (15 fish / group) of fish (initial weight 2.26 ± 0.03 g / fish) in 300 L fiberglass tanks. Fish fed the basal diet (Diet 1, 34.2 IU vitamin D / kg diet) did not show any vitamin D deficient syndrome at the end of experiment, although survival was the lowest among all the dietary treatments. Weight gain (WG), specific growth rate (SGR), feed efficiency ratio (FER) and protein efficiency ratio (PER) increased with increasing dietary vitamin D level up to 393.8 IU / kg diet (Diet 3), and thereafter, level off. Liver vitamin D concentration in the fish increased with increasing dietary vitamin D level up to 1534.1 IU / kg diet (Diet 5), and thereafter, plateaued. Dietary vitamin D level had no significant influence on carcass crude protein, crude lipid and moisture, but significantly influenced the carcass ash, calcium and phosphorus contents (p<0.05). Meanwhile, dietary vitamin D level significantly affected the mineralization of vertebra, operculum and scale. The analyses of serum parameters showed that alkaline phosphatase (AKP), hydroxyproline (HPro), calcium ion (Ca²⁺) and inorganic phosphorus (Pi) content were all significantly affected by dietary vitamin D level. Broken-line regression analysis of weight gain showed that juvenile Japanese seabass require a minimum of 431.0 IU vitamin D / kg diet for maximal growth. The second–order polynomial equation had the best fit for depicting the relation between dietary vitamin D and liver vitamin D contents. Based on the second–order polynomial analysis, Japanese seabass require 2444.4 IU vitamin D / kg diet for maximal liver vitamin D deposition.

Keywords: Vitamin D; Cholecalciferol; Growth; Requirement; Japanese seabass; *Lateolabrax japonicus*

¹ E-mail address: zhanglu_ouc@163.com (Lu Zhang).
Intestinal epithelial cell turnover is an adaptive response to diet-induced enteritis in Atlantic salmon (Salmo salar L)

Elvis M. Chikwati1*, Inger Rudshaug1, Gunn C. Østby1, Fredrik F. Venold1, Thor Landsverk2, Ståle Refstie3, Anne Marie Bakke1, Michael H. Penn1, Åshild Krogdahl1
1Aquaculture Protein Centre (a CoE), Department of Basic Sciences and Aquatic Medicine, Norwegian School of Veterinary Science, P O Box 8146 Dep, NO-0033, Oslo, Norway
2Section of Anatomy and Pathology, Department of Basic Sciences and Aquatic Medicine, Norwegian School of Veterinary Science, P O Box 8146 Dep, NO-033, Oslo, Norway
3Aquaculture Protein Centre (a CoE), Nofima Marine, 6600 Sunndalsøra, Norway

Abstract

Inclusion of plant ingredients in fish feeds is often associated with manifestations of wide ranging gastrointestinal (GIT) disorders. The causes remain elusive. Knowledge regarding intestinal response mechanisms may contribute to the better understanding of the causes and mechanisms behind these disorders. Intestinal epithelial cell (IEC) turnover (proliferation, differentiation, migration and loss) is an important adaptive response of the fish intestine to diverse stimuli such as inflammation, parasitism, chemo- and radio- toxicity.

A 28-day experiment aimed at characterising IEC turnover as an adaptive response to a well-documented diet-induced enteropathy was performed with two groups of Atlantic salmon (Salmo salar L.) post smolts in seawater at two different temperatures of 8 and 12 °C. Two diets were fed, a control fishmeal (FM)-based diet and the experimental diet containing 20% extracted soybean meal partially replacing fishmeal as the protein source. IEC proliferation was investigated by immunohistochemical detection of cells labelled with the proliferation marker 5-bromo-2´-deoxyuridine (BrdU) injected intraperitoneally to all the fish at day 0 of the experiment. Sites of predominant proliferative activity were studied using antibodies against BrdU and the proliferating cell nuclear antigen (PCNA). Studies were conducted on histology samples of the pyloric, mid and distal regions of the intestine collected at 19 time points over the experimental period.

Histologically, FM-fed fish had normal mucosa while soybean meal (SBM)-fed fish had developed distal intestinal enteritis with shortened mucosal folds. Major zones of cell proliferation were in the bases of mucosal folds and BrdU-labelled cells migrated up the mucosal folds to the tip before being lost. Migration rates were temperature and diet dependent. Migration was slow in FM-fed fish with BrdU labelled cells in the distal intestine, for example, having covered 40% and 75% of the mucosal folds in 28 days for the 8 °C and 12 °C groups, respectively. Absolute migrating cell distances over time will also be presented. Migrating cells in SBM-fed fish reached the mucosal fold tips after 15 days post BrdU injection (p.i.) at 8°C and after about 10 days at 12°C. SBM-fed fish seemed to develop additional sites of epithelial proliferation in the inflamed distal intestine from 5 days p.i. and onwards. Findings in this study complement previous studies that observed similar intestinal responses to other insults in suggesting that IEC turnover is a non-specific intestinal adaptive response whose impact regarding adaptation to the pathologic state of SBM-induced enteritis in fish, however, needs further investigation.

Keywords: Intestinal epithelium; Cell proliferation; BrdU; Soybean meal; Atlantic salmon

* E-mail address: elvis.chikwati@nvh.no (E.M. Chikwati).
Responses of rainbow trout clonal lines to plant-based feed evidence genotype x diet interactions

F. Médale*1, M. Dupont-Nivet2, S. Rimoldi1, H.A. Yilmaz1, F. Terrier1, G. Corraze1, E. Quillet2, I. Geurden1

1. INRA, UMR 1060, NUAGE, Laboratory for Aquaculture, Genomics and Nutrition, 64310 St Pee sur Nivelle, France
2. INRA, UMR 1313 GABI, Animal Genetics and Integrative Biology, 78350 Jouy-en-Josas, France

Abstract

This study compares the response of 8 heterozygous clones of rainbow trout to two extreme experimental diets: one devoid of marine ingredients, made of blends of plant sources and vegetable oils (diet V), the other containing fish meal and fish oil as protein and lipid sources (diet M). Triplicate groups of each clone (35 fish per group – 30-40g initial body weight – 17°C) received one of the two diets twice a day to visual satiation for 4 months.

Feeding diet V reduced growth rate of each clonal group due to a significant decrease in both feed intake and feed efficiency. However large differences in responses to the dietary treatments were observed among the clones and significant interactions were recorded for several parameters: feed intake, feed efficiency, growth rate, retention of protein, lipid and energy as well as proportions of some specific fatty acids of whole body. Regarding growth, the genotype x diet interaction is illustrated by differences in body weight ranking of the clones depending on the diets, i.e. some clones displaying high growth when fed diet M had one of the lowest growth rates when fed diet V, while others had the same ranking within each dietary treatment. Diet V lowered protein retention in all groups compared to diet M. This decrease ranged from 12 to 38% depending on the clones. Lipid retention in two clones was similar irrespective of the dietary treatment, while it was lower for all other groups when fed diet V compared to diet M (8 to 28% depending on the genotype). Irrespective of the genotype, total substitution of fish meal and fish oil by plant products resulted in lower proportions of long-chain polyunsaturated fatty acids n-3 and increased proportions of fatty acids typical of vegetable oils. Differences in gains and losses of n-3 highly unsaturated fatty acids among clones suggest differences in the capacity of fatty acid synthesis and/or utilization. The present clonal groups provide thus a valuable experimental material for further understanding mechanisms associated with the improved utilization of plant-based diets in carnivorous fish and may open new perspectives for the genetic selection of fish.

Key-words: Fish meal and fish oil substitution; Nutrient utilization; Body composition

*E-mail address: medale@st-pee.inra.fr (Dr Françoise Médale)
The Role of Animal by-Products in Aquaculture Feeds

Sergio F. Nates*, Kent Swisher
1Fats and Proteins Research Foundation, Inc., 801 N. Fairfax Street, Suite 205, Alexandria, Virginia, 20619 USA
2National Renderers Association, 801 N. Fairfax Street, Suite 205, Alexandria, Virginia, 20619 USA

Abstract

Aquaculture is expanding rapidly throughout the world. The driving forces for this expansion include the need for additional food resources, and the recognition of marine oils and other products as healthy substitutes for other traditional products. New potential aquatic species are being studied and cultured each year creating a need for specialized formula feeds and feed ingredients. However, one of the major challenges faced by aquaculture industries worldwide includes sourcing alternative proteins and oils for animal feeds. Because many aquatic species require rations high in protein, a wide variety of animal and marine meals of very similar appearance are used. Of all the ingredients used in the manufacturing of aquaculture feeds, fish meal appears to be the most critical ingredient as it is required in certain quantities for health and performance of many species. However, supplies of fishmeal are declining and prices are increasing. Worldwide the demand for fishmeal and fish oil has increased to 50% of production compared to only 30% of production in 2003.

Pressures to reduce fish meal consumption, combined with its increasing price, has resulted in research to find candidates for fish meal replacement, and the replacement of fishmeal in aquaculture diets is a major international research priority. The rendering industry in the US collects and processes over 50 billion pounds of by-products from the slaughter and meatpacking industries and transforms them into useful and valuable feed and industrial materials. Rendered animal proteins are especially valuable to the aquaculture feed industry because of their high protein content, digestible amino acid levels, mineral availability (especially calcium and phosphorous), and relatively low cost in relation to their nutrient value. Meat and bone meal (MBM) and poultry by-product meal (PBM) are two potential alternative protein sources because of their high protein content (45-65%) and low cost compared to fish meal, and have been successfully used for replacement of fish meal in diets of many aquatic animals.

In this context, this presentation will attempt to present data on the use of rendered by-products in aquaculture feeds. It will explore the research program funded by Fat and Protein Research Foundation (FPRF), Inc. and the research developed to substitute fish meal and fish oil using animal byproducts. In addition, the North American Rendering Industry Code of Practice, which promotes the safety of animal proteins and rendered fats for feed use through the establishment of recommended industry programs and an accreditation process, will also be presented. Finally, worldwide production data and trends on animal protein meals will be reported.

Keywords: Animal protein meal, Animal by-product; Fat and Protein Research Foundation; FPRF, Meat and bone meal, Poultry by-product meal

*E-mail address: snates@nationalrenderers.com (S. Nates).
Assessment of the Nutritive Value of Processed Animal Proteins for Fish

D.P. Bureau

1 UG/OMNR Fish Nutrition Research Laboratory, Department of Animal and Poultry Science, University of Guelph, Guelph, ON, N1G 2W1, Canada

Abstract

Processed animal protein ingredients, such as blood meal, feather meal, meat and bone meal, and poultry by-products meal, compare favorably cost-wise with many other types of protein sources commonly used in fish feeds. Accurately characterization of the nutritive value of the different types of processed animal proteins available on the market is essential to optimize their use in feeds. Processed animal proteins are produced using different raw materials and cooking and drying equipment and this may result in significant differences in the digestibility and nutritive value of these ingredients. Our understanding of critical parameters is limited, despite the significant research effort invested by animal nutritionists over the past 30 years. Methodological approaches and screening test to meaningfully differentiate ingredients of different nutritive values are still relatively poorly developed.

A significant effect of processing conditions had been observed on the apparent digestibility coefficient (ADC) of protein of blood meals. Studies have also highlighted differences in the digestibility and nutritive value of different grades of poultry by-products meals (e.g. pet food vs. feed-grade). Differences in ADC of protein of feather meals and meat and bone meals from different origins have also been observed in fish. However, there has been not clear demonstration of differences in the nutritive value of different feather meals and meat and bone meals despite anecdotal evidence from aquaculture feed manufacturers to that effect.

To date assessment of the nutritional value of processed animal proteins has mainly focused on apparent digestibility, with main emphasis on proximate analysis components (dry matter, crude protein, lipid, energy) and much less emphasis on specific nutrients, such as amino acids. Different processed animal proteins have been evaluated in hundreds of “practical” feeding trials in which the test ingredients replaced fish meal or other high quality protein sources. In most of these feeding trials, the control diet is formulated with high fish meal levels and/or all essential nutrients, especially essential amino acids, are supplied greatly in excess of requirements. The test ingredient is included at graded levels and effect on growth performance is monitored. Under these conditions, the evaluation of the nutritive value of the “test” ingredients is not very robust nor is it specific enough. There is a need to refine methodological approaches so that focus is on assessment of available nutrient “contribution” of the test ingredients to the diet (i.e. the bioavailability of nutrients in ingredients) rather than “absence of effect” of the test ingredient.

Keywords: Diet, Ingredient; Protein; Digestibility; Nutritive Value; Amino Acids

*E-mail address: dbureau@uoguelph.ca (D.P. Bureau).
O-025

Is ruminant meat and bone meal a vector of BSE transmission through aquaculture feeds??

John E Halver
School of Aquatic and Fishery Sciences, University of Washington, USA

Abstract

The probability that ruminant meat and bone meal is a vector for transmission of bovine spongiform encephalopathy (BSE) through aquaculture feeds from cattle to fish to man involves two assessments.

The first probability hinges on incidence of BSE passing through the fire-wall established by the National Renderers Association to produce MBM for animal feeds. BIORAD presumptive testing coupled with Immunohistochemistry and Western Blot confirmatory tests have eliminated BSE prion presence in MBM made by certified NRA producers of material used for aquaculture feeds.

The second probability for transmission is coupled to the ability of the abnormal prion protein to survive in a poikilothermic animal by passing through the digestive and intestinal wall into the circulatory system and passing the blood brain barrier for invasion of the nervous system. Tests have shown that this has not occurred.

Therefore sequential probability of BSE transmission from cow to fish to man is astronomically small.
Review of use of plant proteins in aquafeeds

Delbert M. Gatlin III*
Department of Wildlife and Fisheries Sciences and Faculty of Nutrition
Texas A&M University System
College Station, Texas 77843-2258

Abstract

Demand for seafood continues to increase at a rate which will require a significant global increase in farmed-fish production over the next 15 years. To meet that demand, feedstuffs that can be used in economically viable and sustainable aquafeeds must be further developed and evaluated. Alternatives to fish meal and fish oil, on which many present aquafeeds are largely based, are required to continue the increase in aquaculture production. Thus, dating back to the summer of 2005, a gathering of international experts interested in aquaculture nutrition occurred in Twin Falls, Idaho to assess the status of plant feedstuffs used in aquafeeds for carnivorous species, and consider approaches to increase advancements in this area. At that meeting, the group organized itself into the Plant Products in Aquafeed (PPA) Working Group with one of its first tasks to compile a review paper concerning the potential of current and future plant-based ingredients to replace fishmeal in aquafeeds. That review paper was published in Aquaculture Research in 2007 (vol. 38, pp. 551-579). Following the initial workshop of the PPA Working Group, another gathering in the Fall of 2005 occurred in which approximately 30 aquaculture and agronomy researchers and representatives from U.S. technical agencies developed an integrated (cross-disciplinary) strategic approach to improve understanding of the factors limiting inclusion of plant feedstuffs in diets for carnivorous fishes. The participants drafted a roadmap of research required to reach defined goals, with accompanying performance measures and targeted outcomes. This strategic plan was published in Reviews in Fisheries Sciences in 2008 (vol. 16, pp. 449-455) and highlights from it will be presented at this workshop.

Keywords: Diet Formulations, Nutrition; Plant Protein Feedstuffs; Research Strategies

*E-mail address: d-gatlin@tamu.edu
In replacing fishmeal, what are we replacing?

M.H. Holme*, V. Crampton, K. Ruohonen, R. Taylor, K. Østerhus and V. Ohnstad
Ewos Innovation, N-4335 Dirdal, Norway

Abstract

Fishmeal has traditionally been the most important ingredient in commercial salmon feed formulations, but fluctuating availability and price as well as concerns over the sustainability of the fishmeal fisheries have resulted in a need for more flexible and rational use of fishmeal. In the search to reduce fishmeal inclusion in feeds, several studies compare fish growth achieved with traditional high fishmeal diets, with growth achieved when feeding diets containing lower fishmeal inclusion. Success is claimed when the low fishmeal feeds support growth equal or better than the control feed. But how can success be assured if we don’t know the quality of the fishmeal we are replacing?

EWOS Innovation has since 2008 conducted extensive studies of chemical and technical parameters of fishmeals from various species and suppliers from all over the world. The testing has also included evaluation of biological performance in a series of growth trials, where a standardized protocol has been used to calculate growth and mortality response as a proportion of the performance achieved with an LT meal from particular supplier. Care has been taken to ensure consistent growth on the reference meal in every trial, and the repeated tank mean weight measures have been modeled with cubic splines in relation to time. Results have shown extensive variation in proportional growth to reference (ranging from 0.6 to 1.2% or reference meal), demonstrating the enormous variability in fishmeal quality. When applying the same methodology to measure growth of fish fed plant protein sources, the conclusion is clearly dependant on what fishmeal has been used in the control diet.

The chemical testing of more than 300 fishmeals have documented large variation in both total protein and amino acid profile among meals. However, our testing shows that although this analysis gives important information on the supplementation needed in order to achieve balanced diet, the amino acid profile is not by itself a good indicator of fish meal quality. This highlights the importance of using high quality fishmeal for scientific testing, as it is clear that the ability of a low fishmeal feed to support growth is more strongly affected by the quality of the chosen fishmeal rather than the quality of the low marine feed under examination.

Keywords: Fishmeal quality, biological performance, low fishmeal diets, amino acid profile

* E-mail address: may.holme@ewos.com (M.H. Holme)
Potential and problems in the use of rendered animal protein ingredients as dietary protein sources for three marine fish species

Wang Yan*
College of Animal Sciences, Zhejiang University, 268 Kaixuan Road, Hangzhou 200090, Zhejiang Province, P. R. China

Abstract

Rendered animal protein ingredients, such as poultry by product meal (PBM), meat and bone meal (MBM), feather meal (FEM) and blood meal (BM), have been demonstrated good dietary protein sources for fishes because their high protein content and balanced amino acid profile. In the past years, we examine the potential of using PBM, MBM, FEM and BM as fish meal substitutes in diets for three marine fish species, cuneate drum, malarbar grouper and Japanese sea bass. Fish meal level in the diets for cuneate drum can be reduced from 35% to 17.5, 24.5% by including PBM and MBM as fish meal substitutes alone, respectively, and can be further reduced to 7.5% by using the blends of PBM, MBM, FEM and BM, without significant negative effect on growth performance, feed utilization and body composition of the fish. In malarbar grouper, dietary fish meal level can be reduced from 50% to 25% by including PBM and MBM alone or by including PBM, MBM, FEM and BM in combination. Fish meal in the diet formulation for Japanese sea bass can be completely removed by including pet grade PBM as fish meal substitute. Our results indicate PBM and MBM are good dietary protein sources for cuneate drum, malarbar grouper and Japanese sea bass, and can be used to replace fish meal at high levels. Failure in using FEM at high levels in diet formulation for cuneate drum and malarbar grouper was found, and we deduce the limitation is mainly attributable to the deficiency of Met and Lys. Relationship between dietary fish meal requirement and dietary protein level is also find in cuneate drum.

Key Words cuneate drum; malarbar grouper; Japanese sea bass; fish meal; rendered animal protein ingredient

* E-mail address: ywang@zju.edu.cn (Y. Wang).
O-029

Evaluation of partial replacement of fishmeal by terrestrial, marine and vegetal protein sources in diets for spotted rose snapper *Lutjanus guttatus*

Crisantema Hernández*1, Ronald Hardy2, Miguel A. Olvera-Novoa3, Paul Varilla1, Alan Santos1, Denisse Marquez1, Martin Valverde1, Patricia Domínguez1, Blanca González1, Maria I. Abdo1.

1Laboratory of Nutrition and Larviculture, Centro de Investigación en Alimentación y Desarrollo (CIAD), Unidad Mazatlán. C.P. 82010, Mazatlán, Sinaloa, México.
2Hagerman Fish Culture Experiment Station, University of Idaho, Hagerman, Idaho 83332 USA
3Centro de Investigación y de Estudios Avanzados del IPN (CINVESTAV), Unidad Mérida. C.P. 97310 Mérida, Yucatán, México.

Abstract

Snappers are commercially important in the tropics and sub-tropics. Snapper command good market prices and in particular red-coloured snappers, such as the spotted rose snapper (*Lutjanus guttatus*) often obtain a premium market price. In Mexico, wholesale prices for whole snapper in 2010 ranged from 8 to 10US$ kg\(^{-1}\). The objective of this research was to study the effect of terrestrial, marine and vegetal protein sources in practical feeds for spotted rose snapper *Lutjanus guttatus*.

The experiment comprised eight isonitrogenous (45% crude protein) and isoenergetics dietary treatments: a basal diet with fish meal FM (herring) as the main protein source, and seven test diets where poultry by-product meal-petfood grade (PBM-GP), porcine meal (PM), meat meal (MM), tuna by product meal (TBM), canola meal (CM), soybean meal (SBM) and corn gluten meal (GM) were included to substitute 30% of the protein on a digestible protein basis and fed to the snapper (mean initial wt. ±SD, 100 ± 5 g) over a 10 week period. Eight fish were stocked into each of 24-120 L tanks and fed three time daily (8:30; 14:00; 17:00 h). At the conclusion of the feeding trial, the dry feed intake was significantly different across treatments where the diets with vegetal ingredient were less palatable than terrestrial or marine-protein diets. PBM-GP, BMM, and CM dietary treatment resulted in equivalent values for final weight, percent weight gain and PER to the fish meal control diet, whereas the PM, TBM and SBM diet showed significantly lower growth performance in comparison with control diet. Survival, FCR, and SGR values were either improved or were not significantly influenced by the replacement of sardine fish meal with either PBM-GM, CM or BMM. Hence, these products can be used to replace partially the sardine fish-meal content of practical diets for *L. guttatus*.

Keywords: rose snapper, Animal protein, Renderer meals, growth, alternative protein

*E-mail address: chernandez@ciad.mx (C. Hernandez).*
Effects of fish meal quality and fish meal substitution by animal protein blend on growth performance GH/IGF-I axis and flesh quality of Japanese seabass (*Lateolabrax japonicus*)

Liang Hu*, M. Xue, L. Wang, X. Wu H. Ge, Y. Zheng
Feed Research Institute, the Chinese Academy of Agricultural Sciences, Beijing 100081, P. R. China

Abstract

An 8-week growth trial was conducted to study the effects of fish meal quality and replacing dietary Peru fish meal (PFM) by animal protein blend (APB with 40% meat and bone meal, 35% poultry by-product meal, 20% spray-dried blood meal and 5% hydrolyzed feather meal) on growth performance and flesh quality of Japanese sea bass (initial body weight = 76.3g ± 0.2g) under digestible ideal amino acid (DIAA) profile. Six isonitrogenous and isoenergetic diets were formulated. A control diet contained 40% PFM was formulated according to the DIAA profile of Japanese seabass, and 20%, 40%, 60% or 80% of PFM were replaced by APB, in which lysine (Lys), methionine (Met), and threonine (Thr) were balanced as control diet with crystallized amino acid, respectively. Besides, a negative control diet used 50% local fish meal (LFM) with high volatile basic nitrogen (VBN) was formulated. The six diets were named as PFM, LFM, APB20, APB40, APB60 and APB80 respectively. Growth performance, body composition, plasma Insulin-like growth factor I (IGF-I) and growth hormone (GH) and flesh textural characters and sensory evaluation parameters were determined.

Growth performance was decreased with higher APB inclusion level, and APB60 and APB80 showed significant higher feed conversion rate (FCR) ($p<0.05$). Fish of APB80 showed lowest final body weight, but highest hepatosomatic index (HSI), which showed hepatic disease to some extent. Fish of LFM group showed significant lower feed intake than that of PFM, but did not show remarkable difference in growth profile. Besides, except fish fed PFM diet, skin fester were found in other 5 groups during the trial, but only 1 and 1 infected fish died in group APB60 and APB80 respectively until the end of the trial. Fresh flesh textural characters determined by Texture Analyzer did not show difference, but differences were found in cooked samples. Plasma IGF-I were reduced in fish of APB40 and plasma GH were highest in APB60 group. The present study showed that APB could replace 40% Peru fish meal at 160mg/kg inclusion level in Japanese seabass diet.

Keywords: Japanese sea bass; animal protein blend; fish meal quality; growth performance; flesh quality; GH/IGF-I axis

*E-mail address: xuemin@caas.net.cn (Min XUE)
Effect of taurine supplement to graded fish meal feed on yellowtail Seriola quinqueradiata in net cage

Shuichi Satoh1*, Daisuke Kondoh1, Yohsuke Futami1, Yutaka Haga1, Yoshihiro Yamamoto2 and Takafumi Oishi2
1 Tokyo University of Marine Science & Tech., Minato, Tokyo 108-8477, Japan
2 Oita Institute of Fisheries, Saiki, Oita 879-2602, Japan

Abstract
Recently, it was reported that taurine is an essential nutrient for marine fish, because marine fish cannot convert sulfur amino acid such methionine and cysteine to taurine due to low activity of cysteine sulfinic acid decarboxylase. If marine fish eat the feed which contains low or no fish meal, fish shows low growth performance and green liver syndrome after several weeks. And it was reported that taurine supplement could avoid such incidence. However, it has not been clear in which fish meal level taurine supplement is efficient. Therefore, a feeding experiment in net cage was conducted with yellowtail to evaluate necessity of supplementation with taurine to graded fish meal level feed. Fish meal based diet (60 %) was arranged as a control feed, and 40 %, 30 %, and 20 % fish meal diets were also formulated to obtain the same protein and calorie content by including soy proteins and corn gluten meal. Taurine was supplemented to the graded fish meal diets to meet the same level of control diet (0.15-0.29 %). Yellowtail (108g) were admitted in experimental net cages (3 x 3 x 3m, 200 fish) and fed the experimental diets for 16 weeks. The fish accepted all diets and ate well, even 20 % fish meal feed were eaten vigorously. After 16 week feeding, there was no significant difference in growth performance among the fish fed the diet containing more than 30 % fish meal regardless with taurine supplementation. However, the fish fed the 20 % fish meal diet without taurine supplementation showed marked reduction in growth, and taurine supplementation improved feed performance to the same level as the other groups. Green liver syndrome was not observed in any groups of this experiment. The results of the experiment demonstrated that it is not necessary to supplement taurine to 30 % fish meal feed if yellowtail eat well. And it is need to supply taurine to 20 % fish meal feed at the same level of fish meal based feed in order to obtain the same growth performance of yellowtail.

Key words: Yellowtail Seriola quinqueradiata, low fish meal feed, taurine supplement

*E-mail address: ssatoh@kaiyodai.ac.jp
Effects of L-carnitine on growth, vitality and RNA/DNA ratio in first feeding larvae of grass carp, *Ctenopharyngodon idella*

Chen Yu-ke¹, Xu Jing¹, Lin Li-li¹, Song Dan¹, Pei Huai-quan¹, Dong Jing¹², Wu Li-fang¹, Ge Chen-xia¹, Zhang Dong-ming¹*

¹Faculty of Animal Science and Technology, Jilin Agricultural University, Changchun 130118, China
²College of Animal Science and Veterinary Medicine, Shenyang Agricultural University, Shenyang 110866, China

Abstract

Effects of L-carnitine enrichment on growth, vitality and RNA/DNA ratio in first feeding larvae of freshwater grass carp (*Ctenopharyngodon idella*) were studied. The experimental larvae were reared in 50L tank with stocking density of 100 fish/L at 25±1°C for 21 days. The newly hatched *Artemia* nauplii, enriched with 4 levels (0, 1, 100, 1000 mg/L) of L-carnitine for 12 h at 25±1°C, were used as starter food for rearing larvae. Results indicated that growth of larvae were significantly (*P*<0.05) improved by feeding 1 mg/L L-carnitine enriched nauplii; Vitality of larvae were improved by feeding 100 mg/L L-carnitine enriched nauplii, but statistically did not show significant differences (*P*>0.05); There was significantly (*P*<0.05) higher RNA/DNA ratio for larvae in 100 and 1000 mg/L L-carnitine enriching groups than in the control, no significant differences (*P*>0.05) were observed on RNA/DNA ratio for larvae between 1 mg/L L-carnitine enriching group and the control. Results suggest that growth performances and survival of freshwater larvae might improved by L-carnitine supplementation.

Keywords: L-carnitine; First Feeding Larvae; Growth; Vitality; RNA/DNA Ratio

* *E-mail address: dmzhang@jlau.edu.cn*
Impacts of dietary hydroxyproline on growth, muscle firmness, collagen and PYD cross-links formation in Atlantic salmon (Salmo salar)

Sissel Albrektsen1*, Ellen Sirnes2, Anders Aksnes1 and Ørjan Hagen2
1 Nofima, Kjerreidviken 16, N-5141 Fyllingsdalen, Norway. E-mail: sissel.albrektsen@nofima.no
2 Bodø University College, Faculty of Biosciences and Aquaculture. Seafood quality group. 8049 Bodø, Norway

Abstract

Hydroxyproline (Hyp) is a non-essential amino acid essential to stabilize the triple helical structure of collagen protein in the connective tissue. As the textural properties of Atlantic salmon is affected by collagen concentration and hydroxylysyl pyridinoline (PYD) cross-links formation in the collagen protein, the aim of the present study was to evaluate dietary impacts of Hyp on alkaline-soluble (a-s) and alkaline-insoluble (a-i) collagen, PYD cross-links formation and muscle firmness in close to harvest size Atlantic salmon (2.4 kg). Three experimental high plant protein diets with a natural low basal Hyp content (1) were produced. Dietary impacts of Hyp administrated in free form (2) and as a bone gelatine product from an animal by-product (3) (Hydroxy Plus™, Sonac) were tested:

1) Control diet without added Hyp (0.33 g Hyp 100 g protein⁻¹)
2) Control diet with Hyp added in free form, free Hyp (1.10 g Hyp 100 g protein⁻¹)
3) Control diet with Hyp added in bone-bound form, bone Hyp, (1.11 g Hyp 100 g protein⁻¹)

Dietary Hyp provided as free Hyp and bone Hyp did not affect growth (SGR = 0.51 ± 0.05%) feed intake and feed conversion of Atlantic salmon in a 12 week feeding trial. During controlled storage of salmon in a cooling chamber (4°C), dietary free Hyp significantly increased pH (day 5), a-i Hyp (Day 5), a-s Hyp (day 5, 9 and 15) and PYD cross-links concentration (Day 9) in the salmon muscle. By instrumental texture analysis, dietary free Hyp improved muscle firmness by 5 – 10 % following 5, 9 and 15 days of storage (ns). Muscle pH, a-i Hyp (collagen containing most of the mature PYD cross-links) and PYD cross-links concentration correlated significantly to muscle firmness (shear force/shear work). Dietary Hyp provided as bone Hyp significantly increased plasma free Hyp and muscle a-s Hyp, indicating that dietary Hyp was highly available to salmon, and further tended to increase a-i Hyp (Day 5 of storage). However, no dietary impact of bone Hyp was found on muscle firmness or PYD cross-links formation in the muscle, indicating a dietary difference by providing Hyp as free Hyp and as bone Hyp. Muscle firmness measured by instrumental texture analyser (shear force and shear work) was significantly influenced by a-i Hyp, PYD cross-links concentration and pH at all samplings during storage for 5, 9 and 15 days, and a negative correlation to fish body weight was also found at all samplings.

Keywords: Hydroxyproline; Atlantic salmon; muscle firmness; collagen; PYD-crosslinks

* E-mail address: sissel.albrektsen@nofima.no (S. Albrektsen)
Nucleotide nutrition in aquatic animals: current knowledge and future applications

Peng Li1*, Sergio Nates2, and Delbert M. Gatlin III3
1 National Renderers Association Asian Regional Office, 21/F Causeway Bay Commerce Bld., 1-5 Sugar Street, Causeway Bay, Hong Kong SAR, China P. R.
2 Fat and Protein Research Foundation, 801 Northfax Street, Suite 205, Alexandria, VA 22314, USA
3 Department of Wildlife and Fisheries Sciences and Intercollegiate Faculty of Nutrition, Texas A&M University, College Station, TX 77843-2258, USA

Abstract
The roles of nucleotides and metabolites in fish diets have been sparingly studied for over 25 years. Beside possible involvement in diet palatability, fish feeding behavior and biosynthesis of non-essential amino acids, exogenous nucleotides have shown promise most recently as dietary supplements to enhance immunity and disease resistance of fish produced in aquaculture. Research on dietary nucleotides in fishes has shown they may improve growth in early stages of development, enhance larval quality via broodstock fortification, alter intestinal structure, increase stress tolerance as well as modulate innate and adaptive immune responses. Fishes fed nucleotide-supplemented diets generally have shown enhanced resistance to viral, bacterial and parasitic infection. However, currently there are numerous gaps in existing knowledge about exogenous nucleotide application to fish including various aspects of digestion, absorption, metabolism, and influences on various physiological responses especially expression of immunogenes and modulation of immunoglobulin production. Additional information is also needed in regard to age/size-related responses and appropriate doses and timing of administration. Recent findings with dietary nucleotides showed mixture of pure nucleotides can enhance growth and feed utilization of red drum and Pacific white shrimp. In addition, the concentrations of various types of nucleotides, nucleosides and oligonucleotides in feedstuffs including fishmeal and rendered animal protein meals including poultry by-product meal and meat and bone meal were measured. Animal protein meals are good source of balanced nucleotides.

Keywords: Nucleotide; Fishmeal; poultry by-product meal; meat and bone meal; animal protein meal

* E-mail address: li@nrahongkong.com.hk (P. Li)
Abstract

Modern finfish aquaculture has great promise in providing high value food, but as a relatively new animal production industry in many countries it faces problems with not only infectious but also non-infectious diseases. The latter include skeletal deformities, cataracts, cardiovascular disease, as well as unspecific ulceration of the intestinal mucosa and various other digestive disorders like intestinal colic in Atlantic cod and gastric dilatation (bloat) in rainbow trout. Furthermore, the occurrence of intestinal tumors in salmonid broodstock has been observed in Norway in recent years. The causes of these production-related disorders are most likely multifactorial in nature, and may include nutritional imbalances, changes in feed composition, intensive growth and/or unfavorable environmental conditions, to name a few. The disorders are often not lethal but raise animal welfare issues, cause production losses, and may increase the susceptibility to secondary disorders and infectious diseases.

In 2008, the Norwegian Food Safety Authority requested the Norwegian Scientific Committee for Food Safety (VKM) to assess if the use of plant ingredients in diets for cultured carnivorous fish fulfils the Norwegian Feed Regulation §7 that specifies that feed commodities should “not induce negative health effects to the animal”. Other aspects to be assessed were if the changes in fish diet ingredient composition seen in recent years were economically feasible, environmentally friendly, and sustainable. Also asked was whether high levels of plant ingredients, both protein and lipid sources, and additions of immunostimulants and other feed additives would in any manner challenge fish health, and if any ingredient should be limited due to its negative effect. Intrinsic in this request was the evaluation of effects of antinutritional factors, contaminants such as mycotoxins, herbicides and pesticides, as well as modern feed processing technology. In particular, plant ingredients which might induce long-term negative effects were to be identified. The request resulted in a report published in the spring of 2009 with the title “Criteria for safe use of plant ingredients in diets for aquacultured fish”. It includes an extensive review of the literature. A summary of the conclusions from report will be presented.

Keywords: Alternative feed ingredients; Plant ingredients; Feed additives; Undesirable substances; Feed production technology

* E-mail address: AnneMarie.Bakke@nvh.no
O-036

Regulation of insulin-like growth factor (IGF) and IGF binding protein gene expression by nutrition and aquaculture related stressors: Implications in nutrient utilization and stress assessment

Cunming Duan*1,2, Qiang Feng2, Yun Li2, Ling Lu2, Xianglei Wang2, and Hiroyasu Kamei1

1 Department of Molecular, Cellular, and Developmental Biology
University of Michigan, Ann Arbor, MI 48109, USA
2 School of Medicine and Pharmacy, Ocean University of China, Qingdao, China

Abstract
Vertebrate animal growth, determined by the rate and duration of the growth process, is genetically controlled, but also strongly influenced by nutritional and environmental factors. This is especially true for ectotherms like fish, which rely on changes in water temperature, photoperiod and food availability to trigger developmental processes such as hatching, metamorphosis (flatfishes, eels) or smoltification (salmonids), sexual maturation and spawning. Information from both environmental cues and the internal state is processed and integrated by the brain for appropriate response through hormonally mediated pathways. A central step in this regulatory network is the growth hormone (GH)-insulin-like growth factor (IGF)-I axis. GH, synthesized in the pituitary gland and secreted into the bloodstream, is of critical importance for postnatal growth. The growth-promoting action of GH is primarily mediated by IGF-I, a polypeptide structurally related to IGF-II and proinsulin. The importance of IGF signaling in fish development and growth has been illustrated by recent genetic studies in zebrafish. IGF-I is expressed in a wide variety of fish tissues with the highest level found in the liver. The hepatic IGF-I expression is under the regulation of GH and nutritional states. This dual regulation of IGF-I represents an interface between nutrients and hormones acting in concert to control animal growth and reproduction. IGFs in blood and other extracellular fluids are present in complexes with several specific IGF binding proteins (IGFBPs). Intriguingly, IGFBP-1, the first identified member of the IGFBP family, is highly induced under a variety of catabolic conditions such as food deprivation, malnutrition, stress, and hypoxia. Recent in vivo studies have indicated that elevated IGFBP-1 under these catabolic conditions serves as a molecular switch by restricting IGF signaling and diverts the limited energy resources away from growth toward those metabolic processes essential for survival. This talk will review recent findings on the molecular regulation of IGF and IGFBP-1 gene expression by nutritional factors and aquaculture related stressors and discuss the possibility of using these endocrine parameters as new tools in nutrient utilization and stress assessment.

Keywords: Hormone, hypoxia, gene expression, growth, fish

E-mail address: cduan@umich.edu (C. Duan)
Organogenesis and particularly skeletogenesis in fish larvae are affected by environmental and nutritional factors. Recent studies established that some nutrients, such as highly unsaturated fatty acids and vitamins influence skeletal development, especially when they are fed to fish from very early stages. Vitamins directly regulate the expression of a large amount of genes coding for cell multiplication, differentiation and osteogenesis. A transcriptomic approach conducted on 6626 distinct genes between 7 and 43 days post-hatching, using heterologous hybridization of a rainbow trout cDNA microarray, revealed 485 genes differentially expressed. Genes corresponding to ossification, such as collagen alpha chain precursor (COLIA), connective tissue growth factor precursor (CTGF), and periostin (POSTN) were up-regulated from 31 day post hatching onward, corresponding to the period of ossification/mineralisation of the vertebral column. Besides, experimental rearing were conducted using diets including controlled levels of different vitamins. A deficiency in dietary vitamin D resulted in skeletal deformities such as kyphosis, scoliosis (vertebral column), pugheadness, or light curvatures of the branchiostegal rays (skull), as well as deformities of the caudal-fin. Transcriptomic study conducted on 6990 annotated genes (REX Marine Genomic Europe) on sea bass cDNA microarray showed that vitamin D deficiency was associated with activation of proliferation process to the detriment of differentiation pathways, that may in great part explain the observed abnormalities. Moreover, quantification of gene activity by qPCR allowed to associate abnormalities in sea bass skeletal development to regulation of some genes by vitamin D, C and A.

Keywords: Vitamin; Fish larval development; Gene regulation.

*E-mail address: Chantal. cahu@ifremer.fr
Preliminary results on the modulation of ACTH-induced expression of stress related genes by polyunsaturated fatty acids in head kidney from European sea bass, *Dicentrarchus labrax*.

Montero, D.*, Negrín-Báez, D., Ganga, R., Navarro, A., Izquierdo, M.S., Afonso, J.M.
Grupo de Investigación en Acuicultura. Universidad de las Palmas de Gran Canaria & Instituto Canario de Ciencias Marinas. Transmontañá s/n, 35416 Arucas, Las Palmas, Canary Islands, Spain

Abstract

Polyunsaturated fatty acids are essential components of cell membranes and have been shown to modulate physiological processes, including transport events throughout cell membrane, membrane associated receptor and enzymatic activities. Besides, dietary fatty acids have been described to alter physiological response to stressful situations. Essential fatty acid deficiencies or utilization of plant oils in fish diets have been previously shown to increase plasma cortisol after stress in different species such as gilthead sea bream or Atlantic salmon.

Previous studies described a direct effect of different fatty acids on *in vitro* adrenocorticotropic hormone (ACTH)-induced cortisol release by interrenal cells of gilthead sea bream demonstrating that both arachidonic acid (ARA) and eicosapentaenoic acid (EPA) promoted cortisol release from interrenal cells. However, as far as we know, there is no evidence on the role of different fatty acids on the *in vitro* modulation of stress-related gene expression in marine fish. To clarify this, European sea bass head kidney, tissue in which interrenal cells are embedded, was maintained in superfusion system for 3 hours to reach the basal levels and perfusion medium was supplemented with 50 μM of different fatty acids, including ARA, EPA, docosahexaenoic (DHA), linolenic (LA) or linoleic (ALA) acids. After one hour of incubation with fatty acids, tissue was stimulated with ACTH at a concentration of 5nM hACTH1-39 during 20 min. Afterwards, perfusion was maintained being tissue and perfused fluid collected at 20, 40, 60, 110, 160 and 250 min for stress-related genes expression and cortisol concentration analysis respectively. The expression of CYP11B, (cytochrome P450 implied in cortisolsyntesis), glucocorticoid receptor, HSP70 and HSP90 genes were measured by qPCR.

Results showed the first evidence of the effect of certain fatty acids on expression of stress-related genes by ACTH-stimulated head kidney cells in fish. N-6 fatty acids and particularly ARA significantly increased (P<0.05) the expression of the stress-related gene studied, whereas ALA seemed to have more effect on expression of genes related with cortisol synthesis, and have less effect on expression of glucocorticoid receptor gene. EPA and DHA seemed to have less effect on those genes. Time-course study and relationship between gene expression and cortisol concentration of perfused fluid are also discussed, describing the modulation of stress related genes by fatty acids and helping to understand the stress response in European sea bass.

Keywords: Stress; Cortisol; Fatty acid; Gene expression; Arachidonic acid

*E-mail address: dmontero@iccm.rcanaria.es (D. Montero)*
Zebrafish Elovl4 elongases: role in biosynthesis of very long-chain fatty acids and expression during embryogenesis

Óscar Monroig1*, Josep Rotllant2, José M. Cerdá-Reverter3, James R. Dick1, Antonio Figueras2, Douglas R. Tocher1

1 Institute of Aquaculture, University of Stirling, Stirling, Scotland, UK
2 Instituto de Investigaciones Marinas, CSIC, Vigo, Pontevedra, Spain
3 Instituto de Acuicultura Torre de la Sal, CSIC, Cabanes, Castellón, Spain

Abstract

Elongases of very long-chain fatty acids (Elovl) are the initial and rate-limiting enzymes responsible for the condensation reaction required for the 2-carbon elongation of fatty acids with aliphatic chains of 16 or more carbon atoms. One of the members of the Elovl protein family, ELOVL4, has been demonstrated recently to participate in the biosynthesis of very long-chain polyunsaturated fatty acids (VLC-PUFA), compounds with unusually long hydrocarbon chains (C ≥ 24) that are relatively abundant in phospholipid molecules of retina photoreceptor cells, testicles and brain in mammals. The study of Elovl4 proteins therefore represents an interesting research area in fish farming, in which altered visual acuity (critical in visual predators such as most cultured fish species), disruptions of brain functioning, and fertility issues of broodstock can affect fish performance and eventually the economical profit of the farm. Many of these physiological aspects can be especially relevant in developing stages, where high amounts of LC-PUFA are required to fulfill demand of forming tissues. The present study has characterised two Elovl4 enzymes, Elovl4a and Elovl4b, from zebrafish Danio rerio, and investigated their expression patterns during embryonic development. Heterologous expression in baker’s yeast showed that both zebrafish Elovl4 proteins efficiently elongate saturated fatty acids up to C36, with 26:0 appearing the preferred substrate as reported for human ELOVL4. Interestingly, activity for the elongation of PUFA substrates was only shown by Elovl4b, which effectively converted eicosapentaenoic (20:5n-3) and arachidonic (20:4n-6) acids to elongated polyenoic products up to C36. Furthermore, zebrafish Elovl4b may be involved in the biosynthesis of docosahexaenoic acid (22:6n-3, DHA) as it had the capacity to elongate 22:5n-3 to 24:5n-3 which can be subsequently desaturated and chain shortened to DHA in the peroxisomes. The distinct functional roles of zebrafish Elovl4 proteins were also reflected in their spatial-temporal expression patterns during ontogeny. Analyses by whole-mount in situ hybridisation in zebrafish embryos showed that elovl4a is expressed in neuronal tissues (wide-spread distributed in the head area), with elovl4b specifically expressed in epiphysis (pineal gland) and photoreceptors in the retina. Similarly, tissue distribution in adults reveals that elovl4a transcripts are found in most tissues analysed, whereas elovl4b expression is essentially restricted to eye and gonads. Overall, the results suggest that zebrafish elovl4b resembles other mammalian orthologs in terms of function and expression patterns, whereas elovl4a may represent an alternative elongase not yet described in vertebrates and whose physiological roles have to be further investigated.

Keywords: Development; elongation; Elovl4; fatty acid metabolism; very long-chain polyunsaturated fatty acids; zebrafish

* E-mail address: oscar.monroig@stir.ac.uk (Ó. Monroig).
Gene expression profiling reveals similar hepatic responses to restricted feeding and extracted soybean meal in diets for Atlantic salmon (Salmo salar L.)

Stanko Skugor\textsuperscript{1,2}, Barbara Grisdale-Helland\textsuperscript{1,3}, Ståle Refstie\textsuperscript{1,3}, Sergey Afanasyev\textsuperscript{1,4}, Jouni Vielma\textsuperscript{5}, Aleksei Krasnov\textsuperscript{1}

\textsuperscript{1}Nofima Marin, P.O.B. 5010, 1432 Ås, Norway
\textsuperscript{2}Department of Animal and Aquaculture Sciences, Norwegian University of Life Sciences (UMB), P.O. Box 5003, Ås 1432, Norway
\textsuperscript{3}Aquaculture Protein Centre, CoE, Norway
\textsuperscript{4}Sechenov Institute of Evolutionary Physiology and Biochemistry, St Petersburg 194223, Russia
\textsuperscript{5}Finnish Game and Fisheries Institute, P.O.B2 00791 Helsinki, Finland

Abstract

We assessed gene expression analyses for studies and diagnostics of nutritionally-induced growth reduction in Atlantic salmon. The four experimental treatments consisted of feeding a fishmeal-based control diet or one including 20\% extracted soybean meal, at full or reduced rations for 54 days. Compared with the fish fed the control diet at full ration, the thermal growth coefficient decreased in the group fed with the control diet at reduced ration by 51\%, the soy diet at full ration by 22.2\% and the soy diet at reduced ration by 67\%. The hepatic gene expression changes were analyzed with cDNA microarray and qPCR. Transcriptomic responses to soy and reduced feeding were highly similar, consistently with the decreased intake of the soy diet. Unexpectedly, joint treatment produced smaller expression changes than single treatments. A search across our gene expression database showed 12 genes with greater regulation in this experiment than in other studies that used the same microarray platform. These included four up-regulated ribosomal proteins and four down-regulated genes for lipid and steroid metabolism. Growth reduction was associated with coordinated down-regulation of genes involved in oxidative and cellular stress responses, xenobiotic metabolism and protein degradation. To conclude, this pilot study revealed significant hepatic gene expression changes induced with nutrition. The responses weakened at the most severe underfeeding however, and the detection of specific markers was problematic. High expression of stress-related genes in the control group suggests that maximum growth rates can be associated with health problems in salmon.

Keywords: Atlantic salmon; growth; soy, reduced ratio; liver; microarray

\textsuperscript{*}E-mail address: stanko.skugor@nofima.no (S. Skugor).
Arginine intake and carbohydrate to lipid ratios affect gene expression of anabolic hormones in largemouth bass, *Micropterus salmoides*

Lina Jin¹, Naisong Chen², Hengyong Zhou, Xiaojie Qiu
Shanghai Ocean University, 999 Hucheng Huan Road, Shanghai 201306, PR China

Abstract

Nutritional factors influence regulation of growth hormone (GH), insulin-like growth factor-I (IGF-I) and insulin (INS) in fish. But so far there are no published studies describing how single indispensable amino acids and different carbohydrate to lipid ratios influence those systems. Therefore, the present study aimed to evaluate whether arginine (Arg) intake and carbohydrate to lipid ratios would affect expression of GH, IGF-I and INS in largemouth bass.

Six isonitrogenous and isolipidic (45.90% crude protein and 12.24% crude lipid of dry diet) diets with varying arginine levels (1.76-2.92% of dry diet) and six isonitrogenous (46% crude protein) and isoenergetic (19 MJ/kg gross energy) diets with varying carbohydrate to lipid (CHO/LIP, w/w) ratios (0.51-5.18) were fed to test fish for 8 weeks. Each diet was randomly assigned to triplicate groups of 35 juvenile fish. Fish were fed by hand to apparent satiation twice daily (08:00 and 16:00). During the experimental period, water temperature was maintained at 28±1°C and the natural light cycle was adapted.

With the elevated levels of dietary Arg, pituitary GH mRNA abundance was up-regulated gradually. A significant up-regulation was 3.88-fold higher in fish fed 2.92% Arg diet compared to fish fed 1.76% Arg diet. Hepatic IGF-I mRNA expression was 4.34-fold and 4.98-fold significantly up-regulated in the fish fed 2.23% and 2.92% Arg diets, respectively. No effect of Arg intake on hepatic INS mRNA expression, however was be found regardless of dietary treatments.

Pituitary GH mRNA level was 2.19-fold and 4.68-fold higher in the fish fed diets with 1.68 and 2.41 CHO/LIP, respectively, than in those fed diet of 0.51 CHO/LIP. But there was significant down-regulation of hepatic IGF-I mRNA expression in the fish fed diet with 2.41 CHO/LIP. Hepatic INS mRNA expression positively correlated with the dietary carbohydrate content; the lowest with 0.51 CHO/LIP was 2.21-fold lower than the highest with 5.18 CHO/LIP.

Dietary Arg levels and carbohydrate to lipid ratios indeed affected the hepatic IGF-I and INS mRNA expression as well as pituitary GH mRNA expression in largemouth bass. This research indicated that the nutritional factors would affect growth performance of largemouth bass through regulating related mRNA expression in the different organs.

Keywords: Largemouth bass; Growth hormone; Insulin-like growth factor-I; Insulin; Arginine intake; Carbohydrate to lipid ratio

* E-mail address: siben2008@hotmail.com (Lina Jin)
Molecular cloning, characterization and mRNA expression of selenium-dependent glutathione peroxidase from abalone *Haliotis discus hannai* Ino in response to dietary selenium, zinc and iron

Chenglong Wu, Kangsen Mai, Wenbing Zhang*, Qinghui Ai, Wei Xu, Xiaojie Wang, Hongming Ma, Zhiguo Liufu
The Key Laboratory of Mariculture (Education Ministry of China), Ocean University of China, 5 Yushan Road, Qingdao 266003, P.R. China

Abstract

A novel selenium-dependent glutathione peroxidase (Se-GPX) was cloned from abalone *Haliotis discus hannai* Ino (HdhGPx) by homology cloning with degenerate primers and RACE techniques. The full length of HdhGPx cDNA was 963 bp with a 669 bp open reading frame (ORF) encoding 222 amino acids and a 101 bp eukaryotic selenocysteine insertion sequence (SECIS) in 3' untranslated region (UTR). It was showed that HdhGPx has a characteristic codon at 235TGA237 that corresponds to selenocysteine (SeC) as U72. Sequence characterization revealed that HdhGPx contains a characteristic GPx signature motif 2 (96LGLPCNQF103), an active site motif (179WNFEKF184). In addition, two potential N-glycosylation sites (112NGTE115 and 132NLTQ135) were identified in HdhGPx. 3D modeling analysis showed that the overall structure of HdhGPx monomer had more similarity to human GPx3 than human GPx1. Relatively higher-level mRNA expression was detected in hepatopancreas, mantle and gonad by real-time PCR assays. The relative expression levels of HdhGPx mRNA in hepatopancreas and haemocytes were detected by real-time PCR in abalone fed with nine different diets containing graded levels of selenium (0.15, 1.32 and 48.7 mg Kg\(^{-1}\)), zinc (6.69, 33.85 and 710.63 mg Kg\(^{-1}\)) and iron (29.17, 65.7 and 1267.2 mg Kg\(^{-1}\)) for 20 weeks, respectively. The results showed that the expression of HdhGPx mRNA statistically higher at adequate dietary selenium (1.32 mg Kg\(^{-1}\)), zinc (33.85 mg Kg\(^{-1}\)) and iron (65.7 mg Kg\(^{-1}\)) than these in low dietary minerals, respectively. But HdhGPx mRNA expression levels were down-regulated by high content of dietary selenium (48.7 mg Kg\(^{-1}\)), zinc (710.63 mg Kg\(^{-1}\)) and iron (1267.2 mg Kg\(^{-1}\)) compared with these adequate dietary minerals, respectively. These results indicated that adequate dietary minerals could increase the mRNA expression of HdhGPx to increase the total antioxidant capacities in abalone.

**Keywords:** selenium-dependent glutathione peroxidase; *Haliotis discus hannai* Ino; cDNA cloning; mRNA expression; minerals

*E-mail address: wzhang@ouc.edu.cn* (W Zhang).
Effect of high carbohydrate dietary on the growth, blood immune parameter and gene expression of heat shock protein 70 of Wuchang bream (*Megalobrama amblycephala*)

Zhou Chuanpeng12*, LIU Bo12, XIE Jun12, GE Xianping12, XU Pao12, LIU Wenbin2

1 Freshwater Fishery Research Center, Chinese Academy of Fishery Sciences, Wuxi 214081, China
2 Wuxi Fishery College, Nanjing Agriculture University, Wuxi 214081 China

Abstract:

The aim of this study was to investigate the utilization of high level carbohydrate dietary in Wuchang bream (*Megalobrama amblycephala*). One hundred and forty Wuchang bream with an average weight of 35g were randomly divided into two groups. One group in triplicate was fed a high carbohydrate diet (high CHO, 53% carbohydrate) and the other was fed a normal carbohydrate diet (Normal CHO, 30% carbohydrate). All fish were fed three times a day (2%-3% body weight) acclimating for 3 weeks in Aquarium with automatic temperature control breeding system with circle water. The growth and the variations of blood cortisol, glucose, alkaline phosphatase, total protein, cholesterol, triglyceride, hepatic superoxide dismutase (SOD), catalase (CAT) and maleicdialdehyde (MDA) content and the relative level of hepatic HSP70 mRNA were investigated. The results showed that the growth gain rate, feed conversing ratio in High CHO group were higher than those of Normal CHO group. However there were no significant differences in the protein utilization efficiency, feeding rate, and the serum glucose, total protein, cholesterol, insulin level between High CHO group and Normal CHO group. The serum cortisol, triglyceride, hepatic MDA content and the relative level of hepatic HSP70 mRNA in the High CHO group were higher than those of Normal CHO group. However the serum alkaline phosphatase, lysozyme and hepatic SOD concentrations in the High CHO group were lower than those of Normal CHO group. These data suggest that high dietary carbohydrate may impact on hepatic hsp70 expression, be negative fish immune ability and growth.

Key words: *Megalobrama amblycephala*; Carbohydrate; Heat shock protein 70(HSP70); Plasma immune parameter; Growth

*E-mail address: 2009213004@njau.edu.cn*
Effect of retinoic acid on the in vitro proliferation, mineralization and related-gene expression of gilthead sea bream Sparus aurata bone-derived cell lines

Ignacio Fernández 1*, Daniel M. Tiago2, Vincent Laizé2, M. Leonor Cancela2 and Enric Gisbert1

1IRTA, Centre de Sanit Carles de la Ràpita, Unidad de Cultius Experimentals. Crta. Del Poble Nou s/n, 43540 Sant Carles de la Rapita (Tarragona, Spain)
2Centro de Ciências do Mar (CCMAR), Universidade do Algarve, Campus de Gambelas, 8005-139 Faro (Portugal)

Abstract
Several studies have recently demonstrated the role of retinoic acid (RA), the active metabolite of vitamin A (VA), in mammalian bone formation and metabolism. In fish, in vivo dose-response nutritional studies have shown that dietary VA affects skeletogenesis. However, mechanisms of RA/VA action remain unclear and the use of fish-derived in vitro cell systems may provide further insights towards specific cellular and molecular events involved. This work aimed to investigate the effects of RA on in vitro proliferation, mineralization and related-gene expression of two gilthead sea bream Sparus aurata bone-derived cell lines, VSa13 (chondrocyte-like) and VSa16 (osteoblast-like). Cells were grown to confluence, treated for 15 days with RA concentrations up to 312.5 μM, and cell viability evaluated using the MTS assay. RA concentrations higher than 12.5 μM were toxic in both cell lines. Proliferative and mineralogenic RA effects were then evaluated using two non-toxic RA concentrations (0.5 and 12.5 μM). Cell proliferation was assessed during 12 days using the MTS assay and ECM mineralization was followed up to 3.5 weeks through von Kossa staining. Comparative analysis of treated versus control cells showed that both RA concentrations (i) induced phenotypic changes and (ii) inhibited proliferation in both cell lines, although to different extent (36-59% in Vsa13 and 17-46% in Vsa16 treated cells respect to control). Interestingly, the comparative analysis of mineralogenic capacity of treated versus control cells showed distinct effects: while 0.5 and 12.5 μM of RA increased mineral deposition by 50 and 62% in VSa13 cell lines, respectively, it inhibited mineralization by 11 and 57% in Vsa16 cell lines, respectively. Gene expression analysis of nuclear receptors RARs and RXRs, MGP, COL1a1, OPN and PCNA were evaluated through real time qPCR. Interestingly, results suggested that chondrocyte- and osteoblast-like proliferation is disrupted by RARα, being also affected the genes encoding for the extracellular matrix proteins (ECM). In addition, RA induced a disruption of gene expression during the mineral deposition phase in more than one nuclear receptor, suggesting that RA affected retinoid nuclear receptors differentially depending on the cell stage and/or duration of RA treatment. Expression of genes encoding ECM proteins along mineralization phase within each RA treatment support the results regarding the mineral deposition found by densitometry analysis of von Kossa staining. Together, those results shows as RA affected proliferation and differentiation chondrocyte- and osteoblast-like cells in a different extent, providing insights into the mechanisms involved in fish bone formation.

* E-mail address: ignacio.fernandez@irta.es
The whole-body fatty acid balance method: advantages, limitations and opportunities

Giovanni M. Turchini1*, David S. Francis1

1 School of Life and Environmental Sciences, Deakin University, PO Box 423 Warrnambool, Victoria, 3280, Australia

Abstract

The whole-body fatty acid balance method (WBFABM) is a simple and reliable method used for the assessment of overall in vivo fatty acid metabolism, including fatty acid ex novo production, oxidation and bio-conversion (elongation and desaturation). Using the method it is possible to track the individual fate of each dietary fatty acid within the body. In theory, the method can potentially be applied to any organism, requiring little more than a gas chromatography unit for fatty acid analysis, appropriate experimental protocols and elementary calculations. Since its conception in 2006, the WBFABM has undergone further development, through critical and constructive peer-revision. Subsequently, the WBFABM has been used in several farmed finfish species feeding trials and broiler chicken feeding trials. Currently the WBFABM is being implemented by various research institutions worldwide and is gaining popularity from animal nutrition scientists. Within the context of fish oil replacement and alternative lipid sources for aquafeed, the WBFABM has been proved to be a simple and effective tool, potentially applicable in any laboratory, which can significantly broaden and deepen the information obtainable from feeding trials. In this presentation, the overall conceptual framework of the WBFABM will be presented, and its advantages and limitations will be discussed. Case studies reporting data obtained implementing the method across several trials will be reviewed and discussed. Future opportunities within the broad context of fish oil replacement and fatty acid metabolism in cultured aquatic species will be highlighted.

Keywords: Fatty acid; Lipid metabolism; Methodology; Oxidation; Elongation; Desaturation

*E-mail address: giovanni.turchini@deakin.edu.au (G. M. Turchini)
The effect of nutrients and light conditions on diatom propagation, and refinement of larval and post larval culture techniques for the abalone *Haliotis asinina* Linne

Jocelyn A. Madrones-Ladja* and Milagros R. de la Peña
Aquaculture Department, Southeast Asian Fisheries Development Center, 5021 Tigbauan, Iloilo, Philippines

Abstract

Mixed diatoms predominantly *Navicula sp.* (63%) was propagated in corrugated plastic plates using different nutrients and light conditions. Result showed that mixed diatoms grew better and faster with agricultural fertilizers (21-0-0, 16-20-0, and 46-0-0) than with technical grade chemical reagents (Sodium Nitrate, Disodium Hydrogen Phosphate, and Ferric Chloride). Similarly, diatoms treated with agricultural fertilizers and cultured under direct daylight had the highest mean density of 15.83 x 10^4 cells cm^-2 and 15.47 x 10^4 cell cm^-2, after 14 and 35 days of culture, respectively, which were significantly different among treatments (P<0.01). Diatom growth in plates treated with technical grade chemical reagents was slow and low in density. During the 50-day propagation period, *Navicula sp.* maintained a high percentage composition of the mixed diatoms in all nutrient-treated plates.

The abalone *Haliotis asinina* Linne larvae settled best in abalone mucus-treated diatom plates when aeration was provided in cultures only starting day 2, day 3, or day 4, and in un-aerated cultures (P<0.01). The mean settlement rates were 59.5 %, 65 %, 61.5 %, 49 %, respectively. Providing aeration to the cultures immediately or 24 h after larval stocking resulted to significantly low settlement rate among treatments (P<0.01). Likewise, plates with initially high-diatom density (18.64 x 10^4 cells cm^-2) resulted to significantly high mean larval settlement rates among treatments (P<0.01) of 9.5 % and 8.2 %, after 7 and 14 days, respectively. In medium-density (8.494 x 10^4 cells cm^-2) and low-density (3.9 x 10^4 cells cm^-2) diatom plates, larval settlement after day 14 was not significantly different from each other (P>0.01). The mean settlement rate of 3.6% was observed in plates without diatom, which was significantly low among treatments (P<0.01). The formation of the first respiratory pore was observed at day 30. The highest mean survival (9.8 %) of abalone post larvae was recorded in high-diatom density plates. This was significantly different from survival rates observed in low-density (4.5%) and without (3.2%) diatom plates cultures, the latter was lowest among treatments (P<0.01).

These results show that agricultural fertilizers can best grow the suitable diatom species for abalone attachment at any light conditions. Larval settlement is best in mucus treated plates with diatom density of > 3.9 x 10 cells ml^-1 and when aeration is provided to cultures only from day 2 after stocking or using no aeration at all. Similarly, post larval survival at metamorphosis observed at day 30 was best in plates with high-diatom density.

**Keywords:** Nutrients; Light conditions, Diatoms propagation; Larval settlement; *Haliotis asinina* Linne

*E-mail address: joladja@seafdec.org.ph*
Early diagnosis of vertebral deformity induced by excessive vitamin A in live marine fish larvae by fluorescent calcein staining

Yutaka Haga1, Saki Masui1*, Yuichiro Fujinami2, Masato Aritaki3, Shuichi Satoh1

1 Department of Marine Bioscience, Tokyo University of Marine Science and Technology, Konan, Minato 4-5-7, Tokyo 108-8477, Japan
2 Miyako Station, National Center for Stock Enhancement, Fisheries Research Agency, Miyako, Iwate 027-0097, Japan
3 Seikai National Fisheries Research Institute, Fisheries Research Agency, 1551-8, Taira-machi, Nagasaki-shi Nagasaki 851-2213, Japan

Abstract

Early diagnosis of bone deformity in cultured fish is urgent subject for better understand mechanism of bone deformity. Our previous study reported application of calcein staining to visualize hard tissue of red sea bream (Haga et al. J. Appl. Ichthyol. in press) and found that calcein successfully visualized ontogenic development of skeletons and urinary calculi in live sea bream larvae. One of the most frequently-observed skeletal deformities in cultured fish is vertebral fusion. It is well-documented that excess vitamin A (VA) induced vertebral fusion in early stage of marine fish, which makes it popular model to study mechanism of vertebral fusion in fish. However, early symptom of vertebral fusion induced by excessive VA in marine fish is unclear. Therefore, the present study investigated whether fluorescent dye calcein could visualize normal and abnormal vertebral development in juvenile Japanese flounder Paralichthys olivaceus. Japanese flounder were fed Artemia nauplii enriched with or without 1 mg/L vitamin A (VA) palmitate for more than 18 hs. and reared until 50 days-post hatching (dph). Retarded growth and significantly higher incidence of vertebral deformity was observed in flounder fed VA (P<0.05). Growth retardation in fish fed excessive VA was evident from 40 dph. In order to trace skeletal development, live flounder larvae at 3-43 dph were stained with calcein and observed under a fluorescent microscope. Calcein staining of live flounder larvae successfully visualized ontogenic ossification of skeleton. However, cartilaginous skeletons such as hypural, distal pterigiophores, and Meckel’s cartilage were not stained by calcein. In addition, calcein also visualized early pathological changes of vertebral column by excessive VA. Calcein staining revealed that abnormal vertebral development was observed in fish as early as 35 dph at 14 mm SL. Much brighter staining was observed in each end of affected vertebral column. Darker calcein staining was observed in completely fused vertebra. Classification of vertebral column ossification suggested that extension growth of vertebral columns along vertebral axis was inhibited in VA treated fish. The present study suggested that fluorescent calcein was useful to diagnose early symptom of vertebral deformity. It was also suggested that fluorescent calcein staining of skeleton of fish larvae can be utilized for selective screening of fish larvae having normal skeletons.

Keywords: Calcein; Deformity; Larvae; Skeleton; Vitamin A

* E-mail address: haga@kaiyodai.ac.jp (Y. Haga).
Molecular characterization of digestive ontogeny and endocrine functions in Atlantic cod (Gadus morhua L.) larvae as influenced by diet

Trond M Kortner1,2*, Ingrid Overrein3,4, Gunvor Øie3, Per-Arvid Wold5, Tora Bardal1, Elin Kjørsvik1 and Augustine Arukwe1

1Department of Biology, Norwegian University of Science and Technology (NTNU), Trondheim, Norway
2Present address: Aquaculture Protein Centre (a CoE), Department of Aquatic Medicine and Nutrition, Norwegian School of Veterinary Medicine, P.O. Box 8146 Dep, NO-0033 Oslo, Norway
3Department of Marine Resources Technology, SINTEF Fisheries and Aquaculture, Trondheim, Norway
4Department of Biotechnology, Norwegian University of Science and Technology (NTNU), Trondheim, Norway
5Department of Hydraulic and Environmental Engineering, Norwegian University of Science and Technology (NTNU), Trondheim, Norway

Abstract

Development of optimal nutrition feeding protocols is a major challenge in intensive marine fish larval rearing, and marine teleost fish undergo significant alterations in morphology, physiology and metabolism during their larval periods. Regulation of gene expression and subsequent molecular processes are believed to be key mechanisms leading to these developmental and physiological changes. However, information on the molecular basis of marine fish larval ontogeny is scarce. To provide an overview of the ontogeny of digestive capability and endocrine functions during Atlantic cod larval development, we continuously monitored the expression of 12 different gene transcripts at 14 different time points (day 3, 5, 8, 13, 17, 20, 26, 30, 33, 36, 39, 42, 46 and 60 post-hatch) using quantitative real-time PCR (qPCR). Larval digestive enzyme activity levels were also measured spectrophotometrically. Cod larvae were fed either Acartia tonsa nauplii, rotifers grown on Rhodomonas baltica or a control rotifer diet, before all larval groups were fed enriched Artemia followed by weaning to a formulated diet.

For the first time, we report gene expression patterns of the most important digestive enzymes and several appetite and growth controlling factors during Atlantic cod larval development. Gene expression patterns and activity levels of digestive enzymes showed differential profiles during larval development, and were also affected by the different feeding regimes. Trypsin and bile salt-activated lipase mRNA levels peaked at approximately day 17 and 25 post-hatch, respectively, and thereafter showing a consistent decreasing pattern until metamorphosis. Amylase mRNA showed a consistent expression from day 3 until day 17 post-hatch, and thereafter showed a major down-regulation. Acyl CoA dehydrogenase and phospholipase A2 mRNA levels increased during larval development. Interestingly, mRNA expression patterns of PLA2 indicate that PLA2 presumably gained an increasingly important role during larval development. Moreover, a positive correlation between transcript profiles for genes coding for digestive enzymes and several appetite and growth controlling factors (neuropeptide Y, orexin, somatostatin, growth hormone, thyroid hormone receptor) were demonstrated. The differential transcript expression patterns observed suggest that gene expression is developmentally regulated and that digestion, somatic growth and development probably are closely coupled. In summary, this study provides candidate genes suited for characterizing ontogeny of digestive capability and fitness in early larval stages, and the results indicate that the ontogeny of digestive capability and its hormonal components may be tied directly to the type and quality of initial and early dietary constituents.

Keywords: Atlantic cod larvae, nutrition, digestion, gene expression, growth, ontogeny

* E-mail address: trond.kortner@nvh.no
Inclusion of crude palm oil in the broodstock diets of Nile tilapia, Oreochromis niloticus, affected tissue fatty acid and vitamin E composition resulting in enhanced reproductive performance

Wing-Keong Ng*, Yan Wang, Yunyun Qian
Fish Nutrition Laboratory, School of Biological Sciences, Universiti Sains Malaysia, Penang 11800, Malaysia

Abstract

Tilapias are currently the second most widely farmed fish in the world with annual production expected to exceed three million metric tons. The intensive farming of tilapia is rapidly expanding and the need to produce sufficient quantities of quality fry is becoming crucial to meet increasing global demands for stocking tilapia farms. The objective of the present study was to evaluate the effects of dietary lipid sources on the reproductive performance of tilapia broodfish. Four isonitrogenous (35% protein) and isolipidic (10%) soybean meal-casein-based diets were formulated with added fish oil (FO), FO and crude palm oil (FO+CPO; 1:1), CPO or linseed oil (LSO) as the lipid source, respectively. Pre-spawning female Nile tilapia (Oreochromis niloticus, GIFT strain) was individually color-tagged, and six females and two males were stocked into a one-ton fiberglass breeding tank. Each diet was fed to two tanks and the reproductive performance of 12 individual female fish for each experimental diet was monitored over 25 weeks for six consecutive spawning.

Female broodfish fed the two CPO-based diets showed significantly (P<0.05) larger gonad sizes and lower intraperitoneal fat compared to fish fed the FO or LSO diets. First spawning occurred earliest in broodfish fed the CPO diet at 30.8 ± 9.9 days compared to 44.1, 45.5 and 76.3 days for fish fed the FO+CPO, FO or LSO diet, respectively. Over the 25 weeks, the highest number of spawning tilapia was observed in fish fed the FO+CPO diet, followed by fish fed the CPO, FO or LSO diet, respectively. Tilapia fed the CPO-based diets produced the highest total number of eggs per fish. The shorter spawning interval of tilapia fed the two CPO-based diets contributed to the higher total number of eggs produced. Mean diameter, volume and weight of unfertilized eggs did not vary among dietary treatments. The fatty acid composition of the gonad, egg and newly hatched fry were influenced by dietary lipid source but evidence of preferential fatty acid conservation and utilization was observed. The gonads, eggs and fry of tilapia fed the CPO diet contained the highest relative concentration of arachidonic acid (ARA) and DHA/EPA ratio. The high total n-3 PUFA observed in the gonads of fish fed the LSO diet, and to a lesser degree the FO diet, seemed to be detrimental to the reproductive performance of tilapia. Markedly higher concentrations of total tocopherols and tocotrienols were found in the egg and fry of tilapia fed the CPO diet. The gonad of the CPO-fed fish contained the highest concentration of alpha-tocopherol. In conclusion, the inclusion of CPO in tilapia broodstock diets can be a cost-effective method to increase tilapia fry production.

Keywords: Alternative Lipids; Palm oil; Fatty acids; Vitamin E; Reproduction; Tilapia

* E-mail address: wkng@usm.my
Development of zebrafish model system to evaluate feed performance of microdiet for marine fish larvae

Li Ji1*, Yutaka Haga1, Toshihiro Nakamura2, Shuzou Ishida2, Toshio Takeuchi1
1 Tokyo University of Marine Science and Technology, 4-5-7 kounann, minato area, Tokyo 108-8477, Japan
2 Taiyo Yushi K.K. Corporation, 2-7 moriya, kanagawa area 221-0022, yokohama, Japan

Abstract

Development of microdiet to substitute live food is essential for mass production of juveniles. However, the use of microdiet for marine fish larvae has been limited by large leaching of nutrients when applied to water environment. In general, feeding experiment of marine fish larvae is limited because of relatively shorter spawning seasons and requirement of larger facility for bloodstock management. Therefore, suitability of zebrafish Danio rerio larvae as a model system for evaluation of microdiet was tested.

Two kinds of peptides were used: fish collagen peptides (FCP) and milk casein peptide (MCP). Two microdiets (FCP and MCP) were formulated to include either of these peptides at 49%. Two groups of zebrafish larvae were fed FCP and MCP from first-feeding stage for 30 days. Body weight, standard length and ratio of myotome height and body length were measured at 5, 10, 20 and 30 days post hatching. Major constitutive peptides leached from diet within 15, 30 and 60 min were separated by gel chromatography and amino acid composition of eluents was determined by HPLC.

Leaching rate of the diet FCP were much larger than MCP at 15, 30 and 60 min. The amino acid analysis indicates that the dissolved peptide in FCP from 15-60 min was 352 mg/g and 781 mg/g. In contrast, the dissolved peptide in MCP from 15-60 min changed from 186 mg/g to 112 mg/g. Zebrafish larvae fed with MCP showed higher survival rate, longer body length, and heavier body weight than those fed FCP. This study suggests that zebrafish could be a suitable model system to evaluate feed performance of microdiet with varying degrees of nutritional leaching for marine fish larvae.

* E-mail address: tinglovekiss@hotmail.com
Development and use of plant based diets for the production of the pacific white shrimp, *Litopenaeus vannamei*.

D. Allen Davis 1*, Daranee Sookying and Luke A. Roy  
1 Department of Fisheries and Allied Aquacultures, Auburn University, Auburn, AL 36849-5419 USA

**Abstract**

Paralleling an increase in the world’s population is an increased need to produce healthy and safe foods. From the perspective of seafood supply, the world’s fisheries have been over exploited and production cannot be expanded. To meet world seafood demand, aquaculture has grown rapidly and currently produces around 50% of the edible seafood supply. However, many of our feed formulations are based on the use of fish meals and oils that are also in limited supply. Consequently, if aquaculture production is to continue to expand we must adopt our formulations to the use of ingredients that are not only sustainable but can be expanded to meet increased demand. If we take shrimp farming as an example, the industry has rapidly expanded to meet demand. However, the increase in marine shrimp production has been accompanied by an increased use of fish meal (as a percentage of the available meal), decrease in shrimp value, and reduced profitability for the farmers in recent years. Facing this scenario, there has been considerable interest in reducing production cost by improved feed management and lower cost diets. Reducing the cost of feed by minimizing the inclusion of expensive ingredients of marine origin, mainly fish meal, is one alternative. Fish meal is the most important and costly protein source in most shrimp feeds, with commercial shrimp formulations commonly including between 25 to 50% fish meal in the diet. In addition to economic concerns, the use of marine ingredients has received considerable attention by the public in terms of perceived sustainability issues. Hence, the reduction and/or removal of fish meal and other marine ingredients are of considerable concern to the industry. With proper replacement strategies, a number of studies have demonstrated that fish meal and marine oil levels can be reduced or eliminated. It is clear that cheaper high quality plant (e.g. combinations of solvent extracted soybean meal, corn gluten meal, distiller’s dried grain with solubles, pea meal) or terrestrial animal protein sources (e.g. poultry meal) can be used to successfully replace fish meal in shrimp feeds without compromising shrimp growth. Economic returns on various dietary formulations are region specific, however, in most areas, the reduction of fish meal levels in production diets will result in reductions in feed costs and improved economic returns on the investment in feeds. Marine fish oils can also be reduced from the feed by using a suitable combination of plant oils and highly unsaturated fatty acids or a quality marine oil at reduced level. Such techniques have been demonstrated but due to current prices this may not reduce overall costs however, it does allow the advertisement of a more environmentally friendly production system. Such modifications to the feed can only be done if the replacement strategy takes into account nutrient requirements of the species in terms of essential amino acids, fatty acids and minerals as well as potential palatability shifts in the diets

**Keywords:** Pacific white shrimp, plant proteins, alternative diets

*E-mail address: davisda@auburn.edu*
O-052


Nick Wade\textsuperscript{1,2,*}, Christian de Santis\textsuperscript{3}, Simon Tabrett\textsuperscript{1,2}, Simon Irvin\textsuperscript{1,2}, Melony Sellars\textsuperscript{1,2}, Brett Glencross\textsuperscript{1,2}, Nigel Preston\textsuperscript{1,2}

\textsuperscript{1} CSIRO Marine and Atmospheric Research, PO Box 120, Cleveland QLD 4163, Australia.
\textsuperscript{2} CSIRO Food Futures National Research Project, North Ryde, NSW, Australia.
\textsuperscript{3} School of Marine and Tropical Biology, James Cook University, Townsville, 4811, Australia.

Abstract

Improved growth rates have been observed in most prawn species as a result of factors such as sufficient food availability, type and quality of nutritional components in diets, inclusion of bioactive compounds and genetic selection. In a study designed to assess the physiological and nutritional mechanisms underpinning the gains seen in a selected stock of Black tiger prawns relative to an unselected strain, we sought to also investigate changes in the underlying genetic pathways controlling muscle development in the same treatments. Key molecular candidates for regulation of muscle development and maintenance in prawns have been isolated from *Penaeus monodon*, including key muscle growth regulators IGF, myogenin and myostatin, as well as muscle specification and maintenance genes mTOR, MyoD and Pax7. Their respective expression profiles were determined using quantitative RT-PCR across the restrictively fed, bioactive-nutrient supplemented or genetically selected animals. Strong growth rate differences were observed in prawns across the treatments, and the molecular expression profiles of some genes was also modified in line with the treatments, thereby identifying the first functional genetic candidates involved in prawn muscle growth. As such, we have begun to define the molecular pathways and key genetic regulators that underpin the growth gains observed in prawns triggered by bioactive feed additives and genetic selection, with the view to understanding in more detail how these effectors work.

Keywords: Nutrigenomics; Shrimp; Bioactives, Muscle, Growth

*Email Address: nick.wade@csiro.au.*
Quantitative dietary arginine requirement of juvenile Pacific white shrimp, *Litopenaeus vannamei* (Boone) reared in low-salinity water

Ming-yan Huai¹,²*, Yong-jian Liu¹, Li-xia Tian¹, An-long Xu¹, Hui-jun Yang¹, Gui-ying Liang¹

¹School of Life Sciences, Sun Yat-sen University, Guangzhou 510275, P.R. China
²Alltech biological products (China) Co.Ltd, Beijing 101407, P.R.China

**Abstract**

An 8-week growth trial was conducted to determine dietary arginine requirement for juvenile Pacific white shrimp *Litopenaeus vannamei* (Boone) with an initial average body weight of 0.47 g reared in low-salinity water (0.50-1.50 g L⁻¹). Six isonitrogenous and isoenergetic semipurified diets (crude protein 360 g kg⁻¹ and gross energy 19.70KJ g⁻¹) were formulated with fish meal and wheat gluten as intact protein source. Pre-coated crystalline amino acids were added to obtain six levels of dietary arginine from 13.9 to 28.6 g kg⁻¹ dry diet. Triplicate tanks (30 shrimps in each tank) were randomly assigned to each diet and the shrimps were fed four times daily. The results showed that the shrimps fed the diet of lowest arginine had significantly lowest growth and feed efficiency. Maximum weight gain was obtained in the shrimp fed the diet of 22.6 g arginine kg⁻¹ diet while higher dietary arginine did not obtain higher growth or feed efficiency. The chemical composition of whole body and muscle, the muscle arginine content and the concentration of triacylglycerol, total protein and urea in haemolymph were significantly affected by the arginine level. However, aspartate aminotransferase and alanine aminotransferase activities in haemolymph were not influenced by dietary arginine levels. Broken-line regression analysis of weight gain in related to dietary arginine showed that optimal dietary arginine requirement for maximum growth of *Litopenaeus vannamei* was 20.8 g kg⁻¹ dry diet (5.78 % of dietary protein).

**Keywords:** *Litopenaeus vannamei*; arginine; Requirement;Growth performance;Low salinity

* E-mail address: huaimy@126.com (Ming-Yan Huai).
Assessing energy and protein requirements of juvenile European lobster Homarus gammarus designed for intensive culture.

Ingrid Lupatsch*, Lydia Cuthbertson and Robin J. Shields
Centre for Sustainable Aquaculture Research, Swansea University, Swansea SA2 8PP, United Kingdom

Abstract
European lobster (Homarus gammarus) is one of the most valuable seafood in the world. As the fishery of lobster alone can not sustain market demand, aquaculture is of great interest to increase supply. The main obstacle for lobster aquaculture has been the high production costs due to their cannibalistic nature and the need for individual rearing compartments. While there have been major developments in automated technology leading towards land based lobster farming, the lack of nutritionally balanced feeds is still one of the limiting factors.

Studies were performed to determine energy and protein needs in growing lobster as the sum of the requirements for maintenance and growth. Requirement for maintenance is considered to be a function of the size of lobster and water temperature and requirement for growth is depending on the weight gain and its energy and protein content. Total daily intake can thus be calculated by incorporating the utilization efficiencies of energy and protein deposition. The consequence of this approach is that protein and energy needs are expressed primarily in terms of absolute demand per body mass and anticipated weight gain and only secondary as inclusion level in the diet.

Maintenance requirements and efficiencies of digestible energy (DE) and digestible protein (DP) were determined by feeding groups of lobsters (initially 1 and 3g, at 18°C) at increasing levels from zero to maximum feed intake. Total energy and protein gain in lobster were determined by comparative body analysis and the relationships between DE intake and energy gain as well as DP intake and protein gain established. The slopes of the linear relationships between intake and gain described the respective efficiencies of energy and protein for growth. The requirement of digestible energy for maintenance was calculated to be DEMaint = 105 J g-1 and of digestible protein DPMaint = 2.4 mg g-1 body mass per day. The efficiency of growth deposition was 0.31 and 0.35 for digestible energy and digestible protein respectively. Through proximate analyses the average energy content of juvenile lobsters was determined as 3.57 kJ g-1 and the protein content as 115 mg g-1 live weight gain.

Using these values together with a suitable growth prediction will allow optimization of practical feeding tables for Homarus gammarus culture.

Keywords: European lobster; Energy requirement; Protein utilization; Maintenance; Feed formulation

*E-mail address: i.lupatsch@swansea.ac.uk (Ingrid Lupatsch)
Regulation of sulfur amino acid pathways by methionine, cysteine and choline in juveniles tiger prawn Penaeus monodon

Lenaïg Richard¹ ², Christiane Vachot¹, Anne Surget¹, Vincent Rigole², Sadasivam Kaushik¹, Inge Geurden¹

¹ INRA, UMR1067 NUAGE Nutrition, Aquaculture and Genomics, F-64310 Saint-Pée-sur-Nivelle, France
² AQUALMA, BP 93 Immeuble SCIM, 4 rue Galliéni, Mahajanga 401, Madagascar

Abstract

Requirements for methionine or total sulphur amino acids (TSAA: methionine + cysteine) have been determined in tiger prawn Penaeus monodon. Besides its involvement in protein synthesis, as a methyl donor, methionine can be transmethylated to homocysteine which can be transformed into cysteine through the transsulfuration pathway. As such, cysteine is often considered a semi-essential amino acid.

Ten semi-purified diets (crude protein: 38% DM) were fed during five weeks to juvenile P. monodon (3.3 ± 0.1 g initial wet weight) to investigate the effects of i) a reduction in TSAA supply (through a methionine reduction of 25 and 50%, keeping the ratio CYS/MET equal to 0.4) and ii) a constant supply in TSAA, with methionine reduction being compensated by cysteine, on the methylation/transsulfuration pathways. Under both conditions, dietary choline was supplied either adequately or in excess. Betaine-homocysteine methyltransferase (BHMT) and cystathionine-β-synthase (CBS) were specifically studied since BHMT catalyses the remethylation of homocysteine with betaine (product of choline degradation) whereas CBS catalyses the first step of transsulfuration, transforming homocysteine into cystathionine.

We found that the activity of BHMT changed according to both choline and methionine supplies. A reduction in methionine supply by 25% significantly reduced the specific activity of BHMT compared to the control treatment (14 vs 32 mU/mg protein), whereas the activity increased with a further reduction of methionine supply by 25% (41 mU/mg protein). Choline in excess was found to reduce significantly the BHMT activity (24 vs 34 mU/mg protein, respectively for excess and control levels). However, when TSAA supply was adequate, no significant effect of additional cysteine or choline could be detected. Regarding CBS, specific activity did not significantly respond to dietary changes.

These first results suggest that the methylation pathway is controlled by methionine rather than TSAA intake. Therefore, it seems that shrimp can use choline supply through an increased BHMT activity to compensate for low methionine intake. Furthermore, since CBS activity was more or less constant, cysteine was probably not in excessive amounts for the shrimps and the cysteine load would have been successfully oriented towards other metabolites such as taurine formation. This should be confirmed by further analyses.

* E-mail address: lrichard@st-pee.inra.fr (L. Richard)
O-056

Effects of Dietary Probiotic Lactic Acid Bacterium on Growth, Survival, Digestive Enzyme Activities and Vibriosis Resistance of the Shrimp Litopenaeus vannamei

Yanhua Huang1,2*, Guoxia Wang1, Wening Huang1, Ye Zhou1, Shangzhi Dong1
1Zhuhai Agriculture Research Center, Zhuhai 519070, China
2Institute of Animal Science, Guangdong Academy of Agriculture Sciences, Guangzhou 510640, China

Abstract
White shrimp Litopenaeus vannamei has become the primary penaeid currently being cultured worldwide. However, the culture of penaeid shrimps has been limited by epidemic infectious diseases including viral infections such as Taura syndrome virus (TSV) and vibriosis due to Vibrio alginolyticus and Vibrio harveyi. The bacterium V. alginolyticus isolated from diseased L. vannamei with whitish musculature has been demonstrated to cause mortality of shrimp, and can decrease its immunity. In recent years, biological control of these diseases, affecting aqua-cultured species especially bacteriological disorders including environmentally friendlier methods such as the use of probiotics, has become an important subject of investigations. The objective of the present study was to evaluate the effects of Lactic acid bacterium (LAB) on growth, survival, digestive enzyme activities and vibriosis resistance of the shrimp Litopenaeus vannamei. Two kinds of LAB (A and B) were administered at doses of 10^9 cfu/g feed to juvenile shrimp, Litopenaeus vannamei for 56 days. Fifty-six days after the start of the LAB feeding, shrimps number and weight of each tank were recorded, and some shrimps were used for digestive enzyme and the left shrimps were challenged with V. alginolyticus (30 µL V. alginolyticus suspension at 3.2×10^8 cfu/mL per shrimp). The cumulative mortality was monitored after injection for 3 days. Results showed that grouper fed LAB B had significantly decreased weight gain ratio(WGR) and special growth rate(SGR) which were 3895.83% and 2.67 (VS 4819.35% and 2.82), respectively, and grouper fed LAB A was not different from the control group on growth. But shrimp fed with LAB had very significantly higher survivals than the control group (99.17% and 100% respectively vs 81.67%). No differences were recorded among groups on feed conversion ratios(FCR), but groups fed LAB was lower than control. Digestive enzyme activity of each groups in the digestive tract was no difference, however protease in the hepatopancreas of treatments had a decline trend compared with that of the control. LAB reduced the shrimp mortality significantly, from 36.37% in the control to 23.33% and 30.0% in the LAB A and B feed, respectively. These results suggest LAB A may be a promising probiotics for shrimp without subsequent risk for human consumption.

Keywords: Lactic acid bacterium; Litopenaeus vannamei; Growth; Survival; Digestive enzyme activity; V. alginolyticus; Resistance

* E-mail address: huangyuh111@126.com (Y.H. Huang)
The effects of different DHA/EPA ratios on the survival, growth and larval development of blue swimmer crab, *Portunus pelagicus* and ornate rock lobster, *Panulirus ornatus* based on live food enrichment

Xugan Wu*, Chaoshu Zeng, Greg Smith, Paul C. Southgate, Mike Hall

1AIMS@JCU, Australian Institute of Marine Science, School of Marine and Tropical Biology, James Cook University, Townsville, Queensland 4811, Australia
2AIMS@JCU and Australian Institute of Marine Science, PMB no.3, Townsville MC, Queensland 4810, Australia

Abstract

Blue swimmer crab (*Portunus pelagicus*) and Ornate rock lobster (*Panulirus ornatus*) are two tropical commercially crustaceans. Because of their large size, high meat yield and delicate flavour, there is an increasing demand for both *P. pelagicus* and *P. ornatus* supply worldwide. For the future commercial production of *P. pelagicus* and *P. ornatus*, optimal hatchery production is essential together with the development of formulated diets. Although previous studies have shown dietary DHA/EPA ratio are very important to crustaceans, particularly for Penaeid prawns, no available information could be found on blue swimmer crab and ornate rock lobster. The current experiments were conducted to evaluate the effects of dietary DHA/EPA ratios on the larval development and body fatty acids composition for both early stage *P. pelagicus* and *P. ornatus*. There were five treatments fed with different DHA/EPA ratios (0.3, 0.6, 1, 2, 3) *Artemia*. Although no significant difference could be found on the survival of early stage larvae for both species, the dramatic lower survival of *P. pelagicus* megalopa could be found on the high and low DHA/EPA ratio treatments. The newly hatched phyllosomas of the ornate rock lobster fed high DHA/EPA ratio (2 and 3) *Artemia* showed a larger carapace length, body dry weight and shorter development time while the reverse trend could be found on the blue swimmer crab. Those results indicated the early larvae of ornate rock lobster had higher DHA/EPA ratio requirements than the blue swimmer crab larvae. Therefore, optimization the fatty acid composition of hatchery food will be an effective means to improve seed quality and quantity for both species.

Keywords: DHA/EPA ratio; Blue swimmer crab; Ornate rock lobster; Larval nutrition

*E-mail address: wuxugan@hotmail.com; xugan.wu@jcu.edu.au (X.G. Wu).*
Solid-state fermentation with *Aspergillus niger* to improve the nutritional value of rapeseed meal as a feed ingredient for Nile tilapia (*Oreochromis niloticus* L.)

Y. Gao1,2,4, M.M. Nabulime2, J.F. Hansen3, V. Denstadli1,2, H.M. Gjøen2, T. Storebakken1,2*

1Aquaculture Protein Centre, Centre of Excellence. 2Department of Animal and Aquacultural Sciences. Norwegian University of Life Sciences (UMB). NO-1432 Ås (Norway)

3Department of Chemistry, Biotechnology and Food Science, UMB. NO-1432 Ås (Norway)

4College of Biological and Environmental Sciences, Zhejiang Wanli University, Ningbo 315100, China.

**Abstract**

The aims of present study were to determine if solid-state fermentation (SSF) is a useful tool to increase the nutritional quality of rapeseed meal, and to evaluate the potential for use of untreated and SSF rapeseed meal in diets for Nile tilapia. *Aspergillus niger* strain ATCC 1577 was used in SSF to produce two batches of fermented rapeseed meal, which were used as the protein source in the diets for the two feeding experiments. In Exp. 1, three moist diets were produced: a diet based on fish meal and soybean meal (SBM-diet), and two diets in which SBM was replaced by rapeseed meal (RSM-diet) or fermented rapeseed meal (FRSM-diet). The diets contained 400 g crude protein kg\(^{-1}\) and 100 g lipid kg\(^{-1}\) in the dry matter (DM). They were fed to 3 groups of tilapias (GIFT strain, 14\(^{th}\) generation, initial body weight: 7.8 g) to apparent satiation twice daily for 37 d. In Exp. 2, three extruded diets (SBM, RSM and FRSM-diets) were produced with the same formulation as used in Exp. 1. Each diet was fed to 3 groups of 54.6-g fish, 3 meals a day for 43 d.

The results showed that the content of crude protein in rapeseed meal was increased from 385.0 to 402.8 and 401.8 g (kg DM)\(^{-1}\) by SSF. The content of total amino acids was reduced from 76 to 71 g (100 g CP)\(^{-1}\). The content of phytic acid (IP6) was reduced from 35.8 to 10.5 and 19.0 g (kg DM)\(^{-1}\), and total glucosinolates were reduced from 1.8 to 0.4 and 0.7 g (kg DM)\(^{-1}\) in Exp. 1. In Exp. 1, the specific growth rate (SGR) of fish fed the SBM-diet was 3.8, which was significantly lower than that of tilapias fed the RSM (4.1) and FRSM (4.2) diets. Feed conversion ratio (FCR) of fish fed RSM-diet (1.69) was significantly higher than that of fish fed the SBM (1.22) and FRSM (1.20) diets. Fish fed RSM-diet had significantly more whole-body crude protein than fish fed the SBM-diet. In Exp. 2, the specific growth rate showed no significant difference among the dietary treatments, while FCR differed obtained all 3 dietary treatments (SBM, 1.08; RSM, 1.23; FRSM, 1.34). The nitrogen retention of fish fed SBM was significantly higher than that of fish fed the RSM and FRSM diets, and tilapias fed the FRSM-diet had significantly lower nitrogen retention than those fed the RSM-diet. The digestibility of nitrogen in fish fed SBM-diet was higher than that in fish fed the RSM and FRSM diets. The digestibility of Mg was significantly higher in fish fed the SBM and FRSM diets than that in tilapias fed RSM.

In conclusion, SSF reduced contents of glucosinolates and phytic acid, as well as amino acids in rapeseed meal. SSF of rapeseed meal improved the feed conversion when used in a moist diet, while it impaired the protein utilization and feed conversion when used in an extruded diet. The differences in nutritional value in the two experiments may partly be rationalized by differences in fermentation of the rapeseed meal, and partly by the two methods of feed production used.

**Keywords:** Solid-state fermentation; *Aspergillus niger*; rapeseed meal; Nile tilapia; Specific growth rate; Feed conversion ratio; Digestibility.

* E-mail address: trond.storebakken@umb.no
Effect of discontinuous administration of $\beta$-glucan and glycyrrhizin on the growth and immunity of Pacific white shrimp *Litopenaeus vannamei*

Nan Bai*, Kangsen Mai, Wenbing Zhang, Hongming Ma, Qinghui Ai, Xiaojie Wang
Key Laboratory of Mariculture (Ministry of Education), Ocean University of China, Qingdao 266003, PR China

Abstract
A six-week growth trial was conducted to compare the effects of different feeding strategies of dietary immunostimulants on the growth and immunity of white shrimp *Litopenaeus vannamei* (4.70±0.20g). Shrimps were fed with diet containing glycyrrhizin continuously, containing $\beta$-glucan continuously, discontinuously (seven days with diet containing $\beta$-glucan following seven days with diet without $\beta$-glucan; two days with diet containing $\beta$-glucan following five days with diet without $\beta$-glucan), alternately with diet containing glycyrrhizin (seven days with diet containing $\beta$-glucan following seven days with diet containing glycyrrhizin) and were sampled every 2 days. The results showed that continuous administration of $\beta$-glucan (0.2 g kg$^{-1}$ diet) or glycyrrhizin (0.02 g kg$^{-1}$ diet) brought about immunity fatigue. The total haemocyte count (THC), phenoloxidase (PO) activity, superoxide anion and superoxide dismutase (SOD) activity of shrimps fed with $\beta$-glucan continuously were significantly higher than those of shrimps fed with diet without $\beta$-glucan (basal diet) on the 5th, 5th, 3rd, 3rd day, respectively. The THC, PO activity and SOD activity of the shrimps fed with diet containing glycyrrhizin were significantly higher than basal level on the 13th, 13th, 3rd day, respectively. The immune parameters of the shrimps of other three treatments were elevated and significantly higher than those of shrimps fed with basal diet at the same time as the shrimps fed with $\beta$-glucan continuously. However, with the experiment went on, there were no significant differences in THC, superoxide anion and SOD activity between shrimps fed containing dietary $\beta$-glucan continuously and those fed diet without $\beta$-glucan on the 29th day. No significant difference was found in PO activity on 35th day. The significantly elevated THC, PO and SOD of the shrimps fed with diet containing glycyrrhizin were no significant differences with those of shrimps fed with basal diet on the 25th, 35th, 27th day, respectively. Discontinuous or alternate strategy could eliminate immunity fatigue. Compared with control group, immune parameters of the shrimps fed with $\beta$-glucan discontinuously and alternately with glycyrrhizin were still significantly higher than basal level during the experimental period. Shrimps fed with immunostimulants all showed significantly higher specific growth rate (SGR) compared with basal level. In all groups, the highest SGR was observed in the shrimps of the group that feeding with $\beta$-glucan two days following basal diet five days. As can be seen from the experiment, discontinuous administration of $\beta$-glucan or alternate administration of $\beta$-glucan and glycyrrhizin is more suitable for shrimps to maintain a high immunity than continuous administration with dietary $\beta$-glucan. The strategy that feeding with $\beta$-glucan two days following basal diet five days is most suitable for L. vannamei.

Keywords: Pacific white shrimp; $\beta$-glucan; glycyrrhizin; immunity; immunity fatigue

*E-mail address: bainan668@163.com (N.Bai)
Using Near-InfraRed Spectroscopy (NIRS) to predict the digestible protein and energy value of grain – The lupin case study

Brett Glencross1,*, Wayne Hawkins2, Peter Burridge2, David Evans3, Neil Rutherford3, Peter McCafferty4, Ken Dods4, Rhys Hauler5 and Sofia Sipsas2
1CSIRO Marine and Atmospheric Research, Cleveland, QLD 4163, Australia
2Department of Agriculture and Food, South Perth, WA 6161, Australia
3Department of Fisheries, Hillarys, WA 6025, Australia
4Skretting Australia, Cambridge, TAS 7017, Australia

Abstract

Over a five-year period, we performed 10 independent digestibility experiments to examine the digestibility of protein and energy from 136 different samples of lupin meal from either Lupinus angustifolius or L. luteus. Lupin samples were obtained from the Australian National Lupin Breeding Program’s germplasm lines and selected on the basis of maximal crude protein variability using existing crude protein near-infrared spectroscopy (NIRS) calibrations. Chemically measured crude protein values varied from 232 to 613 g/kg dry basis. Gross energy values ranged from 18.7 to 21.6 MJ/kg DM. Other compositional parameters assessed included amino acids, total lipids, ash, total carbohydrates, cellulose, hemicellulose and lignin.

The digestibility of protein and energy was assessed in rainbow trout (Oncorhynchus mykiss) using the diet-replacement ingredient assessment method, where the test ingredient comprised 30% of each test diet. Digesta was collected using faecal stripping techniques. Digestible protein values ranged from 193 to 595 g/kg DM and digestible energy values ranged from 6.0 to 17.7 MJ/kg DM. The digestible protein and energy values were then assessed using multiple regression techniques to determine which compositional parameters accounted for the majority of the variability. Crude protein content was observed to be the most dominant single factor in affecting digestible protein levels. However, multiple regression analysis supported that protein and lignin content combined were the strongest predictors of digestible protein value, explaining close to 60% of the variability in this parameter. Digestible energy values were heavily reflective of the factors influencing digestible protein value. This study demonstrates that within one raw material type, not only does significant variability exist in the digestible value of the raw materials, but it is also possible to identify compositional features of that raw material that are intrinsically influencing its own digestible value. Predictive equations have been developed that explain this variability.

Assessment of these samples using NIRS shows that there are certain wavelengths that correlate with crude protein and lignin content of the lupin kernel meals. Applying a dual assessment to both of these parameters, based on the predetermined relationship, it has been possible to develop a calibration to predict both digestible protein and energy content of lupin kernel meals. This calibration can now be used to assess lupin meals prior to formulation to minimise variability of digestible protein and energy content of diets in which they are included.

Keywords: Grain, Digestibility, Fishmeal replacement, Salmonid

* E-mail address: Brett.Glencross@csiro.au
Effect of a finishing diet strategy on EPA and DHA restoration in rainbow trout, *Oncorhynchus mykiss*, previously fed dietary palm fatty acid distillate at two different water temperatures.

**Basseer M. Codabaccus**, **Wing-Keong Ng**, **Peter D. Nichols**, **Chris G. Carter**

1. NCMCRS, University of Tasmania, Launceston, Tasmania, Australia
2. Fish Nutrition Laboratory, School of Biological Sciences, Universiti Sains Malaysia, Penang 11800, Malaysia
3. CSIRO Food Futures Flagship and Division of Marine and Atmospheric Research, Hobart, Tasmania 7000, Australia
4. Tasmanian Aquaculture and Fisheries Institute, University of Tasmania, Hobart, Tasmania, Australia

**Abstract**

Fish oil (FO) replacement in aquafeeds for salmonids has become a necessity due to the rise in the price of FO dictated by the market imbalance between demand and supply. Vegetable oil (VO) is the most studied substitute for FO; however, the lack of the health-benefitting omega-3 long chain polyunsaturated fatty acid (ω3 LC-PUFA) in VO remains a major short-coming. Feeding fish on a finishing FO diet after grow-out on a VO diet is one strategy to restore the ω3 LC-PUFA content in fish, most particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). We investigated the restoration of EPA and DHA in rainbow trout fed a finishing FO diet for four weeks following a grow-out period of eleven weeks at two different water temperatures and fed on diets where FO was replaced by 50% or 75% palm fatty acid distillate (PFAD). We evaluated whether the fatty acid (FA) composition of fish tissues could be explained by the dilution model and/or other processes. The flesh of rainbow trout fed the 75%PFAD diet showed a decrease of 54 and 51% in EPA, compared to the FO-fed fish, while DHA decreased by 39 and 29% at 15 and 20°C, respectively. For whole carcass, a reduction of 46 and 54% in EPA was observed, while DHA was reduced by 36 and 40% at 15 and 20°C, respectively. The decrease in EPA and DHA percentage composition for both flesh and whole carcass was lower in fish fed the 50%PFAD diet compared to fish fed the 75%PFAD diet irrespective of water temperature. EPA was restored to 82 and 68% in fish flesh, while DHA restoration was 78 and 76% at temperatures 15 and 20°C, respectively, for fish fed on 75% PFAD. Restoration of EPA for whole carcass was 91 and 72%, while DHA was restored to 93 and 76% at temperatures 15 and 20°C, respectively, for fish fed on 75%PFAD. There was no significant difference (P<0.05) in EPA and DHA percentage composition between fish fed 50%PFAD and then the finishing diet or a FO diet for both flesh and whole carcass. A regression analysis between observed and predicted FA values showed that the dilution model was a good predictor of final percentage FA composition for flesh and whole carcass at both temperatures. A four week finishing period was sufficient for restoration of EPA and DHA in rainbow trout previously fed on 50% PFAD for eleven weeks.

**Keywords:** Finishing diet strategy; Dilution model; Fatty acids; Rainbow trout; Vegetable oil

*E-mail address: mohamedc@utas.edu.au*
Responses of common carp (Cyprinus carpio L.) to DL-methionine supplementation at different feeding strategies

Frieder J. Schwarz1,*, Andreas Lemme2, L.C. Nwanna1, Abdallah Metwally1
1Department of Animal Sciences, Section Animal Nutrition, Technische Universität München, Liesel-Beckmann-Str. 4, D-85350 Freising, Germany
2Evonik Degussa, Rodenbacher Chaussee 4, D-63457 Hanau-Wolfgang, Germany

Abstract

In fish nutrition feed formulation including plant proteins needs further research on dietary amino acid (AA) balance. DL-Methionine belongs to the important supplemental AA. However, for carp as a stomach-less fish possible interactions between the utilization of supplemented free AAs and feeding strategy are still in discussion. Therefore, in a two-factorial trial the supplementation of DL-Methionine to a Met-deficient diet was investigated comparing two-meal feeding to continuous feeding of carp (Cyprinus carpio L.). A total of 20 tanks (each with 9 carp with a mean initial live weight (LW) of 239g/fish) were assigned to 6 different treatments. Half of them were fed a Met-deficient diet (Schwarz et al. 1998) containing 0.45% Met and 0.85% Met+Cys in dry matter (DM) while the other carp were fed a DL-Methionine supplemented diet with 0.86% Met and 1.26% Met+Cys in DM. The mean protein and fat contents of the diets were 39% and 7% in DM. Fish were fed six days a week using the following strategies. I: to apparent satiation by hand twice a day (8.00 a.m., 5.00 p.m.; 4 tanks each), II: continuously by automatic feeders (twice daily over a 6h period) using the same amount as I (pair feeding; 3 tanks each) and III: continuously as II, but with a constant amount of an average of 1.4% feed of LW (3 tanks each). For the latter treatment feed quantity was adjusted fortnightly per tank after carp weighing. The experimental period lasted 82 days. The two-factorial analysis of variance indicates overall significant effects of dietary methionine supply and feeding strategy (p<0.05) but no interactions with one exception (feed conversion, FCR). DL-Methionine supplementation increased feed intake (4.05g vs. 4.56g), final LW (419g vs. 481g), average daily gain (2.21g vs. 2.88g), and improved FCR (1.92g/g gain vs. 1.60g/g gain). This effect was most pronounced at twice daily feeding. Continuously feeding (II, III) raised average daily gain (2.19g vs. 2.78g) and improved FCR (2.11g/g gain vs. 1.53g/g gain). DL-Methionine supplementation significantly (p<0.05) increased the protein content in the carcass at the expense of fat content. The free AAs of plasma (Met, Lys, Arg, Ser) are effected by dietary methionine supply similar to Schwarz et al. (1998). DL-Methionine supplementation to Met deficient diets positively affects performance parameters of carp.

Keywords: DL-Methionine, Feeding strategy; Carp

* E-mail address: schwarzf@wzw.tum.de (Frieder J Schwarz).

Reference

Plant-based protein source and processing affect gastrointestinal inflammatory markers and growth rate in rainbow trout, *Oncorhynchus mykiss*

Graeme S. Mansfield¹*, Stephanie A. Nilson¹, Atul R. Desai², Janet E. Hill², Murray D. Drew¹, and Andrew G. Van Kessel¹

¹ Department of Animal and Poultry Science, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK, Canada, S7N 5A8
² Department of Veterinary Microbiology, Western College of Veterinary Medicine, Saskatoon, SK, Canada

Abstract

To discern the effects of plant-based protein inclusion and processing level on the health and performance of rainbow trout (*Oncorhynchus mykiss*), six separate trials were performed, each with 22 fish allocated to five treatment groups with three replicates (n = 1980). Three plant-based protein sources: soybean, pea, and canola were included in the experimental diets at an inclusion rate of 0 to 30%. These ingredients were evaluated at two distinct processing levels, a meal and a purified protein concentrate, giving six experimental ingredients in total. Expression of inflammatory markers (transcript copy number per 50 ng total RNA) including proliferating cell nuclear antigen (PCNA), immunoglobulin M (IgM), interleukin-1 beta (IL-1β), interleukin-8 (IL-8), and interleukin-10 (IL-10) was evaluated in distal intestinal tissue (n=9 per treatment), collected after eight weeks on feed, by quantitative PCR. Significant (P<0.05) pairwise Pearson correlation coefficients are reported for gene expression, specific growth rate (SGR), and inclusion level of feed ingredients. SGR was negatively correlated with IgM (R=-0.308) and IL-1β (R=-0.292). Dietary inclusion of soybean meal (SBM) was negatively correlated with SGR (R=-0.299) and positively correlated with all proinflammatory markers including PCNA (R=0.482), IgM (R= 0.706), IL-1β (R=0.867), and IL-8 (R=0.297). Pea meal (PM) inclusion level was positively correlated with the anti-inflammatory marker, IL-10, but was not significantly correlated to SGR. Canola meal (CM) inclusion level was negatively correlated with SGR (R=-0.403) and tended (P=0.066) to be negatively correlated with IL-8 (R=-0.252). No correlations were observed between the inclusion level of any protein concentrate ingredient and either SGR or the inflammatory markers. The results indicate that while increasing dietary levels of SBM or CM reduce growth performance in rainbow trout, only SBM inclusion is associated with intestinal inflammation. Dietary inclusion of PM or any of the protein concentrate ingredients appeared to be well tolerated.

**Keywords:** Rainbow trout; Gene Expression; qPCR; Intestinal inflammation

* E-mail address: graeme.mansfield@usask.ca (G.S. Mansfield).
Effect of sodium diformate and extruder temperature on nutrient digestibility in rainbow trout fed barley protein concentrate-based diets

Thea Morken1*, Olav F. Kraugerud1, Frederic T. Barrows2, Mette Sørensen1, 3, Trond Storebakken1, Margareth Øverland1
1 Aquaculture Protein Centre, Centre of Excellence, Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, P.O. Box 5003, N-1432 Ås, Norway.
2 U.S. Department of Agriculture, Agricultural Research Service, Hagerman Fish Culture Experiment Station, 3059-F National Fish Hatchery Road, Hagerman, ID, 83332, USA.
3 Nofima Marine, P.O. Box 5010, N-1432 Ås, Norway.

Abstract

Barley protein concentrate (BPC), a by-product from ethanol production, is a promising protein source for aquaculture feeds mainly due to its high protein content (~55%). Organic acids salts have been reported to promote growth and health performance in warm-blooded monogastric animals due to its antimicrobial effects. Previous studies with Atlantic salmon (Salmo salar) have shown that addition of formic acid salt to a plant-based diet improved protein digestibility. Furthermore, interactive effects of heat treatment and formic acid salt supplementation on amino acid digestibility have been observed.

The objective of this experiment was to investigate the effect of sodium diformate (Na-diformate) and increasing extruder temperatures on nutrient digestibility in rainbow trout (Oncorhynchus mykiss) fed BPC-based diets. Triplicate groups of fish were fed a plant-based diet with 270 g kg-1 of BPC, with or without 10.6 g kg-1 Na-diformate. The diets were extruded at 110, 126, and 141ºC in a twin screw extruder, resulting in a total of six diets. Apparent digestibility coefficients (ADCs) of nutrients were determined by faecal stripping using yttrium oxide as an inert marker.

ADCs of essential amino acids ranged from 82.5 to 92%, which is high. Increasing extruder temperatures resulted in a significant linear increase in ADCs of crude protein (P<0.001), the essential amino acids arginine (P<0.01), valine (P<0.05), and several non-essential amino acids (P<0.05). Moreover, ADCs of crude fat (P<0.05), starch (P<0.01), and organic matter (P<0.001) increased linearly with increasing extruder temperatures. Addition of Na-diformate significantly increased ADC values of crude protein (P<0.001), all essential and non-essential amino acids (P<0.05), crude fat (P<0.05), and organic matter (P<0.001). In general, ADCs of crude protein, most amino acids, crude fat, starch, and organic material increased with the supplementation of Na-diformate to diets. Increasing extruder temperatures from 110 to 141ºC in diets containing no Na-diformate had no effect on ADCs of individual amino acids, while adding Na-diformate to the diets gave a significant increase in ADCs of arginine, cyst(e)ine, and glycine with increasing extruder temperatures. These findings demonstrate that increasing extruder temperatures and supplementation of Na-diformate increases nutrient digestibility of BPC-based diets in rainbow trout.

Keywords: barley protein concentrate; digestibility; extrusion; sodium diformate; rainbow trout.

* E-mail address: thea.morken@umb.no (T. Morken).
Dietary potassium diformate protects against heat-induced reduction of protein digestibility in a mixture of full-fat soy and wheat when used in extruded diets for Atlantic salmon (*Salmo salar* L)

Trond Storebakken¹*, Gerd Marit Berge², Margareth Øverland¹, Karl D. Shearer¹, Marie Hillestad³, Åshild Krogdahl⁴

¹Aquaculture Protein Centre, Centre of Excellence. Department of Animal and Aquacultural Sciences. Norwegian University of Life Sciences. P.O. Box 5003, NO-1432 Ås, Norway
²NOFIMA Marine, NO-6600 Sunndalsora, Norway
³BioMar AS, NO-7484 Trondheim, Norway
⁴Aquaculture Protein Centre, Centre of Excellence. Department of Basic Science and Aquatic Medicine. Norwegian School of Veterinary Science. P.O. Box 8146 Dep, NO-0033 Oslo, Norway

Abstract

The aims of the present experiment were to determine if expander heat treatment of a mixture of wheat and full-fat soybeans, and subsequent addition of potassium diformate (KDF) to the diets, prior to extrusion of the feed affected nutrient digestibilities, intestinal enzyme activities and soybean-meal-induced enteritis in Atlantic salmon. Full-fat, raw soybean was milled, mixed with milled wheat in a 1:0.6 ratio (w/w), and pre-treated at 100, 110, 116 and 122°C using an expander. The expander treated mixture of soy and wheat was included at 400 g kg⁻¹ into an extruded salmon diet with, or without supplementation of KDF (12 g kg⁻¹). Each diet was fed to two groups of salmon for determination of digestibility of macronutrients and individual amino acids. Trypsin and bile salt were assessed in the contents of the pyloric and distal regions of the intestines. Activities of leucine aminopeptidase (LAP) and soybean meal-induced enteritis were quantified in the tissues of the distal intestine.

No detectable differences in amino acid composition of the mixture between soy and wheat were related to increasing temperature during the expander treatment. Regression analysis demonstrated two main effects. Firstly, apparent digestibility of most amino acids was improved by KDF addition to the feed prior to extrusion. Secondly, digestibility coefficients were first improved and then reduced (Asp, Thr, Pro, Cys, His) or improved to a constant value (Ser, Glu, Val, Lys) with increasing expander pre-treatment temperature when KDF was not added. Digestibilities were improved with increasing expander pre-treatment temperature for two amino acids, Arg and Met. When KDF was added, Digestibility of Asp, Ser, Cys, Met and Lys increased in a linear manner with increasing pre-treatment temperature, while those of Glu, pro, Gly, Ala, Val, Ile, Leu, Tyr and His increased in a quadratic manner, with higher values for the higher expander temperatures than for the diets without KDF added. Neither digestibilities of fat, starch nor ash, trypsin and bile salts in contents of pyloric and distal intestine, nor activity of LAP or histological indices in the distal intestine were significantly affected by expander temperature or KDF supplementation.

In conclusion, addition of KDF prior to extrusion resulted in improved digestibility of amino acids. Furthermore, dietary KDF counteracted reactivity that had been induced by prior expander heat treatment, thereby improving digestibility of amino acids in ingredients that had been pre-treated at the highest temperatures.

Keywords: Atlantic salmon, digestibility, soybeans, heat treatment, potassium diformate (KDF)

*E-mail address: trond.storebakken@umb.no (T. Storebakken).*
The optimal pellet quality is a trade-off between durability and responses in the fish

T. Synnøve Aas¹,⁴*, Bendik F. Terjesen¹, Trygve Sigholt³, Marie Hillestad³, Jørgen Holm⁴, Ståle Refstie¹,⁶, Grete Baeverfjord¹, Kjell-Arne Rørvik², Mette Sørensen²,⁶,⁷, Maike Oehme¹,⁵, Gaojie He⁵,⁷, Torbjørn Åsgård¹,⁵
¹ Nofima, NO-6600 Sunndalsora, Norway
² Nofima, Postboks 5010, NO-1432 Ås, Norway
³ BioMar AS, Nordregt 11, NO-7011 Trondheim, Norway
⁴ BioMar A/S, Mylius Erichsensvej 35, DK-7330 Brande, Denmark
⁵ Centre for research based innovation in aquaculture technology (CREATE), SFI, SINTEF Sealab, NO-7645 Trondheim, Norway
⁶ Aquaculture Protein Centre (APC), CoE, P.O.Box 5003, NO-1430 Ås, Norway
⁷ Norwegian University of Life Sciences (UMB), Department of Animal and Aquacultural Sciences, P.O. Box 5003, NO-1432 Ås, Norway

Abstract

Handling, storing and transport of feeds lead to attrition of the pellets. Undersized particles represent loss and poor utilisation of feed resources. Thus, feeds with high physical pellet quality are demanded. However, the physical properties affect the nutritional value of feeds. In a 48 days trial, two feeds, with high (feed A) or low (feed B) water stability, were fed to rainbow trout (Oncorhynchus mykiss) kept in sea water (salinity 32-34 ppm, temperature 12 °C, O2-saturation >80%). The feed intake was 23% higher in trout fed feed B than in those fed feed A. Introducing a daily period of lowered salinity (< 10 ppm), temperature (6 °C) and oxygen saturation (50-60%) resulted in a severe drop in feed intake in both dietary groups. However, the apparent digestibility of macronutrients was highest in feed A, but at fluctuating environment the difference between the dietary treatments was small. Furthermore, the stomachs of trout fed feed B, particularly of those kept at stable environment, contained large amounts of free water and oil, which could potentially lead to fat belching. Both apparent digestibility and free water and oil in the stomach seemed to be related to feed intake, and were thus indirectly caused by feed and environmental conditions. In another trial, pellet degradation of three commercial feeds (12 mm pellet size) during pneumatic conveying, which is commonly used in cage culture to transport the feed from the storage silo to the sea pens, was measured. The three feeds, chosen especially for having different physical properties, showed different degradation patterns during conveying. Furthermore, high air speed (m/s) in the conveying system, which is used to spread the feed pellets well in the cage, increased pellet degradation, whereas high feeding rate (kg/min) protected the pellets from breakage. In all runs, less than 1% of the sample ended up as small particles. In conclusion, feed intake appears to be a main factor when evaluating pellet quality, but several other factors, such as pellet durability, nutrient digestibility and fat belching must be taken into consideration when searching for the most economical and sustainable pellet quality.

Keywords: Physical pellet quality; Pellet degradation; Feed intake; Nutritional value of feed

* E-mail address: synnove.aas@nofima.no (T.S. Aas)
Feed, feeding and environment safety in aquaculture

XIE S.*
State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, the Chinese Academy of Sciences, Wuhan, Hubei, 430072, P. R. China

Abstract

With fast increase in aquaculture, there comes the environmental problems which have received most attentions. The pollution from aquaculture includes nitrogen and phosphorus loading, unexpected chemicals and organisms. Poor water environment not only resulted in poor growth efficiency of fish, but also in poor fish quality and high risk of diseases.

This paper reviews the present advances on the researches on improving environment safety.

Reducing nitrogen and phosphorus discharging

Ingredient selection and pre-treatment: As fish shows different utilization on different feed resources of different proteins, lipids, carbohydrates, proper ingredient selection could help to increased the growth efficiency. Pre-treatment of extrusion or additive enzymes could increase the utilization of some nutrients and decrease the effects of anti-nutritional factors.

Diet formulation: Proper diet formulation is the most important way to decrease the nutrient discharging. Nutrient requirement is basic for diet formulation. Up to now, most requirement values were obtained at chemical composition. In order to achieve better feed utilization, digestible nutrient requirement should be re-evaluated. Balanced nutrients formulation should be careful with the contents and absorption of different nutrients and their interactions. Suitable additives could increase feeding rate, feed utilization and therefore decrease waste production. Unexpected additives should be reduced or prohibited.

Feed processing: Optimal feed processing including grounding, heating, coating etc. could obtain better physical and chemical situation of pellet to meet the requirement of fish and also reduce the effect of anti-nutritional factors.

Feeding strategy: Feeding strategies including suitable feeding table of which feeding frequency, feeding rhythm, feeding rate should be considered. Modeling the feeding rate or nutrient requirement could help to reduce feed waste and nutrient discharging. In those fish who shows compensatory growth, variation of feeding rate and/or feeding duration, variation of different diets could help to improve feed utilization.

1. Evaluating waste production

In order to manage the farming system and treat the waste water, evaluation of waste production from fish farm is very important. Different models including nitrogen and phosphorus budget, growth model and bioenergetic model were used to calculate waste production.

2. Research focus on environment safety

Researches on fish nutrition and feed processing should be focused on not only fast growth but also low waste discharging. New biotechnology on fish breeding could help to select new species/strains which show improved feed utilization and lower environmental pollution.

Keywords: Feed; Feeding; Environment safety

* E-mail address: sqxie@ihb.ac.cn (S. Xie).
A model for sustainable cage farming in semi closed water systems

Anders K. Kiessling1,2*, Eva Brännäs1, Jana Pickova6, Lars Edebo3, Odd Lindahl4, Anders Alanärä1, Kristina Sundell2, Matilda Olstorpe8, Erik Sandblom5, Afaf Kamal-Edin6, Corine Sandström9, Muhamed Muminovic12, Leif Norrgren10, Lars Ove Eriksson1, Johan Schnürer6, Jan Erik Lindberg7, Torbjörn Lundh7

1 Department of Wild Life, Fisheries and Environment, Swedish University of Agricultural Sciences, 901 83 Umeå, Sweden (SLU).
2 Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, Norway.
3 Cewatech, Sweden.
4 The Royal Swedish Academy of Sciences, Sweden.
5 Department of Zoology, Gothenburg University, Sweden.
6 Department of Food Science, SLU, 7 Department of Animal Husbandry, SLU., 8 Department of Microbiology, SLU, 9 Department of Chemistry, SLU, 10 Department of Veterinary Sciences, SLU.

Abstract

Feed is both the single largest cost to the farmer but also the major factor affecting the environmental impact of fish farming, including production and transport of the feed as well as effluence from the farm during production. However, the same arguments apply to all intensive animal production. On the one hand, fish are certainly our most efficient farmed animal in the sense of nutrient utilisation and farming space required. On the other, intensive fish farming offers new challenges not faced by terrestrial animal farming in minimizing the environmental impact.

The fact that the carrying capacity of all ecological systems is limited is gaining general acceptance, as is the insight that most plant- or animal-based feed sources suitable for farmed animals, including fish, are also suitable for human consumption. This insight leads to the realisation that the only sustainable alternatives are scenarios in which farmed animals become net contributors by a transfer of “non-human” food resources into human ones in an ecologically sound way. The effluent problem in intensive fish farming is often solved by dilution, i.e. location to less sheltered areas. However, this does not solve the problem of sustainable feed sources or the increasing need to recycle valuable nutrients in the effluent back into the food production.

The limited water exchange from the Baltic basin, being the ultimate recipient of the whole of Fennoscandia, excluding the narrow Atlantic coast, makes the approach of dilution impossible. On the other hand it offers the possibility of regaining lost nutrients. In this paper we present a model for sustainable salmonid cage farming of the Baltic and its tributary utilizing three different eco-services to provide environmental friendly feed sources, of none or low human interest. This model makes a total account of nutrients showing that each ton of produced fish remove 62 kg of N (6 kg P), in the form of contaminated fish (later detoxified) and non-food blue mussels, from the heavily eutrophic Southern Baltic, but only released 39 kg in the artificial oligotrophic environment of Swedish power dams. The remaining protein (42% DW of feed) is obtained from fungi (Rhizopus oryzae) mycelium produced on pulp effluents. In addition to the model will the first data from experiments, including both growths, physiological, digestion, gut histological and immunological responses of Arctic charr fed different mixes of these sources be presented, including effects on gut transport and gut microbial composition.

Keywords: Sustainable; Recycling; New feed sources

* E-mail address: anders.kiessling@umb.no (A.K. Kiessling).
O-069

Influences of low salinity and dietary fatty acids composition on biosynthesis potency of highly unsaturated fatty acids in red sea bream Pagrus major

Md. Al-Amin Sarker\(^*\), Yutaka Haga\(^1\), Md. Shah Alam Sarker\(^1\), Misako Miwa\(^1\), Yoji Yamamoto\(^2\), Goro Yoshizaki\(^1\), Shuichi Satoh\(^1\)

\(^1\)Department of Marine Bioscience, Tokyo University of Marine Science and Technology, 4-5-7 Konan, Minato-ku, Tokyo
\(^2\)School of Aquatic and Fishery Science, University of Washington, 1122 Boat St., Box 355020, Seattle, WA 98105, USA

Abstract

Fish are a vital dietary source of n-3 highly unsaturated fatty acid (HUFA) which is essential to the health of vertebrates. Fatty acid desaturase and elongase are critical enzymes in the biosynthetic pathways of HUFA from \(\alpha\)-linolenic acid (18: 3n-3). Water salinity and dietary fatty acids are suggested to influence HUFA bio-synthesis in marine fish. This study was conducted to investigate the effects of salinity and dietary fatty acid on growth performance, fatty acid composition and fatty acid desaturase and elongase expression in red sea bream (Pagrus major). Two diets were formulated with fish oil (FO) alone (FO diet) and the 67% fish oil substituted with vegetable oil (VO diet). Duplicate groups of red sea bream (51.1±2.1g) were reared in 60L glass tanks for 12 weeks at three different salinities (15ppt, 20ppt and 33ppt). Growth performance and proximate composition were examined after rearing. Fatty acid composition in whole body and liver of fish were analyzed by using a gas-liquid chromatography. Total RNA was isolated from the liver, and cDNA was synthesized by AMV first-stand cDNA synthesis kit. Expression of fatty acid desaturase and elongase in liver was examined by PCR after 12 weeks of feeding trial. There is no significant difference in growth performance and proximate parameters among all treatments. Content of HUFA such as eicosapentaenoic acid (EPA, 22:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) in whole body showed no notable difference in any salinity the fish fed same diet. However, EPA and DHA content were significantly higher in liver of fish at 15ppt salinity in comparison with 33ppt salinity. The EPA and DHA content of fish reared 15ppt were 1.6-1.8 fold higher in liver total lipid compared to fish reared in 33ppt. Fatty acid desaturase transcript was successfully detected by RT-PCR in liver of fish fed VO diet at 15ppt salinity but neither at 20ppt nor 33ppt salinity. Fatty acid elongase gene was also clearly expressed in liver in the all treatments. These results suggested that red sea bream might synthesize HUFA from VO in liver and expression of fatty acid desaturase was stimulated at low salinity.

Key words: Water salinity, Red sea bream; Highly unsaturated fatty acid (HUFA); Desaturase; Elongase

\(^*\)Email address: maa_sarker@yahoo.com (M.A. Sarker)
Effect of within-day feeding strategies on Nile tilapia fry performance: step towards deliver diets vary in nutrients proportion in terms of meals timing

Ashraf Suloma¹, Osama M. El–Husseiny¹, Rania S Mabroke¹*, TAHOUN, A. M²
1Animal Production Department, Faculty of Agriculture, Cairo University, Egypt.
2Animal Production Dept., National Research Center, Cairo, Egypt

Abstract

This study was conducted to investigate if mixed feeding schedule can have benefits in terms of meals timing. We experimentally tested our hypothesis that the low utilization of different nutrients may be due to delivering them at wrong time of day. Experiment was designed to deliver equal amount of nutrients (protein, lipid, carbohydrates) to fish in all treatments in spite of different protein schedules. Fish daily ration was divided into two equal meals (morning (am) and afternoon (af)) to examine the effect of three daily protein schedules on tilapia performance. Three diets (20 % - 30% - 40%) were employed to achieve different protein schedules as follows: (regular feeding A: 30% am - 30% af), (B: 20% am - 40% af) and (C: 40% am - 20% pm). Fish maintained under protein schedule B showed the highest growth performance followed by regular feeding A. The schedule B had the best feed conversion followed by regular feeding A. According to these results feeding tilapia with constant protein diet during the day increase loses of unutilized protein to water which cause economical loses. Results showed that tilapia fish utilize dietary protein more efficiently during afternoon period. More work is needed to detect the right time for presenting the right amount of different nutrients.

Keywords: Within-day; Feeding strategies; Tilapia; Protein

* E-mail address: suloma2001@yahoo.com.
Fish nutrition, health and welfare: An overview of their interrelationships

Delbert M. Gatlin III*
Department of Wildlife and Fisheries Sciences and Faculty of Nutrition, Texas A&M University System, College Station, Texas 77843-2258

Abstract

It is well established that proper nutrition is essential for maintenance of normal growth and health of all animals including various aquatic species. As such, nutritious diets and appropriate feeding regimes play critical roles in intensive aquaculture. In recent years, heightened attention has been given to the development of nutritional strategies and dietary supplements that positively influence immunity and disease resistance of cultured organisms to lessen dependence on chemotherapeutics and reduce the economic losses from disease-related mortality. Increased attention also has been given to the welfare of aquacultured fish which directly relates to the development of integrated strategies to reduce susceptibility to various stressors and diseases, as well as enhance the overall health of fish.

Dietary formulations with certain nutrients included in excess of their minimum required levels for normal growth, as well as supplementation of non-nutritive immunostimulants have become recognized as potential means of enhancing immunocompetence and disease resistance of various aquatic species. A wide variety of non-nutritive immunostimulatory compounds including microbial cells or cellular fractions such as brewer’s yeast, beta-glucans, peptidoglycans, chitin and oligonucleotides have been evaluated with several aquatic species. Probiotics or live microbial diet additives and prebiotics, which are non-digestible components that alter the gastrointestinal tract conditions to promote certain favorable bacteria, also have become more extensively studied with aquatic species in recent years. Various responses of the non-specific (innate) and adaptive immune systems may be up-regulated by these dietary components, leading to enhanced disease resistance.

Specific examples concerning application of these various nutritional strategies to enhance immunocompetence and disease resistance in aquaculture will be presented. Further advancements in these areas should allow nutritional modulation of stress and immune responses to enhance the health and welfare of fish in aquaculture.

Keywords: Nutrition; Health; Immunostimulant; Welfare

*E-mail address: d-gatlin@tamu.edu
A short and medium term study on healthy and sensitized Atlantic salmon, *Salmo salar* L., fed genetically modified maize

J. Gu1*, A.M. Bakke1, N.H. Sissener2, Å. Krogdahl1
1 Aquaculture Protein Centre (a CoE), Department of Aquatic Medicine and Nutrition, Norwegian School of Veterinary Science, P.O. Box 8146 Dep, N-0033 Oslo, Norway
2 National Institute of Seafood and Nutrition Research (NIFES), P.O. Box 2029 Nordnes, N-5817 Bergen, Norway

Abstract

Since the contribution of genetically modified (GM) plant crops such as soybeans and maize to total global harvests are increasing, the safety of GM plants in diets needs to be assessed. Maize and maize gluten are common sources of starch and protein, respectively in commercial diets for fish. The objective of this study was to evaluate the effects of whole meal GM Bt-maize on healthy and sensitized (soybean meal-induced enteritis) Atlantic salmon in the short (30 days) and medium (90 days) term. The post-smolt salmon, approximately 100-125 g initial body weight, were fed fishmeal-based diets containing 1) 20% non-GM maize, 2) 20% GM maize, 3) 20% non-GM maize with 15% extracted soybean meal, or 4) 20% GM maize with 15% extracted soybean meal for 30 days and 90 days, triplicate tanks per diet group. Growth performance, haematology, plasma clinical chemistry, organ weights and histomorphology, and proliferating cell nuclear antigen (PCNA) level in the intestinal tissue were evaluated. With the exception of a soybean meal induced reduction in condition factor (P<0.05) after 30 days of feeding, no significant differences in body weight, body length or condition factor related to diet were detected. For organosomatic indices, no significant differences were caused by GM maize, but decreased relative weights of the pyloric caeca, mid intestine, distal intestine and liver were induced by soybean meal. Neither haematology nor white blood cell counts differed between fish fed the GM maize or non-GM maize diets at either time point, but MCV (mean cell volume) was decreased by soybean meal inclusion at 30 days. No differences in histomorphology of head kidney, spleen, liver, pyloric ceaca or mid intestine were related to any diet. As expected, however, enteritis of distal intestine was caused by soybean meal at both 30 and 90 days. There were no significant differences in plasma clinical chemistry caused by GM maize at either time points, but alkaline phosphatase (P<0.05) was significantly increased and bile acids, cholesterol, and free fatty acids (P<0.01-0.05) were lowered in fish fed the two soybean meal-containing diets at 90 days. Western blot analysis of PCNA content in tissue homogenate, which has been suggested as a non-specific biomarker for toxic exposures, was reduced in the distal intestine (P<0.05) of GM maize compared to non-GM maize fed fish at 30 days but not at 90 days. It was significantly increased (P<0.01) in fish fed the soybean meal-containing diets at both 30 and 90 days. These results indicated that those fish fed soybean meal were successfully sensitized. No adverse effects of GM (Bt) maize have been detected in healthy or sensitized Atlantic salmon in short and medium term. Further analyses are pending.

Keywords: Genetically modified maize, Atlantic salmon, alternative feed ingredients, soybean meal

* E-mail address: jinni.gu@nvh.no (Jinni Gu).
O-073

Continuous light induces bone resorption and affects vertebral morphology in Atlantic salmon (*Salmo salar* L.) fed a phosphorous deficient diet

Per Gunnar Fjelldal¹*, Erik-Jan Lock³, Tom Johnny Hansen¹, Rune Waagbø³, Anna Wargelius², Laura Gil Martens³, Adel El-Mowafi⁴ & Robin Ørnsrud⁴*

¹ Institute of Marine Research (IMR), Matre Aquaculture Research Station, N-5984 Matredal, Norway.
² Institute of Marine Research (IMR), Nordnesgaten 50, N-5005 Bergen, Norway.
³ NIFES, National Institute of Nutrition and Seafood Research, Strandgaten 229, N-5004 Bergen, Norway.
⁴ EWOS Innovation, Dirdal, N-4335 Dirdal, Norway.

Abstract

The aim of the present experiment was to investigate the combined effects of photoperiod and mineral malnutrition on mineral metabolism and development of vertebral bodies in Atlantic salmon post-smolts. An experiment was performed with quadruple groups of salmon reared under 24 h continuous light (LL) or 12 h light/dark (LD) per day, and fed diets with insufficient P (0.4 % available P, LP) or sufficient P (0.8% available P, HP) for 79 days. Plasma profiles of P, Ca, and vitamin D metabolites, along with vertebral bone ALP (marker for osteoblasts) and TRACP activity (marker for osteoclasts), Col XI and MMP13 gene expression and mineral content (% of bone dry weight), and mechanical stiffness and morphology (length / dorso-ventral diameter) of vertebra were measured. After 79 days of feeding, the vertebrae of the LP-LL group was significantly more compressed (lower length/dorso-ventral diameter ratio) than those of the other groups. Continuous light increased plasma 25 (OH)D₃ and bone TRACP activity, and decreased bone Col XI expression. A low P diet increased plasma calcitriol and bone ALP activity, but decreased bone TRACP activity, MMP13 expression, and bone mineral content and stiffness. The results show that combined effects of LP and LL induced morphological changes in the vertebral bodies, which may be related to a reduced bone strength resulting from impaired mineralization by low dietary P, an altered collagen structure (Col XI α 1↓) and increased bone resorption (TRACP↑) imposed by continuous light.

The present work presents novel findings on the effect of photoperiod and dietary P on osteoclast and osteoblast activity, and vitamin D endocrinology.

Keywords: Atlantic salmon, vertebrae, deformities, vitamin D, calcitriol, ALP, TRACP

*E-mail address: robin.ornsrud@nifes.no*
Effects of increasing concentrations of the mycotoxins deoxynivalenol, zearalenone or ochratoxin A in diets for Atlantic salmon (Salmo salar) on growth performance and health

S. Döll1*, G. Baardsen2, P. Möller2, W. Koppe2, I. Stubhaug2 and S. Dänicke1
1Bundesallee 50, 38116 Braunschweig, Germany
2 Skretting Aquaculture Research Centre AS, Sjøhagen 3, 4016 Stavanger, Norway

Abstract

Deoxynivalenol (DON) and zearalenone (ZON) are mycotoxins produced by fungi of the genus Fusarium. Their particular importance arises from the frequent occurrence of toxicological relevant concentrations on grain worldwide. Besides its known effects as protein synthesis inhibitor and its immunomodulatory properties, the presence of high concentrations of DON in feed results in decreased voluntary feed intake and reduced growth especially in pigs. ZON exhibits structural similarities to estrogen, leading to symptoms summarized as hyperestrogenism which can be observed in a species dependent manner. In contrast to the Fusarium toxins, which are produced on field, ochratoxin A (OTA) is produced by so called storage fungi (e.g. Aspergillus ochraceus) and is known as renal toxin. However, little information on the effects of those mycotoxins on performance and health of salmon are available and no evidence based recommendations on maximum concentrations of those toxins in feed for salmon exist. A general recommendation on guidance values of DON in complementary and complete feedingstuffs of 5mg/kg (at 88% dry matter) was given by the European Commission (2006/576/EC). Concerning ZON and OTA only guidance values for feed materials of 2 mg ZON/kg and 0.25mg OTA/kg exist. Therefore a feeding trial was carried out to investigate the effects of those mycotoxins on the performance and health of Atlantic salmon. A total of 36 tanks (1m diameter) with 22 fish each were distributed to 12 feeding groups (3 tanks/diet). Dietary treatments included control group, containing background levels of mycotoxins (0.3mg DON, 0.03mg ZON, 0.0mg OTA/kg), 4 increasing concentrations of DON (0.8 – 3.7 mg/kg), ZON (0.06 – 0.77 mg/kg), and 3 increasing concentrations of OTA (0.09 – 0.33 mg/kg). Animals had a mean initial weight of 405 ± 31g and were fed ad libitum for 15 weeks. At the end of the experimental period 10 fish per tank were sampled for blood plasma for clinical chemistry. From 3 fish per tank organ weights were recorded and tissue samples were prepared for histological examinations. Feeding the diet containing 3.7 mg DON/kg resulted in a significant decrease in feed intake of 20% as compared to control. As the feed conversion ratio in this group was significantly increased by 18% the effect amounted to a 31% decrease of the specific growth rate. In addition, DON-dose dependent alterations of plasma enzymes hint at adverse effects on the liver, which will be validated by histological investigations. Feeding diets containing increasing concentrations of ZON or OTA did not affect feed intake, growth or health parameters. The results of this experiment demonstrate that current recommendations on DON in feed do not prevent adverse effects on performance and health of Atlantic salmon.

* E-mail address: Susanne.Doell@fli.bund.de
Energy evaluation of fish feeds and ingredients: a net energy approach

J.W. Schrama*1, I. Geurden2, L.T.N. Heinsbroek1, J.A.J. Verreth1, S.J. Kaushik2
1. Aquaculture and Fisheries Group, Wageningen University, PO Box 338, 6700 AH Wageningen, The Netherlands
2. INRA, UMR 1067, NUAGE, Nutrition, Aquaculture and Genomics Unit, 64310 St Péé sur Nivelle, France

Abstract

Current and future fish diets are facing increased variability in ingredient composition due to fishmeal and -oil replacement and due to a diversification in available ingredients for fish feeds. Consequently dietary macronutrient content might alter, including the carbohydrate fraction. For pigs, energy evaluation is done on a net-energy (NE) basis. In such systems, maintenance is assumed constant and the potential for energy retention is derived from the amount of digestible protein (DP), digestible fat (Dfat) and digestible carbohydrates (Dcbh) in feeds/ingredients. This study aimed to assess if for fish, the energy retention potential of diet(ingredient) can be estimated from the intake of digestible protein, fat and carbohydrate. A data set was used containing 8 experiments on Nile tilapia, Oreochromis niloticus (in total 68 groups of fish) done at 26-28°C in which digestibility of nutrients as well as complete energy balances were measured. Carbohydrates content of feed and faeces were calculated as nitrogen fat free extract (NFE). Initial body weight, growth rate, retained energy (RE) ranged between 40 to 140 g, 0.7 to 4.0%, and 6 to 328 kJ/kg0.8/d, respectively. Intake of DP, Dfat, Dcbh and digestible ash (Dash) ranged between 2.0 to 12.0 g/kg0.8/d, 0.3 to 5.7 g/kg0.8/d, 0.5 to10.8 g/kg0.8/d, and 0.2 to 0.8 g/kg0.8/d, respectively. The net energy value of digestible nutrients was described by RE = -48.7(±2.64) + 12.2(±0.52)*DP + 36.7(±0.99)*Dfat + 11.9(±0.43)*Dcbh (R²=0.99). Thus the efficiency of energy utilization of digestible nutrients for energy retention were estimated to be 52%, 93% and 68% for respectively DP, Dfat and Dcbh, being slightly lower for DP and Dcbh compared to pigs. The residuals analysis indicated that digestible ash intake negatively affected RE (P<0.05). Dash effects on RE disappeared if a fixed effect of experiment was included into the equation. This fixed effect of experiment (P<0.001) indicates the presence for differences in energy requirements for maintenance between experiments. However, the inclusion of a fixed effect of experiment did not affect the NE estimates of the macronutrients (i.e., the regression coefficients). The results and use of net energy evaluation for fish are discussed in relation to: fish species (data sets on trout and turbot currently under construction); husbandry system; the aspect of variations in maintenance. This study suggests that a net energy evaluation for Nile tilapia, does not strongly differ from applied net energy evaluation systems in pigs.

Keywords: Feed evaluation; Energy metabolism; Comparative aspects; Nutrient utilization.

*E-mail address: Johan.Schrama@wur.nl (Johan Schrama)
Effect of dietary lipid sources on growth performance and immune parameters of Darkbarbel catfish, *Pelteobagrus vachelli*

Xueqin JIANG, Erchao LI, Liqiao CHEN *
School of Life Science, East China Normal University, Shanghai, 200062 China

Abstract

An 80 days trial was conducted to evaluate the effects of dietary lipid sources on growth performance and immune parameters of Darkbarbel catfish, *Pelteobagrus vachelli*. Five isonitrogenous and isoenergetic diets were formulated with different lipid sources including fish oil (FO), soybean oil (SO), lard oil (LO), mixed oil 1 (MO1, FO:SO 1:3) and mixed oil 2 (MO2, FO:SO 3:1), and were fed to juvenile Darkbarbel catfish (average weight 1.02g) twice a day with triplicates for each treatment. The survival rate of each tank was all 100%. The highest weight gain value was found in FO group, and group MO1 lied in the second, followed by MO2, SO, and LO whose weight gain value was the lowest. Condition factor and special growth rate of fish both followed the similar pattern of weight gain. But no significant differences in these three parameters were found in all the treatments (P > 0.05). Compared with diet SO and LO, diet MO2, FO and MO1, within an increasing trend, had significantly enhanced the activities of immune parameters (complement C3, C4 and IgM) (P<0.05), but no significant differences were observed in plasma lysozyme activity (P>0.05). All these findings could suggest that although dietary lipid sources make no differences on the growth performance for Darkbarbel catfish, plasma immune parameters were significantly affected, and a small total replacement of fish oil with vegetable oil could enhance the immune responses of fish.

Keywords: Darkbarbel catfish; growth; immune parameters

*E-mail address: lqchen@bio.ecnu.edu.cn (Liqiao Chen)*
Effect of feeding Atlantic salmon (Salmo salar L.) a diet enriched with stearidonic acid from parr to smolt on growth and n-3 LC-PUFA biosynthesis.

Basseer M. Codabaccus1, 2, Andrew R. Bridle1, Peter D. Nichols2 and Chris G. Carter3
1NCMCRS, AMC, University of Tasmania, Bag 1370, Launceston, TAS 7250 Australia
2CSIRO Food Futures Flagship, Marine and Atmospheric Research, GPO Box 1538, Hobart, TAS 7001, Australia
3Marine Research Laboratories, Tasmanian Aquaculture and Fisheries Institute, University of Tasmania, Private Bag 49, Hobart, TAS 7001, Australia

Abstract

Vegetable oils (VO) have become the predominant substitute for fish oil (FO) in aquafeeds, however, the resultant lower concentrations of (n-3) long-chain (≥C20) polyunsaturated fatty acids [(n-3) LC-PUFA] in fish has put their use under scrutiny. The need to investigate new oil sources exists. This research tested the hypothesis that in Atlantic salmon a high intake of stearidonic acid (SDA) from Echium oil (EO) would result in increased (n-3) LC-PUFA biosynthesis and deposition due to lower competition for ∆6 desaturase. Comparisons were made to diets containing canola oil (CO) and FO for 112 d in freshwater followed by 84 d in seawater after smolting. EO diet resulted in a higher eicosapentaenoic acid (EPA) than CO diet in seawater. Fatty acid mass balance of freshwater fish indicated higher biosynthesis of eicosatetraenoic acid (ETA) and EPA in EO fish compared to fish on the other diets with a 2 fold increase in the biosynthesis of (n-3) LC-PUFA due to dietary SDA. In seawater, biosynthetic activity was markedly reduced in all treatments, but there was higher biosynthesis of ETA in EO fish and appearance of all desaturation and elongation products along the (n-3) pathway. Given the biosynthetic precursors of (n-3) LC-PUFA, present in both CO and EO fish, no additional dietary (n-3) LC-PUFA were required for smoltification. SDA enriched plant oils might be a more suitable FO substitutes than conventional VO due to higher SDA content, higher total (n-3) and improved (n-3)/(n-6) ratio.

Keywords: Echium oil; Stearidonic acid; Atlantic salmon; Biosynthesis; Fatty acid

* E-mail address: mohamedc@utas.edu.au
Estimating dietary lysine requirements for live weight gain and protein deposition in juvenile rainbow trout (*Oncorhynchus mykiss*)

S. Wang\(^1\), P.M. Encarnacao\(^2\), R.L. Payne\(^3\), and D.P. Bureau\(^2\)*

\(^1\) Nutrition Laboratory, Institute of Aquatic Economic Animals, School of Life Sciences, Sun Yat-sen University, Guangzhou 510275, P. R. China

\(^2\) Department of Animal and Poultry Science, University of Guelph, Guelph, ON, N1G 2W1, Canada

\(^3\) Evonik-Degussa Corporation, Kennesaw, GA, 30144, USA

**Abstract**

A 12-week feeding experiment was conducted to study the effects of dietary lysine level on growth and nutrient utilization of juvenile rainbow trout (initial body weight 5.8±0.2 g). Eight isonitrogenous and isoenergetic (50% crude protein, 20MJ digestible energy kg\(^{-1}\)) practical diets were formulated to contain graded levels of lysine ranging from 1.28 to 3.40% of diet (dry matter (DM) basis) in 0.30% increments. Each diet was hand fed to near-satiety to four groups of fish (15 fish/tank) reared 15°C in a recirculation system. Fish were weighed every 28 d to determine weight gain and feed efficiency. Five fish were sampled per tank at the end of the trial for proximate analysis and analysis of amino acid composition. Results were analyzed by ANOVA and different parameters were analyzed using different curve fitting models to examine shape of the response and estimate lysine requirement. Estimate of requirement for the non-linear models was defined as the level for achieving 95% of maximum response.

Live weight gain, feed efficiency (gain:feed), body protein and lipid concentrations (% wet weight), as well as protein and lysine depositions (g/fish) were significantly affected by the dietary lysine concentrations (*P<0.01*). Increasing lysine resulted in increase in weight gain, feed efficiency and body protein concentration and decrease in body lipid concentration. Estimates of lysine requirements (% in diet DM) based on live weight gain using the different fitted curve models were: asymptotic, 2.11; exponential, 2.68; polynomial, 2.23; broken line regression, 2.19 % diet DM. Based on protein deposition, estimates of lysine requirements using the different models were: asymptotic, 2.44; exponential, 3.15; polynomial, 2.41; broken line regression, 2.22. These results strongly suggest that lysine requirement for maximizing protein deposition is higher than that for maximizing live weight gain. This may have significant implications on final product quality and yield. This issue deserves to be examined more closely.

**Keywords:** Diet, Lysine; Requirement; Trout; Growth; Protein; Model

*E-mail address: dbureau@uoguelph.ca (D.P. Bureau).*
Feeding Atlantic salmon a diet based on decontaminated fish oil during a full production cycle; effect on POP levels, long chain omega-3 fatty acids, vitamins, fillet quality and bone deformities.

Erik-Jan Lock1*, Pål A. Olsvik1, Bente E. Torstensen1, Anders Goksøyr2,3, Johan Johansen4, Trygve Sigholt6, Nanne Joerum6, Jan-Vidar Jakobsen7, Rune Waagbo1, Marit Bjørnevik6, Per-Gunnar Fjelldal1, Olav Breck10, Marc H.G. Berntssen1

1 National Institute of Nutrition and Seafood research (NIFES), P.O. Box 2020, Nordnes, 5817, Bergen, Norway
2 Department of Molecular Biology, University of Bergen, Bergen, Norway
3 Department of Biology, University of Bergen, P.O. Box 7800, N-5020, Bergen, Norway
4 Gildesskål, Research Station, GIFAS, Gildeskål, Norway
5 BioMar, 7484 Trondheim, Norway
6 Skretting ARC, Stavanger, Norway
7 EWOS Innovation, N-4335 Dirdal, Norway
8 HBO, Faculty of Biosciences and Aquaculture, 8049 Bodø, Norway
9 Institute of Marine Research (IMR), Matre Aquaculture Research Station, N-5984 Matredal, Norway
10 Marine Harvest Norway AS, P.O. Box 4102, Dreggen, NO - 5835, Bergen, Norway.

Abstract

Oily fish is an important source of health promoting nutrients such as the very long chain omega-3 fatty acids (VLC-n3) and fat soluble vitamins. It is also well known that oily fish may contain potentially hazardous contaminants. For farmed fish, the inclusion of fish oil in the fish feed is the main source of VLC n-3, but it could also contain contaminants dependant on its geographical origin. Decontamination techniques have now been developed that effectively remove persistent organic pollutants (POPs) from fish oils. However, fat soluble vitamins are also partially removed during this process. The aim of the present study was to assess whether the use of decontaminated oils in partial replacement feed would give the same aquacultural performance, fish health and quality when compared to non decontaminated oils. Fillet levels of potentially hazardous POPs and health beneficial VLC n-3 fatty acids in market size fish and early biochemical toxicological markers of dioxin like exposures are determined. Furthermore, vitamin status is recorded to see if the loss by decontamination could be compensated for by adding retinyl acetate and vitamin D3 to the diets. In order to mimic today’s vegetable oil inclusion practice, half of the oil used was rapeseed oil while the other half was fish oil. Atlantic salmon was fed for 18 months on diets based on decontaminated (Group A) or non-treated (Group B) fish oils until market size (~5kg) was reached. The level of known POPs such as dioxins, dioxin-like polychlorinated biphenyls (DL-PCBs), non dioxin-like PCBs, poly brominated diphenyl ethers (PBDE), and organochlorine pesticides (OCP), as well as fatty acid composition and fat soluble vitamins were analysed in fish oils, the two diets, and Atlantic salmon fillet. The development of known production related diseases (fin and skin erosion, bone deformity, cataract) as well as fillet quality parameters (slaughter weight, fillet colour, gaping, texture and sensory) of Atlantic salmon fed the two diets were assessed. The fillet levels of POPs in market size fish were reduced by 68-85% in Group A, while the concentration of very long chain omega 3 was reduced by 4-7%. The equal fillet vitamin A and D levels confirmed the efficacy of the compensatory vitamin supplementation. No differences in biomarkers of dioxin-like component exposures, such as hepatic gene transcription of CYP1A or AhR2B, CYP1A protein expression and 7-ethoxyresorufin O-deethylase (EROD) activity, were observed between the groups. No differences in growth performances or fillet quality parameters between the two groups were found, however a slight decrease of bone deformities was seen in Group A compared to Group B. Atlantic salmon reared on decontaminated feeds had a sum dioxin and DL-PCB concentrations that were comparable with terrestrial food products such as beef, while the marine omega-3 fatty acids level and fat soluble vitamins remained within the levels found in Atlantic salmon in general.

*E-mail address: elo@nifes.no
Lipid peroxidation, protein oxidant and antioxidant status of muscle, intestine and hepatopancreas for juvenile Jian carp (*Cyprinus carpio* var. Jian) fed graded levels of myo-inositol

Weidan Jiang, Lin Feng, Yang Liu, Jun Jiang, Kai Hu, Shuhong Li, Xiaoqiu Zhou, Gangfu Chen

Abstract

Lipid peroxidation, protein oxidant and antioxidant status of muscle, intestine and hepatopancreas in juvenile Jian carp (*Cyprinus carpio* var. Jian) fed graded levels of myo-inositol (MI) (163.5, 232.7, 384.2, 535.8, 687.3, 838.8 and 990.3 mg/kg diet) for 60 days were investigated. Total tissue malondialdehyde (MDA) and protein carbonyl (PC) content showed a downward trend to a point (*P* < 0.05). Conversely, total tissue anti-hydroxyl radical (AHR), catalase (CAT), glutathione-S-transferase (GST), glutathione peroxidase (GPx), glutathione reductase (GR) activities and glutathione (GSH) content were generally higher in MI-supplemented diets than MI-unsupplemented diet (*P* < 0.05). Muscle and intestinal superoxide dismutase (SOD), and intestinal anti-superoxide anion (ASA) were increased by MI supplementation (*P* < 0.05), whereas these parameters in the other tissue showed no alterations (*P* > 0.05). These results indicated that antioxidant status was improved, and lipid peroxidation and protein oxidant were depressed in muscle, intestine and hepatopancreas by MI.

*E-mail address: zhouxq@sicau.edu.cn*
How apparent is the apparent digestibility of plant proteins by a carnivorous marine fish: the use of protein gel analysis to characterize dietary and faecal proteins of turbot, *Psetta maxima*

Leon T.N. Heinsbroek¹*, Cris Wijnen ², Jan Cordewener², Twan America², Ingrid M. van der Meer², Johan W. Schrama¹ and Johan A.J. Verreth¹

¹ Aquaculture and Fisheries Group, Wageningen University, PO Box 338, 6700 AH, Netherlands. ² Plant Research International, Wageningen University, PO Box 16, 6700 AA, Netherlands

Abstract

With the growth of aquaculture and of aquaculture feed production the need for alternative protein sources becomes ever more urgent. A first step to evaluate the potential of alternative protein sources is the determination of the protein (nitrogen) digestibility. As this has to be repeated for the multitude of ingredients and fish species, the objective of the present study was to investigate a more generic method to characterize dietary proteins and how they influence the digestion process.

An experiment was conducted with turbot, *Psetta maxima*, of 150-200g (18°C, 15 ppt). The turbot were fed 6 weeks with a reference diet, containing only fish meal protein (FM), or a test diet, in which 30% of the protein was replaced by one of 11 plant protein sources. The plant protein sources used were soybean meal (SBM), soya protein concentrate (SPC), rapeseed meal (RSM), pea protein concentrate (PePC), sunflowerseed meal (SFM), wheat gluten meal (WGM), conventional wheat distillers grains (DGc), experimental wheat distillers grains (DGe), algae meal (ALG) and potato protein concentrate (PoPC).

Quantitatively, nitrogen digestibility was linked to the nitrogen budget (nitrogen intake, growth and excretion). For a more qualitative characterization of the proteins and the digestion process, ingredients, diets and faeces were analyzed with SDS-PAGE gel electrophoresis with subsequent analysis of some protein bands with LC-QTOF-MS (liquid chromatography coupled to mass spectrometry).

Nitrogen digestibility of the FM diet was 90.2±0.5%. Nitrogen digestibility of the plant proteins varied from 97.7±1.6% (SPC) to 2.4±5.8% (DGe). Growth, nitrogen excretion and oxygen consumption were all strongly correlated with digestible nitrogen intake, indicating that at the used inclusion levels digestibility was limiting nitrogen utilization.

SDS gel electrophoresis of diets and the secreted faeces samples demonstrated that the majority of all proteins from the feed had been digested. The diet samples displayed multiple bands. The pattern of most test diets corresponded with the FM diet, where additional protein bands were visible that corresponded to the most abundant proteins in the test ingredients, e.g. conglycine in SBM and SPC, patatine in PoPC. In the DGc diet only weak additional bands were found, while in the DGe diet no protein bands were found, not even from the 70% FM in this diet. In the faeces samples only a few protein bands appeared on the gels with abundant bands at 15, 18, 23 and 30 kDa, the last one could be identified as trypsine. With each of the faecal samples a strong staining was found in the front of the gel, indicating the presence of a significant amount of peptides with a mass of < 5 kDa. In order to analyse the peptide composition of the faecal samples we have started a detailed quantitative LC-MS approach, which is in progress.

*E-mail address: leon.heinsbroek@wur.nl (L.T.N. Heinsbroek)
Histological and ultrastructural changes in sea bass (*Dicentrarchus labrax*) larvae muscle in response to high dietary content of DHA

Mónica B. Betancor1, Mª José Caballero1, Tibiábin Benítez-Santana1, Reda Saleh1, Eyad Atalah1, Javier Roo1 and Marisol Izquierdo1

1Grupo de Investigación en Acuicultura (GIA), Universidad de Las Palmas de Gran Canaria and Instituto Canario de Ciencias Marinas, P.O. Box 35200 Telde, Canary Islands, Spain

Abstract

It is widely known that highly unsaturated fatty acids (HUFA) are beneficial for fish larvae in terms of growth, survival and resistance to stress. Nevertheless, these fatty acids are highly susceptible to oxidation and so, a higher addition of antioxidants must be included in formulated diets. Vitamin E is considered to be one of the most efficient physiological antioxidant protections for fish being a structural component of cell membranes. In previous studies we observed dystrophic alterations in muscle of 48 day-old sea bass fed imbalanced DHA and vitamin E diets for 2 weeks. In order to better understand the whole pathological process associated to free radicals, a deeper histological study was performed feeding 14 day-old sea bass larvae with microdiets containing different ratios of DHA/vitamin E (1/150, 5/150 and 5/300) for three weeks. Larvae were sampled each week and fixed in 10% buffered formalin or in 2.5% glutaraldehyde 0.2M phosphate buffer. Longitudinal paraffin sections of these larvae were employed to study the incidence of muscle dystrophy among dietary treatments. Larvae fed diet 1/150 showed no lesions in contrast to larvae fed diet 5/150 and 5/300 where the highest incidence of muscle lesions was observed (around 19%). Semithin sections showed that focal lesions consisted of degenerated fibres and an extensive sarcoplasm vacuolization affecting both red and white muscle. Ultrathin sections of these degenerating muscle fibres showed a diffuse dilatation of the sarcoplasmic reticulum, disorganized miofibres and autophagic vacuoles eventually containing myelin figures and dense bodies. Besides, some monocyte - macrophage cells were observed among lesioned fibres as numerous satellite cells. The results of the present study reinforced the conclusions observed in our previous study and demonstrate the pathological potential of free radicals in sea bass larvae musculature, which could not be attenuated by dietary vitamin E. The implication of other nutrients related to cell protection against oxidative stress is being studied at present.

Keywords: Sea bass larvae, oxidative stress, muscle dystrophy, ultrastructure.

*E-mail address: monica.betancor102@estudiantes.ulpgc.es (M.B. Betancor)*
Efficiency of Methionine Utilization of Rainbow Trout (Oncorhynchus mykiss)

Wai Yee Ho 1*, Andreas Lemme 2, Katheline Hua 1, Margaret Quinton 1, Dominique P. Bureau 1
1 Department of Animal and Poultry Science, University of Guelph, 50 Stone Rd. East, Guelph, Ontario, N1G 2W1, Canada
2 Evonik Degussa GmbH, Rodenbacher Chaussee 4, D-63457 Hanau (Wolfgang), Germany

Abstract

Methionine is frequently the first limiting essential amino acid in fish feed formulations. Feeds are commonly supplemented with DL-methionine (DL-Met) to meet requirements. While methionine requirement of a number of fish species has been examined, surprisingly few studies have examined the efficiency of methionine utilization (retention). Information on the efficiency of utilization of amino acids is required to develop reliable factorial models of essential amino acid requirements for fish. In addition, supplemental amino acids are occasionally believed to be poorly utilized by aquatic animals. Direct assessment of the retention by fish of supplemental amino acids would be valuable. The goal of this study was to investigate the efficiency of utilization of DL-Met by rainbow trout fed diets with graded levels of this supplemental amino acid.

A 16-week dose-response growth trial was conducted with rainbow trout reared at 15°C. A practical control diet (34% digestible protein and 21 MJ/kg -1 digestible energy) was formulated to meet all known nutrient requirements of rainbow trout, but be deficient in methionine (0.42% dry diet). Three experimental diets were prepared by supplementing the control diet with graded levels of DL-Met; 0.15%, 0.30%, and 0.45% of diet (dry matter basis). The four (4) experimental diets were hand fed to near-satiety to three groups of 15 juvenile rainbow trout (initial mean weight =5.5g fish⁻¹) reared in a recirculation aquatic system. The fish were sampled at the beginning and at the end of the trial for analysis of their amino acid composition.

Weight gain (g/fish), growth rate expressed as TGC (thermal-unit growth coefficient, g 1/3/degree day), and protein gain (g/fish) increased non-linearly (p<0.05). At marginally deficient methionine concentrations (<0.72% diet), methionine retention (g/fish) was largely a linear function of methionine intake (g/fish). The efficiency of DL-Met utilization was estimated to be about 48%. This value is comparable to the estimate (52%) of Gaylord and Barrows (2009) derived from an experiment involving supplementation of nutritionally deficient diets with multiple essential amino acids. The efficiency of utilization of methionine appears to be lower than that of lysine (ca. 70%, Rodehutscord et al., 2000) in rainbow trout. This suggests that significant amounts of methionine may be diverted toward other important metabolic functions (methyl donor groups, taurine synthesis, etc.) besides protein deposition.

Keywords: Methionine; Trout, Nutrient Utilization, Metabolism, Requirement

* E-mail address: who@uoguelph.ca (W.Y. Ho).
Abstract

In order to study the reason of the difference of the utilization of dietary carbohydrate in grass carp and tilapia, a study was undertaken to detect the glucose tolerance and carbohydrate metabolism in both fish. Results showed that plasma glucose and triglyceride in both fish increased significantly, but the values in tilapia were higher than in grass carp. The time of high plasma glucose and triglyceride levels in grass carp is longer than in tilapia. The time of tilapia returning plasma glucose and triglyceride levels to baseline was faster than grass carp. The activities of GK and PK in tilapia were higher than in grass carp, and the high levels of enzymes lasted longer than grass carp. All the results indicate that glucose tolerance and the ability of carbohydrate metabolism in tilapia are both higher than those in grass carp.

Keywords: Grass carp; Tilapia; Glucose tolerance; Enzymes

* E-mail address: gaokaijian@yahoo.cn
Effects of dietary digestible carbohydrate on growth, blood biochemical indices, glycogens, digestive enzymes and carbohydrate metabolic enzymes of Jade Perch (Scortum barcoo)

Qi Ye¹, Sun Xiang-jun², Liang Yong-jun², Song Li-wei¹, Qiao Xiu-ting¹*
¹ Tianjin Key Laboratory of Aqua-Ecology and Aquaculture, Fishery Science Department, Tianjin Agricultural University, Tianjin 300384, China
² Beijing Fisheries Research Institute, Beijing 100068, China

Abstract:
The Jade Perch (Scortum barcoo) was introduced from Australia which is suitable for many culture model. In order to evaluate its utilization characteristics of carbohydrate, nine iso-protein (36.5% crude protein) and iso-lipid (8% crude lipid) experimental diets which containing different sources of digestible carbohydrate [glucose(A), sucrose(B), dextrin(C) and starch(D) —the four factors] and levels were designed with the L₉(3⁴) orthogonal design. Triplicate groups, each with 35 experimental fishes, and average initial body weights of 77.90 ±3.28g, were fed with one of the nine test diets for 8 weeks in 27 cages. Growth performance, blood biochemical indices (plasma glucose and triglyceride), glycogen, digestive enzymes (amylase and protease) and carbohydrate metabolic enzymes (GK, HK and PK) were examined at the end of the experiment period. The results showed that dietary starch and dextrin were the most important influencing factors to growth performance, plasma glucose, liver glycogen, amylase and carbohydrate metabolic enzyme (HK and PK) of Jade Perch (P<0.05), followed by sucrose and glucose. Growth performance and feed utilization had the best effect when digestible carbohydrate in diets was at the second level, while plasma glucose and triglyceride, liver glycogen, amylase, HK and PK had the maximum content or activity when digestible carbohydrate was at the third level. These results indicated that Jade Perch utilize starch and dextrin better than sucrose and glucose, when orthogonal combination was D₂C₂B₂A₁, that is starch and dextrin at 8%, sucrose at 6%, glucose at 1%, total digestible carbohydrate at 23%, Jade Perch had the best growth performance and feed utilization, while high digestible carbohydrate could cause its related physiological indexes elevation.

Keywords: Jade Perch (Scortum barcoo); Digestible carbohydrate; Growth performance; Orthogonal design

* E-mail address: qxt6510@yahoo.com.cn (Qiao Xiu-ting).
Modelling oil digestibility of Atlantic salmon feeds

Ewos Innovation, N-4335 Dirdal, Norway

Abstract

Oil digestibility in salmonid feeds is known to depend largely on temperature and the proportion of saturated fatty acids of the total fatty acids. However, the best functional form of the models describing these effects or any effects of other potential variables can be considered unclear and uncertain with no strong theoretical foundation. Still, the model choice especially of describing any nonlinear relationships, such as using polynomials, has a major impact and is crucial to the model outcome and its predictive capabilities, i.e. its usefulness in practice. Polynomials, for example, often lead to spurious shapes of effects, for example second-degree polynomial forces a maximum response with subsequent decrease that may not be evident from the data at all, and thus give unrealistic predictions. The model uncertainty, i.e. the uncertainty in choosing the model, is rarely considered in fish nutrition modelling. We present a case study of applying Bayesian model averaging to account for this extra source of variability in the modelling of oil digestibility of Atlantic salmon feeds in seawater. Such models are expected to lead to wider prediction intervals but more realistic predictive coverage. Instead of using one model the outcome will be an average of several, maybe tens of models based on their posterior probabilities. We used data from published literature as well as our own unpublished studies accounting for 155 cases with temperature varying from 4.5 to 15.0°C, the proportion of saturated fatty acids from 12 to 39% of dietary fatty acids and dietary lipid from 20 to 36% of diet. Additional variables, such as proportions of monounsaturated and polyunsaturated fatty acids, dietary protein level and fish weight, were considered as potential predictors or necessary covariates. Saturated fatty acids were further broken down to individual fatty acids C14, C16 and C18. Each predictor was considered as a linear term and as a cubic spline presentation to account for possible nonlinear forms of effects in the potential model space.

*E-mail address: kari.ruohonen@ewos.com (K. Ruohonen)
Strategies to increase highly unsaturated omega 3 fatty acids in rainbow trout fed canola, flax and camelina oils

K. M. Randall1*, M. J. T. Reaney2, M. D. Drew1
1 Department of Animal and Poultry Science, 51 Campus Drive, University of Saskatchewan, Saskatoon, Canada
2 Department of Food and Bioproduct Sciences, 51 Campus Drive, University of Saskatchewan, Saskatoon, Canada

Abstract

The world’s demand for fish oil already exceeds supplies, necessitating the development of a suitable replacement. Previous work in our laboratory showed that the addition of high levels of antioxidants (vitamin E and butylated hydroxytoluene; BHT) to flax oil increased the levels of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in fillets of rainbow trout compared to fish receiving flax oil with no antioxidants. The objective of the present study was to confirm that the use of antioxidants results in increased production of EPA and DHA in rainbow trout fed canola, flax or camelina oils. A 16-week trial was conducted to evaluate lipid changes in rainbow trout fillets. The experiment was a 4 x 2 factorial arrangement of treatments 4 vegetable oils (fish, canola, flax and camelina oils) and 2 levels of antioxidants (none or 7.5 g/kg BHT and 12.5 g/kg Vitamin E). Twenty four tanks of triploid rainbow trout (initial weight 260 g; N = 10 per tank) were randomly assigned to each diet. After completion of the trial, fillets from 2 fish per tank and diets were analyzed for fatty acid composition using gas chromatography. Results indicated that addition of antioxidants to all of the oils increased their oxidative stability indices. The diets containing canola, flax or camelina oils were completely devoid of the long-chain unsaturated fatty acids including arachidonic acid (AA), EPA and DHA. The diets containing fish oil had significant amounts of EPA and DHA. This indicates that the AA, EPA and DHA present in fillets from fish fed the vegetable oil diets are produced from conversion of linoleic (LA) and alpha linolenic acid (ALA) present in canola, flax and camelina oils. The levels of EPA and DHA in fillets from fish fed the vegetable oil diets were significantly lower (P < 0.05) than in the fillets from fish fed the fish oil diet. However, the addition of antioxidants to the oils resulted in a significant increase in DHA in trout fillets. The level of EPA was also increased by the addition of antioxidants although the increase was not significant (P > 0.05). These results suggest addition of antioxidants may increase the production of EPA and DHA in rainbow trout fed diets containing vegetable oils replacing fish oil.

Keywords: Rainbow trout; Vegetable oils; Antioxidants; Eicosapentaenoic acid; Docosahexaenoic acid

* E-mail address: kyla.randall@usask.ca (K. M. Randall)
O-088

Quantifying the dietary biotin requirement and its effect on growth, survival, welfare, and reproduction of zebrafish *Danio rerio*

Rodrigue Yossa Nouaga*, Pallab K. Sarker¹, Donald M. Mock² and Grant W. Vandenberg¹

¹Groupe de Recherche en Recyclage Biologique et Aquiculture, Département des Sciences Animales, Université Laval, Québec, QC, G1V0A6, Canada
²Department of Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, 4301 W. Markham Street Slot 516, Little Rock, AR 72205, USA

Abstract

The effect of increasing supplementation of biotin in diet was studied in order to determine the dietary biotin requirement for growth, survival, welfare, and breeding of zebrafish *Danio rerio*. Juvenile zebrafish (0.1358 ± 0.0014 g) were fed six purified diets containing 0, 0.01, 0.1, 0.5, 1.5, and 2.5 mg biotin kg⁻¹ diet, in triplicate tanks for 12 weeks. Fish fed the control diet (no biotin) showed (P<0.05) lower weight gain, SGR, and PER than the fish fed the diets supplemented with biotin. The highest (P<0.05) weight gain, SGR, and PER was obtained with the feed containing 0.5 mg biotin kg⁻¹ diet, followed by 1.5, 2.5, 0.1 and 0.01 mg biotin kg⁻¹ diet. Liver glycogen was highest (P<0.05) with the diets containing 0.1, 0.5, 1.5, 2.5 mg biotin kg⁻¹ diet, followed by 0.01 mg biotin kg⁻¹ diet, and lower with the control diet. Despite the severe biotin deficiency signs such as retarded growth, anorexia and convulsion recorded on fish receiving the control diet and 0.01 mg biotin kg⁻¹ diet, there was not significant difference in survival among the treatments; mean = 94%. The broken-line analysis showed that the optimum dietary biotin requirements for maximal weight gain, PER and FCR are 0.227, 0.323 and 0.200 mg kg⁻¹ diet, respectively. The highest (P<0.05) fish fecundity was obtained with 2.5 mg biotin kg⁻¹ diet, and the lowest with 0.01 mg biotin kg⁻¹ diet. The highest (P<0.05) wet weight of eggs was obtained with 0.5 mg biotin kg⁻¹ diet, followed by 1.5, 0.1 and 2.5 mg biotin kg⁻¹ diet, and the lowest with 0.01 mg biotin kg⁻¹ diet. The highest (P<0.05) number of living larvae at 7 dpf was obtained with 0.01, 0.1 and 2.5 mg biotin kg⁻¹ diet, followed by 1.5 mg biotin kg⁻¹ diet, and the lowest with 0.5 mg biotin kg⁻¹ diet. This study provides evidence that the dietary biotin requirement of zebrafish for maximum growth and optimum welfare is about 0.23 mg biotin kg⁻¹ diet but that the effects of biotin deficiency on reproduction are complex and require further study.

Keywords: zebrafish; biotin; dietary requirement; growth; survival; welfare; reproduction

*Email address: rodrigue.yossa-nouaga.1@ulaval.ca*
O-089

Effects of dietary copper sources and levels on performance, plasma antioxidant activities and relative copper bioavailability in *Carassius auratus gibelio*

Xian-ping Shao 1,2*, Wen-bin Liu 1*, Chao Liang 3, Kang-le Lu 1, Wei Xia 1, Yang-yang Jiang 1

1 Laboratory of Aquatic Nutrition and Ecology, College of Animal Science and Technology, Nanjing Agricultural University, Nanjing 210095, P R China; 2 Fisheries College, Nanjing Agricultural University, Wuxi 214081, P R China; 3 Heritage Dongjiang Weiyingyang Additives Co., Ltd, Shenzhen 518057, P R China.

Abstract

The experiment was conducted to determine the effects of dietary copper sources and levels on performance and plasma antioxidant activities in crucian carp, *Carassius auratus gibelio*, as well as the bioavailability of copper (Cu) from tribasic copper chloride (TBCC) or copper amino acid relative to copper sulphate when supplemented to crucian carp. Two hundred and eighty crucian carps (18±1 g average body weight) were randomly assigned to 10 treatments. Treatments consisted of 0, 3, 6 or 9 mg supplemental Cu kg⁻¹ diet from TBCC, copper amino or copper sulphate. Growth performance and plasma antioxidant activities were analyzed after a 55-day period of feeding. Weight gain were higher in fish fed diets supplemented with 3~6 mg Cu kg⁻¹ than those fed with 9 mg Cu kg⁻¹ from TBCC or copper amino acid; while fish consuming 6~9 mg Cu kg⁻¹ from copper sulphate got higher weight gain than those fed with this copper of 3 mg kg⁻¹ (*p*<0.05). FCR in fish consuming diet supplemented with 3 mg Cu kg⁻¹ from TBCC was lower than those consuming the same copper concentration from either copper amino acid or copper sulphate. Fish fed diets supplemented with 6 mg Cu kg⁻¹ got higher SGR than those fed diet with no supplementation of copper (*p*<0.01). No obvious differences of PER and hepatosomatic index were found in fish fed diets supplemented with 6 mg Cu kg⁻¹ among different copper sources. Fish consuming diet supplemented with 6 mg Cu kg⁻¹ from TBCC had the highest condition factor among the ten treatments. Plasma ceruloplasmin and CuZnSOD activities were higher in fish fed diet with 9 mg Cu kg⁻¹ than those consuming other diets. Plasma GSH-Px was highest in fish fed the diet with 3 mg Cu kg⁻¹ from copper sulphate, second highest in fish fed the diet with 6 mg Cu kg⁻¹ from TBCC, and then in fish fed the diet with 9 mg Cu kg⁻¹ from copper amino acid. Bioavailability of Cu from TBCC and copper amino acid, relative to copper sulphate, were estimated from plasma ceruloplasmin using single linear regression and a slope ratio technique. Compared with Cu sulphate (1.00), relative bioavailabilities of Cu from TBCC and copper amino acid were 1.15 and 1.05 based on plasma ceruloplasmin, respectively. This study showed that TBCC is more bioavailable to crucian carps than copper sulphate.

Keywords: Tribasic copper chloride (TBCC); copper amino acid; copper sulfate; *Carassius auratus gibelio*

*E-mail address: njaushxp@yahoo.com.cn (X.P. Shao); wbliu@njau.edu.cn (W.B. Liu)
Feed and feeding affect quality and safety of aquaculture products, with emphasis on salmonids

Rune Waagbø1*, Bente E. Torstensen1, Lise Madsen1 & Marc H.G. Berntssen1
1NIFES, PO Box 2029 Nordnes, N-5817 Bergen, Norway

Abstract

The global seafood production from aquaculture now exceeds that from wild catches, illustrating the increasing importance of aquaculture in the future supply of seafood. With limitations in the global marine ingredient pool, a steady increase in production volume in aquaculture of 8-10% per year may be challenged depending on available sustainable alternatives of plant and land animal origin. Measures on sustainable aquafeeds are often given by the public term “net production of fish” (“fish in-fish out”) with main focus on production. However, feed and feeding also affect fish welfare and the seafood product. Increased use of non-marine feed ingredients will alter the seafood composition of both nutrients and contaminants. Health benefits of traditionally fish meal and fish oil farmed salmonids are well acknowledged by the authorities and consumers, predominantly because they represent a rich source of marine n-3 long chain polyunsaturated fatty acids (PUFA). Fish oil based aquafeeds, however, is also a main source of fat soluble environmental pollutants such as dioxins, PCBs and organochlorine pesticides, while fish meal is the main source for elements. The use of vegetable oils is known to lower the load of these persistent organic pollutants (POPs) in both feed and farmed fish, but also introduce new contaminants normally not associated with farmed fish, such as poly aromatic hydrocarbons and mycotoxins. Using plant ingredient in aquafeeds will also lower nutrients associated with seafood, such as vitamin D, selenium, selected B-vitamins, as well as non-essential nutrients such as cholesterol, thus introducing new uncertainties for product quality aspects. Marine n-3 PUFA:s from fish, including farmed Atlantic salmon, represents 86% of the total n-3 intake in a modern Western diet. A general reduction of the n-3 PUFA:s and elevated levels of 18:2n-6 fatty acids in farmed salmonids by use of plant based diets represent a more unfavorable dietary n-3/n-6 ratio. On the other hand, benefits include reduced levels of marine contaminants. The overall health effects of consumption of oily fish such as Atlantic salmon are therefore a trade-off between beneficial and potentially harmful compounds mediated through the dietary regimes. It may be discussed how this change affects the development life style disorders in a longer perspective. In future, new modeling tools will assist the aqua industry to produce seafood according to markets preferences and scientifically based advices on beneficial health effects. This includes tailoring farmed products by use of finishing diets (n-3 fatty acids, micronutrients) and reducing or eliminating contaminants through decontamination of fish oils.

Keywords: seafood, quality, safety, POPs, vitamins

* E-mail address: rwa@nifes.no (R. Waagbø)
O-091


Magny S. Thomassen1*, Mari Gaarder1, Turid Mørkøre2, Kjell-Arne Rørvik2, Eva Veiseth-Kent3, Ragni Ofstad3, Diane Bahuaud1

1 Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, P.O.Box 5003, 1432 Aas-UMB, Norway
2 NOFIMA, P.O.Box 5010, 1432 Aas, Norway
3 NOFIMA, Osloveien 1, 1432 Aas, Norway

Abstract

Soft texture and muscle gaping are among the most severe quality problems in production of farmed Atlantic salmon. As in meat tenderization, endogenous proteases are believed to play a significant role also in fish muscle degradation. The objective of the present studies has been to learn more about the significance of lysosomal cathepsins and the calpain/calpastatin systems in farmed Atlantic salmon muscle degradation. Effects of dietary n-6 fatty acids from rapeseed oil and very high levels of n-3 fatty acids (EPA and DHA) on cathepsin and muscle integrity were studied. Total replacement of fish oil by rapeseed oil did not lead to any significant changes on muscle integrity (myofibre-myofibre or myofibre-myocommata detachments), cathepsin B or L total activities or gene expressions. Very high levels of n-3 fatty acids in the diet did, however, result in a higher percentage of myofibre-myofibre detachments already 0h post mortem, and higher cathepsin B gene expression. Pre-slaughter long-term crowding stress (24 h) was further found to give increased cathepsin B+L activities, enhanced myofibre-myofibre and myofibre-myocommata detachments and muscle softening. Muscle pH was significantly correlated to myofibre-myofibre and myofibre-myocommata detachments, fillet firmness and cathepsin B+L total activity. In a second feeding trial, the combination of pre-slaughter stress and changes in diet composition was studied. Diets with the addition of a bioactive fatty acid (Tetradecylthioacetic acid – TTA) or boosts of free amino acids (Arginin and Glutamate) were used. In pre-slaughter stressed salmon these dietary changes led to reduced cathepsin B activity. Effects on muscle integrity (histology) are presently being investigated, and results will be presented. The calpain/calpastatin system also seem to be involved in the softening of salmon muscle, as observed in a study on muscle texture in different Atlantic salmon families. In summary, our results strongly indicate a significant influence of these protease systems in salmon muscle softening, and have revealed effects of diet composition, pre-slaughter stress as well as the family background of the salmon.

Keywords: Atlantic salmon; rapeseed oil; EPA; DHA; TTA; amino acids; stress; cathepsin; calpain; calpastatin; muscle quality

*E-mail address: magnth@umb.no*
O-092

Evaluation of sensory quality indices and freshness assessment of Nile tilapia O. niloticus fed recycled food waste material

Gabriel Gana Bake¹, Masato Endo¹, Atsushi Akimoto², Naoko Hamada-Sato*³, Toshio Takeuchi*¹
¹. Department of Marine Biosciences, Tokyo University of Marine Science and Technology, 4-5-7 Konan, Minato, Tokyo 108-8477, Japan.
² Nippon Formula Feed Mfg. Co., Ltd. 3-9-13 Moriya, Yokohama, Kanagawa 221-0022, Japan
³. Course of Safety Management in Food Supply Chain, Tokyo University of Marine Science and Technology, 4-5-7 Konan, Minato, Tokyo 108-8477, Japan

Abstract

The use of recycled food waste material in aquaculture as an ingredient in fish diet is a recent development brought about by the advancement of technology. In our previous study, we established the suitability of recycled food waste materials from food industry waste (FIW) and soy sauce waste (SSW) as an ingredient in the diet of Nile tilapia Oreochromis niloticus. This study was designed to evaluate the effects of inclusion of recycled food waste materials from food industry waste (FIW) and soy sauce waste (SSW) on the sensory quality indices, freshness assessment and body composition of tilapia using K values, microbial viable cell count and electric nose as the evaluation tools. Five experimental diets were formulated at 0 and 20-22% inclusion level of recycled food wastes. The diets were designated as D1: 0% of recycled food waste, D2: 20% inclusion of FIW, D3: 20% inclusion of FIW and SSW, D4: 20% inclusion of FIW and tryptophan, and D5: 22% inclusion of SSW. The formulated diets were fed to tilapia from the onset of exogenous feeding for 32 weeks. Fishes used for this experiment range from 95 – 210 g in body weight.

The result from the body composition shows that D1 had higher carcass protein, while D3 had the highest lipid and there was no significant difference in the carcass moisture and ash contents among all treatments. Except for D4 that was slightly higher than the control, the microbial viable cell counts for other treatments were lower than the control. There was a gradual linear increase of the K value in all the treatments. D3 had the highest K value while D5 had the lowest. However, there was no significant difference in the fish fed the experimental diets based on the smell test conducted using the electric nose.

From the result of this study, we concluded that using 20% inclusion of recycled food waste materials (FIW and SSW) in the diet of tilapia had no negative effect on the flesh of the fish; hence, recycled food waste could be a good alternative ingredient to aquaculture.

*E-mail address: gabbygana@yahoo.co.uk
O-093

Effects on performance and product quality in Atlantic salmon fed diets reduced in organic pollutants

Jan Olli1, Bente Ruyter2, Turid Mørkøre2, Harald Breivik3, Aimo Oikari5, Aleksei Krasnov2, Olav Thorstad4, Åshild Krogdahl6, Gunnar Berge8 and Torbjørn Åsgård7*

1 AVS Chile SA, Casilla 300, Puerto Varas, Chile
2 The Norwegian Institute of Food, Fisheries and Aquaculture Research (Nofima), P.O. Box 5010, NO-1432 Ås, Norway
3 Neperdo™ Biomarine, Porsgrunn, Norway
4 Pronova, BioPharma P.O Box 2109, NO-3202 Sandefjord, Norway
5 University of Jyväskylä, P.O.Box 35, FIN-40014 Jyväskylän yliopistoy
6 Norwegian School of Veterinary Science, Oslo, Norway.
7 Pronova, BioPharma P.O.Box 420, 1327 Lysaker, Norway.
8 The Norwegian Institute of Food, Fisheries and Aquaculture Research (Nofima), NO-6600 Sunndalsøra, Norway

Abstract

Oily fish, including Atlantic salmon, are due to their content of omega-3 fatty acids, considered healthy for human consumption. However, marine raw materials used in fish diets are considered a major source of persistent organic environmental pollutants (POPs). Advice regarding fish consumption has therefore been complicated by reports that some species are burdened with potentially harmful levels of POPs, such as dioxins, PCBs and brominated flame retardants (BFRs). Despite the rather low levels of environmental pollutants found in farmed Atlantic salmon, there is still some concern about the levels found. One should therefore aim at reducing the level of POPs in aquacultured fish, in order to strengthen the “healthy image” of the product. As a way to reduce these POPs in farmed fish, Pronova

BioPharma Norge AS has developed a short path distillation process using a volatile working fluid efficiently removing POPs from fish oils. Potential beneficial effects in the farmed fish from reducing the POPs in the feed oil, were tested in a study lasting from post smolt size to slaughter size at Gildeskål Research Station (GIFAS), Norway. The fish were given feeds containing purified or not purified fish oil, four replicates per treatment. The effects on growth, health and fillet quality were studied. There was a tendency for better growth and feed utilisation in the group receiving feed containing purified oil. This was particularly the case during periods of high growth when both the growth rate and feed utilisation rate were significantly better for the fish that received the feed containing purified oil. The salmon that received the feed containing purified oil appeared to better tackle the handling stress during the slaughtering process, measured as a delayed pH reduction after killing. Other stress markers showed the same tendencies. All the fish in the trial maintained a good red colour and firmness, but there was a tendency that fish that received the feed containing purified oil had a firmer texture. The difference in texture was most pronounced after freezing. The proportion of salmon with soft texture was then halved in comparison to the fish that received unpurified oil.

Keywords: Lipid purification; Atlantic salmon; Growth; Health; Stress; Fillet texture.

* E-mail address: torbjorn.asgard@nofima.no
Toxicological, physiological responses and residue of aflatoxin B1 in tilapia (Oreochromis niloticus × O. aureus)

Deng Shi-xi*, Tian Li-xia, Liu Fu-jia, Jin Sheng-jie, Liang Gui-ying, Liu Yong-jian
Fish nutrition & safety laboratory, Institute of Aquatic Economic Animals, School of Life Science, Sun Yat-sen University, Guangzhou 510275, China

Abstract
Currently, the increasing use of aflatoxin-contaminated plant ingredients in feeds creates the economic and health risks to farm animals and human due to the cumulative effects of aflatoxin B1 (AFB1) and its metabolites in animal tissues after AFB1 exposure. In the present study, the toxicological, physiological responses and residue of AFB1 were compared in control tilapia (Oreochromis niloticus × O. aureus) and tilapia given to 85, 245, 638, 793 and 1641 μg AFB1/kg of feed for 20 weeks. The AFB1 was obtained from mildewed peanut meal fermented by Aspergillus flavus (NRRL 3357). The results showed that the gradual aflatoxicosis effects on tilapia were in a dose- and duration-dependent manner. A diet with 245 μg AFB1/kg of feed or higher doses could reduce the growth performance of tilapia significantly (P < 0.05) for 20 weeks, compared to the control group. Tilapia given to 638 μg AFB1/kg of feed or higher doses exhibited lower levers of the plasma total proteins and albumin concentrations, and liver cytochrome P450 1A ethoxyresorufin-o-deethylase (EROD) activity than the control tilapia. Liver histopathological damage, such as eosinophilic materials accumulation, increased inflammation and vacuolar degeneration, were observed in last 3 groups. Residual AFB1 was cumulated in the liver of tilapia after exposure, while it was undetectable in muscle. We concluded that AFB1 in diets could affect tilapia health, though tilapia could sustain as high as 1641 μg AFB1/kg of feed for 20 weeks without residual AFB1 in edible tissues.

Keywords: Aflatoxin B1; Tilapia; Responses; Residue; Long-term

* E-mail address: edls@mail.sysu.edu.cn
Dietary enzymes in aquaculture

Viviane Verlhac Trichet* & Ester Santigosai Culi
DSM Nutritional Products France, Animal Nutrition & Health Research Centre, BP 170, 68305 Saint-Louis, France

Abstract
The increasing use of alternative protein sources and new ingredients in aquaculture has lead to even more relevance of dietary enzymes such as phytases, carbohydrases and also proteases in the industry. This presentation will review the current knowledge on the effect of dietary enzymes in cold and warm water fish as well as in marine and fresh water fish, considering the different enzyme categories including lipases whose effect have been very seldom studied. It has been clearly demonstrated that the optimization of aquaculture diets using vegetable protein sources requires the use of phytase which hydrolyses plant phosphorus (phytate) making it available to the fish thereby maximizing performance while at the same time minimizing phosphorus excretion into the environment. For the other enzyme categories with less specific substrate, it has been shown that they enhanced nutrient such as protein and lipid digestibility in some cases. Enhancement of energy digestibility has also been demonstrated in several studies. Those studies involved different enzymes either single or multi-enzymes products. Nutrient retention, performance and feed conversion ratio have also been evaluated in several studies in relation to feeding dietary enzymes to various fish species of commercial interest. Results are variable depending on application conditions, feeding duration, dose and nature of the enzyme and also depending on the fish species studied. There is still quite a lot of research needed: besides phytase, the mechanism of action of the other enzyme categories are not well known and strongly depends on the nature of the substrate involved. Therefore it is also of great importance to characterize the different substrates in order to better identify the potential of the different enzymes. Defining the physical characteristics (pH, temperature, stability) of the enzyme in order to adjust to feed processing conditions as well as fish intestinal conditions are also of importance. Nevertheless, some studies already demonstrate the potential of enzyme application in aquaculture feed to reduce water pollution by improving nutrient retention and reducing discharge in the environment.

Keywords: dietary enzymes, carbohydrase, protease, phytase, fish, plant protein

* E-mail address: viviane.verlhac@dsm.com (V. Verlhac Trichet).
Applications of DVAQ® in aquaculture

Fanyi Meng
Diamond V Mills, Inc., Cedar Rapids, Iowa, USA

Abstract

Due to a number of food-related incidents outbreaks worldwide, consumers have become increasingly concerned about the food safety. Accordingly, the safety of feed additives is more and more important. Diamond V Yeast Culture® is a fermented complex product (Diamond V Mills, Inc., Cedar Rapids, Iowa, USA) specifically developed for animal feeds, which is a safe, all-natural feed ingredients with a pleasing aroma and two years self life. It shares a unique role in animal nutrition, largely centering on balance populations or activities of the bacteria that colonize the intestinal of animals. It also helps optimize digestibility of animal feeds. DVYC® is already widely used in animal feeds. Benefits of DVYC® in terrestrial animals include improving nutrient digestibility, utilization and animal growth performance, and enhancing animal’s immune functions and anti-stress capacity. DVAQ® is one product of DVYC family exclusively manufactured for aquatic animal diets, which has excellent sensory characteristics and the particle size. In recent years, the effectiveness of DVAQ® has been demonstrated in different species of aquatic animals, such as white shrimp (Litopenaeus vannamei), Chinese mitten crab (Eriocheir sinensis), channel catfish (Ictalurus punctatus), Wuchang bream (Megalobrama amblycephala), Japanese flounder (Paralichthys olivaceus) and turbot (Scophthalmus maximus). This review provides an overview of DVAQ® applications in aquaculture. Studies has shown that the aquatic animals fed with DVAQ® diets showed significantly higher growth performance and lower feed conversion ratio than the animals fed basal diets. The supplementation with DVAQ® also showed considerable improvement in the immune functions and intestinal histology of different aquatic animals. In addition, the environmental quality of white shrimp farms was also improved by feeding diets with DVAQ®. Future studies should be conducted to examine these effects and their mechanisms of DVAQ® in more variety of aquatic animals.

Keywords: Yeast culture; DVAQ®; Aquaculture; Aquatic animals

*E-mail address: mengfy@diamondv.com.cn
Effects of different supplementation levels of extracted soy peptide on growth performance and tolerance to high temperature stress in juvenile Japanese flounder, *Paralichthys olivaceus*

Janice A. Ragaza¹*, Saichiro Yokoyama², Manabu Ishikawa², Shunsuke Koshio²

¹ Food Function and Nutrigenomics Laboratory, Faculty of Agriculture, Kagoshima University, Kagoshima, Japan
² Laboratory of Aquatic Animal Nutrition, Faculty of Fisheries, Kagoshima University, Kagoshima, Japan

Abstract

The experiment was designed to examine whether different supplementation levels of extracted soy peptide (SPEP) to soy protein concentrate (SPC) diet could improve growth performance, amino acid body composition, and tolerance to high temperature stress in juvenile Japanese flounder (*Paralichthys olivaceus*). Four isonitrogenous and isolipidic SPC diets and one fishmeal (FM) diet (positive control D1) were prepared with increasing SPEP supplementation levels of 0% (D2), 2% (D3), 5% (D4), and 10% (D5), respectively. Triplicate groups of twenty juveniles (1.29±0.01 g, mean ± SD) were stocked in 100-L polypropylene tanks and were fed twice a day to apparent satiation level for 42 days. The present study demonstrated that fish fed D5 grew faster than those fed diets 2, 3, and 4, respectively, although growth was significantly lower in fish fed D5 than that in fish fed D1. The feed conversion ratio and protein efficiency ratio showed the same trend as that of growth performance, but feed intake was not significantly (P > 0.05) different among diets. Except for some amino acids, whole body total and free amino acid compositions of flounder were not altered by SPEP supplementation in the diets. In the lethal heat stress test, fish fed D5 demonstrated the highest value of LT₅₀, which was significantly (P < 0.05) longer than that of fish fed other diets. Furthermore, LT₅₀ values of fish fed diets D3 and D4 were significantly (P < 0.05) longer than fish fed D1. Heat shock protein 70 family (HSP70s) was highest in the gill, liver, and skin of fish fed D5, which was significantly (P < 0.05) higher than that of fish fed other diets after sub-lethal stress exposure. Moreover, HSP70s values of fish fed D3 and D4 were significantly (P < 0.05) higher than fish fed basal and control diets. Time-course sub-lethal heat shock exposure results demonstrated that level of HSP70s significantly (P < 0.05) decreased among all groups during recovery period, but did not return to normal and initial state after 24 hours. Based on the overall performance of the fish, SPEP can be efficiently absorbed and utilized by flounders, but more than 10% supplementation might be needed to catch up the performance of fishmeal based diet in the condition applied in this study.

Keywords: Soy peptide; Japanese flounder; Heat shock proteins; Median lethal time; Heat stress

* E-mail address: janice_a_ragaza@yahoo.com
Effects of dietary prebiotics on intestinal morphology and digestive enzyme activities of juvenile hybrid striped bass, *Morone chrysops* x *M. saxatilis* and red drum, *Sciaenops ocellatus*.

J. Alejandro Buentello*1,2, Camilo Pohlenz1, Maritza Anguiano1, and Delbert Gatlin III1,2

1Department of Wildlife and Fisheries Sciences and 2Intercollegiate Faculty of Nutrition. Texas A&M University System, College Station, Texas 77843-2258, USA.

Abstract

Two separate feeding trials were conducted to evaluate the effects of including commercially available prebiotics in the diets of juvenile hybrid striped bass (HSB) and red drum (RD). The basal diet in both trials was formulated to contain 40% crude protein, 10% lipid, and 3.4 kcal estimated digestible energy/g, and no supplemental prebiotics to serve as the negative control. Three commercially available prebiotics [fructooligosaccharide (FOS), Bio-MOS®, containing mannanoligosaccharide (MOS), and transgalactooligosaccharide (TOS)] were incorporated at 1% of diet; whereas, GroBiotic®-A was provided at 1 and 2% in test diets at the expense of cellulose. The experiments were conducted according to established procedures in 110-L aquaria maintained indoors in a climate-controlled laboratory. Each diet was fed to apparent satiation twice per day to three replicate groups of 15 juvenile fish. The average initial weight was 10.9 ± 0.2 and 5.1 ± 0.3/fish for RD and HSB, respectively. Both trials continued for 8 weeks. At weeks 0, 4 and 8 three randomly selected fish from each aquarium were anesthetized, measured for total length, weighed and their gastrointestinal (GI) tracts dissected, measured for length and preserved by either flash freezing in liquid nitrogen or immersing in 10% neutral buffered formalin. Formalin-preserved GI tracts were dissected into anterior, middle and distal sections and subsequently submitted for standard histological preparation and examination including paraffin embedding, thin sectioning (3-5 µm), glass mounting and hematoxylin and eosin staining. For each intestinal sample, measurements (µm) of fold length (FL) and width (FW), enterocyte height (EH) and microvilli height (MH) were recorded. Frozen intestinal samples were freeze dried and homogenized in cold 50–mM Tris–HCl buffer. After centrifugation, supernatants were utilized for spectrophotometric analysis of pepsin, trypsin, chymotrypsin, aminopeptidase, α–amylase, lipase and both acid and alkaline phosphatases. Results of the histological evaluation indicate that the inclusion of prebiotics at 1% of diet was adequate to elicit structural changes in the GI tract of both species. Increases in FL, FW, EH and MH were more pronounced in the anterior (proximal) intestine for both RD and HSB. The most significant changes were produced by GroBiotic®-A and MOS supplementation. However, the inclusion of prebiotics at 1% of diet did not induce changes in the activities of the selected enzymes. Therefore, prebiotics appear to be preferentially used in the proximal intestine and enhanced structure and function of the GI tract mucosa.

Keywords: Prebiotics; Intestinal structure; Digestive enzymes; Red drum; Hybrid striped bass

*E-mail address*: abuentello@tamu.edu, cpholenz@neo.tamu.edu, chicamay09@yahoo.com, d-gatlin@tamu.edu
O-099

Effects of supplemental enzymes on apparent nutrient digestibility in rainbow trout (Oncorhynchus mykiss) fed plant-based diets

Johanne Dalsgaard¹, Niels Harthøj Hjermitslev², Kim Schøn Ekmann², Viviane Verlhac³, Per Bovbjerg Pedersen¹, Mikkel Klausen⁴

¹Technical University of Denmark, DTU Aqua, Section for Aquaculture, The North Sea Research Centre, P.O. Box 101, DK-9850 Hirtshals, Denmark
²BioMar A/S, Mylius Erichsensvej 35, DK-7330 Brønde, Denmark
³Aquaculture Research Group, Animal Nutrition & Health Research – CRNA, DSM Nutritional Products, BP 170, 68305 Saint-Louis cedex, France
⁴Novozymes A/S, Krogshoejvej 36, DK-2880 Bagsvaerd, Denmark

Abstract

Exogenous enzymes are widely applied in feed for monogastric animals including pigs and poultry as a means to increase the nutritional value of viscous grains by reducing the anti-nutritional effects of primarily non-starch polysaccharides (NSPs). In comparison, there is very limited information on the effects of enzymes in fish feed apart from phytase. Phytase works by hydrolyzing phytic acid, and numerous studies have documented that phytase supplementation increases phosphorus availability in fish fed diets with high inclusion levels of plant proteins. Plant derived proteins are increasingly used in fish feed due to growing demands for and high price variations in fish meal, but high inclusion levels in diets for carnivorous fish are hampered by a great variety of anti-nutritional factors (ANFs), which reduce nutrient utilisation. Exogenous dietary enzymes may potentially help to alleviate these effects, and the objective of the present study was to evaluate the effects of supplementing protease and pectinase to a diet containing approximately 30% soybean meal, rapeseed meal or sunflower meal on nutrient digestibility in juvenile rainbow trout (Oncorhynchus mykiss). Digestibility trials were carried out using a modified Guelph set-up. Rainbow trout were fed the un-supplemented diets and enzyme supplemented plant-based diets in triplicates for three weeks. While moderate effects of the enzymes on nutrient digestibility were obtained with sunflower and rapeseed meal based diets, both enzymes significantly increased (P < 0.05) the apparent digestibility coefficients (ADCs) of protein, lipid and nitrogen-free extract (NFE) in soybean meal based diets. Hence, the study provided preliminary results on the potential of protease and pectinase to increase the nutritional value of proteinaceous feed ingredients for fish. However, it also reinforces the complexity of using exogenous dietary enzymes and that more research is required to better understand the mechanisms of actions.

Keywords: Digestibility; Pectinase (RONOZYME® VP); Protease (RONOZYME® ProAct); Plant protein; Rainbow trout

* E-mail address: jtd@aqua.dtu.dk
Supplementation of Methionine with 2-Hydroxy-4-Methylthio-butanoic Acid (HMTBA) in Low Fish Meal Diets for Fish and Shrimp

Anant S. Bharadwaj*, Jesus A. Venero, Hong Cao, Andrew F. Giesen, Mercedes Vazquez-Añon, and Craig L. Browdy
Novus International, Inc., 20 Research Park Drive, St. Charles, MO 63304 USA

Abstract

A significant proportion of global fish meal production is presently being used in fish and shrimp diets. Concerns over fluctuating costs, increasing demand, limits to expansion of the industry, and future sustainability have prompted efforts to reduce fish meal use in dietary formulations. Plant proteins are abundantly available, relatively inexpensive and have been viewed as ideal for fish meal and cost reduction in feeds for aquatic animals. However, plant meals may be deficient in one or more amino acids and one such area of concern is methionine which often tends to be limiting in low fish meal diets.

Supplementation with crystalline amino acids has enabled fish meal extension with plant meals without much loss in performance and productivity. However, their use may be limited in some aquatic feeds due to losses by leaching, especially when fed to slow-feeding animals such as shrimp, flatfish, and sturgeon. Different strategies such as coating, micro-encapsulation, and binding to intact proteins have been employed to reduce leaching losses but their widespread use is constrained by cost and limited commercial availability.

An alternative compound that has been used successfully as a methionine supplement is 2-hydroxy-4-methylthio-butanoic acid (HMTBa). Two molecules of HMTBa are chelated to a calcium ion resulting in a water stable form of methionine activity that is less prone to leaching losses and suitable for inclusion in aquaculture feeds. This analog of methionine is enzymatically convertible to L-methionine and the enzymes for these processes are found throughout the animal kingdom having been isolated from the tissues of several species of fish and shrimp.

Several studies have demonstrated that HMTBa can be utilized as a source of methionine for fish and shrimp. Furthermore, the lower leaching of this methionine source from feeds ensures that it is available to culture species and not lost to the environment. This presentation will provide an update of research demonstrating the use of HMTBa in the diets of aquatic animals. Research has demonstrated that HMTBa can be used effectively in reducing fish meal in feed formulations allowing for the reduction of feed costs without sacrificing performance.

Keywords: Methionine source; HMTBa; Fish and shrimp; Fish meal and cost reduction

* E-mail address: anant.bharadwaj@novusint.com
Effect of increasing dietary doses of the lupin alkaloid sparteine on growth performance, body composition and histology of Rainbow Trout (Oncorhynchus mykiss).

Edison Serrano1,3*, Trond Storebakken1, Aliro Borquez3, Michael Penn2, Karl D. Shearer1, H. Patricio Dantagnan3 and Liv Torunn Mydland1.

1Aquaculture Protein Centre, Centre of Excellence, Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, Aas, Norway.
2Aquaculture Protein Centre, Centre of Excellence, Department of Basic Sciences and Aquatic Medicine, Norwegian School of Veterinary Science, Oslo, Norway.
3Escuela de Acuicultura, Universidad Católica de Temuco, Temuco, Chile.

Abstract

This study evaluated the effects of an alkaloid commonly occurring in lupins, sparteine, on growth performance, organ histology and body composition in rainbow trout. Eight fish meal based extruded diets with increasing concentration of sparteine alkaloid (0, 50, 100, 250, 500, 1000, 2500 and 5000 mg/kg of diet) were fed to triplicate groups of fish (initial body weight of 61 g) for 62 days. Survival was not affected by the dietary treatments. Weight gain, feed conversion efficiency and feed intake decreased in a quadratic mode (P<0.0001) for sparteine levels above 100 mg/kg of diet, although no effects were observed at the lowest inclusion levels (≤100 mg/kg of diet). Whole body composition showed a quadratic depletion in lipid and dry matter; whereas the protein and ash content was not affected. No evidence of any tissue alteration attributable to sparteine alkaloid intake was found in liver, kidney, spleen, middle and distal intestine. Nevertheless, organ somatic indices were affected by the dietary treatments. At sparteine levels above 1000 mg/kg of diet, the weight of intestines and the liver relative to body weight decreased, whereas the weight of the spleen increased. However, these effects were probably associated with low feed intake and starvation, rather than dietary intake of sparteine. These results indicate that dietary sparteine alkaloid primarily reduce palatability and does not impair the health of the fish. Thus, due to the bitter taste of sparteine, the level should be kept below 100 mg/kg in salmonid diets to avoid a reduction in feed intake.

Keywords: Rainbow trout; Alkaloids; Sparteine; Growth performance; Histopathology; Body composition

*E-mail address: edison.serrano@umb.no
Use of probiotics in beluga (*Huso huso*) and Persian sturgeon (*Acipenser persicus*) aquaculture. Which bacteria *Lactobacillus curvatus* or *Leuconostoc mesenteroides* should we choose?

Fatemeh Askarian¹², Armin Kousha²³, Wahida Salma², Einar Ringø²*  
¹ Islamic Azad University, Savadkooh Branch, Mazandaran, Iran.  
² Norwegian College of Fishery Science, Faculty of Biosciences, Fisheries and Economics, University of Tromsø, Norway.  
³ Islamic Azad University, Ghaemshahr Branch, Iran.

Abstract  
Chemotherapeutic agents have been banned for disease management in aquaculture systems due to the emergence of antibiotic resistance gene and enduring residual effects in the environments. Instead, microbial interventions in sustainable aquaculture have been proposed, and among them, the most popular and practical approach is the use of probiotics. The aims of the present study was to investigate the effect of two lactic acid bacteria (LAB), *Lactobacillus curvatus* and *Leuconostoc mesenteroides*, originally isolated from gastrointestinal (GI) tract of beluga (*Huso huso*) and Persian sturgeon (*Acipenser persicus*), respectively, on growth, survival and digestive enzymes (amylase, lipase and protease) activity and the population level of LAB in the GI tract of sturgeon. Thirty 20-L fiberglass tanks supplied with fresh water for each sturgeon species were used. The treatments included 10 different groups; control, separate supplements of *L. curvatus* and *Leu. mesenteroides* at three different counts (2×10⁹, 5×10⁹ and 9×10⁹ colony forming units [CFU] gram⁻¹ food) and three combinations of the LAB (2×10⁹ + 2×10⁹, 5×10⁹ + 5×10⁹ and 9×10⁹ + 2×10⁹ CFU gram⁻¹ food). The bacteria used in the present study were added in lyophilized form to chopped Chironomidae. In the beluga study, highest growth rate, survival and improved protease, amylase and lipase activity was noted in the rearing group fed 9×10⁹ *L. curvatus* gram⁻¹ food. In Persian sturgeon the inclusion level of 2×10⁹ *Leu. mesenteroides* had similar positive effect on growth rate, survival and enzyme activities. Lowest growth rate, survival and reduced protease, amylase and lipase activity in beluga was observed by using 9×10⁹ *Leu. mesenteroides* while in Persian sturgeon inclusion of 9×10⁹ *L. curvatus* had the most visible negative effect on enzyme activity. The ability of LAB to colonise the digestive tract seems to involve host specificity and our bacteriological results is highly relevant for future probiotic studies in sturgeon. However, future studies are needed and future directions will be discussed.

Keywords: Probiotics; Sturgeon; Growth; Digestive enzymes; Lactic acid bacteria

* E-mail address: Einar.Ringo@uit.no
Differing effects of saponin supplementation to plant meals in formulated feeds for Atlantic salmon

Michael H. Penn1*, Elvis M. Chikwati1, Fredrik Venold1, Junior Madibana Molatelo1, Ellen Hage1, Anne Marie Bakke1, Jens Rohloff2, Arne Guttvik3, Marie Hillestad3 and Åshild Krogdahl1

1 Aquaculture Protein Centre (a CoE), Department of Basic Sciences and Aquatic Medicine, Norwegian School of Veterinary Science, P.O. Box 8146 Dep, NO-0033 Oslo, Norway
2 Plant Biocentre, Department of Biology, Norwegian University of Science and Technology, NO-7491 Trondheim, Norway
3 BioMar AS, Nordre gt 11, NO-7011 Trondheim, Norway

Abstract

Dietary full-fat or solvent extracted soybean meal causes dose dependent enteritis in the intestine of Atlantic salmon (Salmo salar L.), though the causative agent(s) have not been conclusively identified. Saponins are believed to be involved, but it appears additional component(s) are required. Peas may also cause a similar condition but at higher diet inclusion levels. This study investigated the effects of supplementing soy saponins to diets containing selected plant meals on growth and digestive function in Atlantic salmon.

An 80 day feeding trial was conducted using post smolt Atlantic salmon (mean weight 270 g). Twelve diets were formulated to a DP/DE ratio (digestible protein (g/kg)/ digestible energy (MJ/kg)) of 20, dietary protein and energy varying according to the nutrient densities of the fish meal replacers. Five plant protein sources (corn gluten, CG; pea protein concentrate, PPC; sunflower meal, SFM; rapeseed meal, RSM; and horse bean, HB) were used at high inclusion levels with (+S) or without saponin supplementation (2g kg⁻¹). Two additional diets containing wheat gluten with saponin supplementation (WG+S), and soybean meal (SBM) without saponin supplementation were included in the experiment. Fish were sampled after 28 days for histology and after 80 days for growth, nutrient digestibilities, intestinal histology, brush border enzyme (BBE) activity and trypsin activity in digesta.

All groups doubled in weight over the experimental period except RSM diet groups. The PPC+S group experienced significantly lower growth compared to the PPC group. As expected, marked distal intestine histological changes as well as increased trypsin and reduced BBE activities were observed in SBM fed fish. Histological changes were also observed in the PPC fed fish, which worsened with saponin supplementation. PPC+S fed fish also had markedly reduced BBE and increased trypsin activities in the distal intestine. No significant effects of saponin supplementation were noted in the other groups. Additional growth and nutrient digestibility results are presented elsewhere (see abstract by Venold et al.).

This work corroborates previous findings on the negative effects of high PPC inclusion in feeds for Atlantic salmon and provides additional evidence for the involvement of saponins. The results also suggest that saponins differ in their effects when combined with different plant meals. Caution must be used when including high levels of certain plant meals in formulated diets for Atlantic salmon, or when combining meals depending on the source and the presence of saponins in various meals.

Keywords: soybean meal, pea protein concentrate, Atlantic salmon, enteritis

*E-mail address: michael.penn@nvh.no
Histidine supplementation to plant protein used diet improves growth and freshness of muscle in yellow tail *Seriola quinqueradiata*

Saichiro Yokoyama1*, Yoshinori Sotoyama1, Toshihiro Nawata2, Manabu Ishikawa1, Shunsuke Koshio1

1 Laboratory of Aquatic Animal Nutrition, Faculty of Fisheries, Kagoshima University, 4-50-20 Shimoarata, Kagoshima 890-0056, Japan
2 Kyowa Hakko Bio Co., Ltd. 1-6-1 Ohtemachi, Chiyoda-ku, Tokyo 100-8185 Japan

Abstract

Requirements of essential amino acid (EAA) in marine finish are altered by difference of dietary protein sources. If plant protein source such as defatted soybean meal is used as alternative dietary protein source, it may bring to shortage of dietary EAA for marine finish. Supplementation of some EAA to plant protein used diet has been known to improve growth performance and health status of marine finfish. Histidine (His) is believed to be EAA of most marine species, however, effect of His supplementation with plant protein source on growth performance in marine finfish has not been researched. In addition, His is known to have buffering ability in muscle, closely related to freshness of edible part in fish after sacrificed. Therefore, we evaluated the efficacy of dietary His supplementation on growth performance and freshness of muscle in juvenile yellow tail *Seriola quinqueradiata*. Test diets were formulated to contain 20% soybean protein, and supplemented four levels (0, 0.5, 1.5 and 2.0 % in dry diet) of histidine hydrochloride. Feeding trial was conducted using the test diets for yellow tail (3.6 g) for 42 days. After the feeding trial, growth performances (percent weight gain, specific growth rate, feed intake and feed efficiency ratio) were calculated. Blood chemical constituents were determined as health parameter of test fish. Chemical compositions such as crude protein, crude lipid, crude ash and amino acid compositions of test fish were also measured. To grasp changes in freshness of edible part, test fish were sacrificed after the feeding trial and muscle K-value and pH were measured in time courses during 72h storage under constant condition. After the 42 days feeding trial, fish fed diets with His supplementation showed significantly (p<0.05) higher percent weight gain, specific growth rate and feed efficiency ratio compared to those in fish fed diet without His supplementation. Blood triglyceride (TG) content tended to decrease with increasing His supplementation, while these TG contents were within the normal range usually observed in this fish species. His content of whole body from His 2.0 supplemented group was slightly higher than that of another groups. Muscle pH did not show marked change during the storage. Lower K-value was observed in muscle from His supplemented group at 72h compared to that from fish fed diet without His supplementation. These results suggest that His supplement improves growth performance and freshness of yellow tail fed plant protein used diet.

Keywords: Histidine; Yellow tail; Essential amino acid; Growth performance; freshness

*E-mail address: yokoyama@fish.kagoshima-u.ac.jp (S. Yokoyama).
Effect of dietary crystalline or microcapsule amino acid supplementation on growth and plasma total free amino acids of common carp *Cyprinus carpio*

Leng Xiang-jun1*, Luo Yun-xian1, Xie Jun2, Li Xiao-qin1, Wu Xiao-feng1
1Ministry of Education Key Laboratory of Exploration and Utilization of Aquatic Genetic Resources, Shanghai Ocean University, Shanghai 201306, China;
2Pearl River Fishery Research Institute, Chinese Academic of Fishery Sciences, Guangzhou 510380

Abstract
The present experiment was conducted to investigate the effect of crystalline or microcapsule lysine and methionine supplementation into practical diets on growth performance, and the total plasma free amino acids of common carp *Cyprinus carpio*. Four diets were prepared, which were high fish meal (FM) diet with 15% FM in first half and 10% FM in second half, low FM diet with 10% FM in first half and 5% FM in second half, low FM diet added with crystalline or microcapsule lysine and methionine, which had the same lysine and methionine content as high FM diet respectively. Common carp (5.3±0.5g) were fed the four diets with triplicate for 8 weeks. Growth rates(GR) of the four groups were 929.7%, 831.6%, 853.4%, 903.7%, feed coefficient(FC) were 1.21, 1.34, 1.30, 1.24 respectively. Compared with low FM group, adding crystalline lysine and methionine in diet had no significant effects on growth performance, but the growth rate was improved by 8.67% and FC decreased by 0.10 \((P<0.05)\) by adding microcapsule lysine and methionine in diet. Fish fed on low FM added with microcapsule lysine and methionine in diet. Plasma total free amino acids (TFAA) concentration at 0, 1, 2, 3, 4, 5h after feeding were determined, and the highest concentration of TFAA occurred at 3h after feeding for high FM diet, low FM diet added without or with microcapsule lysine and methionine, but absorbing peak occurred at 2h after feeding for low FM diet added with crystalline lysine and methionine, which means the absorbing peak was advanced. Leaching loss of crystalline lysine and methionine were 69.8% and 53.1%, microcapsule lysine and methionine were 19.8% and 2.6%, which indicated leaching loss of crystalline amino acid was significantly decreased by microcapsuling. Results above showed that growth rate of common carp could be improved, FC decreased by adding microcapsule lysine and methionine in low FM practical diet, but not affected by crystalline lysine and methionine.

Keywords: common carp; crystalline amino acid; microcapsule amino acid; growth; total plasma free amino acids

* E-mail address: xjleng@shou.edu.cn (X.J. Leng).
POSTERS

PRESENTED AT THE

14th INTERNATIONAL SYMPOSIUM ON
FISH NUTRITION & FEEDING

MAY 31--JUNE 4, 2010

QINGDAO, CHINA
Replacement of wheat flour by macaroni wastes in diets of rainbow trout, *Oncorhynchus mykiss*

A. Mirza Aghazadeh¹*, M.S. Yousefi², R. Malekzadeh Viayeh³

1. Department of Animal Sciences, Faculty of Agriculture, Urmia University, Urmia- Iran
2. Iranian Fisheries Organization, Fatemi Ave. Tehran - Iran
3. Artemia and Aquatic Animals Research Institute, Urmia University, Urmia-Iran

Abstract

The objective of this study was to evaluate the effect of total and partial substitution of dietary wheat flour (WF) by macaroni wastes (MW) on growth and feed conversion ratio (FCR) of rainbow trout, *Oncorhynchus mykiss*. Four iso-nitrogenous and iso-energetic diets replacing 0, 5, 10 and 15 percent WF by MW were formulated. Each diet was randomly allocated to 4 groups of fish in 12 cages and each cage was stocked with 25 fish (initial weight 125 ± 3g). Fish were fed twice daily for 7 weeks. The water temperature ranged from 17 to 19°C and dissolved oxygen concentration was measured as 7 – 7.9 ppm during the experimental period. There was no mortality over the course of the study. The results of the present study show that partial or total substitution of WF with MW in diets of the fish does not affect growth and feed efficiency. Although there were no significant differences (p>0.05) in body weight gain (BWG) and feed efficiency among all treatments, but numerically, the lowest (219.43g/fish) and highest (228.39g/fish) weight gain were found with fish fed 15% MW and 10% MW diets, respectively. After 7 weeks of rearing, when the fish reached a size of around 219 grams, the lowest (1.29) and highest (1.43) feed efficiencies were in fish fed 15% WF (control) and 10% MW diets, respectively. Similar results were also obtained for specific growth rate (SGR) data, that means, there were no significant differences in SGR between the control fish and that of fish fed the test diets. However, unlike BWG and FCR, the lowest (1.099) and highest (1.55) SGR were in the fish fed 15% WF and 5% MW diets. It is concluded that replacing up to 15% of WF with MW in the feeds of rainbow trout until the size of 228.39g has no marked effect on its growth performance.

* E-mail address: a.aghazadeh@urmia.ac.ir.
Use of maggot meal for substitution of fish meal in rainbow trout diets

Feng Lu1*, Shuichi Satoh1, Yutaka Haga1, Toshifumi Wakayama2, Koichi Yamaguchi2, Taro Akiyama2

1 Laboratory of Fish Nutrition, Department of Marine Biosciences, Tokyo University of Marine Science and Technology, Tokyo 108-8477, Japan
2 BBB corporation, Japan

Abstract

In aquaculture fish meal is using as a major ingredient for protein source in the world. Many feed ingredients have been investigated in an attempt to replace fish meal in the fish diets. Maggot meal also has high protein content and other biological value can be used as a potential protein source. Maggot, the larva of housefly (Musca domestica) is used for preparing maggot meal. This study aimed to investigate the effect of partial or total substitution of maggot meal on the growth performance of rainbow trout. Low fish meal diet (15% anchovy meal, 26% defatted soybean meal, and 15% corn gluten meal) was arranged as control diet, and fish meal was substituted at the rates of 33, 66, 100% with maggot meal, and soybean meal and corn gluten meal were also replaced partially with maggot meal. Fish oil was also formulated properly in order to meet essential fatty acid requirement. Twenty five fish with a mean initial weight of 28.1g were stocked in 14 tanks with duplicate per treatment, and fed the experimental diets for 12 weeks. The fish grew very well in all treatment and no mortality was observed. Especially, the fish fed the diet where soybean meal and corn gluten meal were substituted with maggot meal showed the best growth performance, and partial replacement of fish meal with maggot meal also showed better performance than that on control diet. Even total substitution of fish meal with maggot meal did not show any adverse effect on growth performance. The result of the experiment demonstrated that maggot meal might be good ingredient for substitution of fish meal for rainbow trout if essential fatty acid is satisfied in the feed.

Keywords: maggot meal, growth performance, rainbow trout, fish meal

*E-mail address: lf_6219@yahoo.com
Isolation and Screening of Tannin or Phytic Acid Degrading Microbes

Youqing Miao, Qinghui Ai, Kangsen Mai, Wei Xu, Wenbing Zhang, Xiaojie Wang, Zhiguo Liu

1The Key Laboratory of Mariculture (Ministry Education of China), Ocean University of China, Qingdao 266003, P.R. China

Abstract

Studies were conducted to isolate and screen tannin or phytic acid degrading microbes. One hundred and twenty-two purified strains of microbes (33 strains of bacteria, 54 strains of yeasts and 31 strains of moulds) were isolated from soil and rotten fruits using enrichment-culture technique. After screening and purification, 11 strains of tannin-degrading microbes were totally obtained using the method of selective media, including 2 strains of bacteria, 1 strain of yeast and 8 strains of moulds. Thirty-seven strains of phytic acid degrading microbes were obtained with another selective medium, including 10 strains of bacteria, 23 strains of yeasts and 4 strains of moulds. Based on the measurement and composition of the diameters of the colonies and hydrolyzed zone, moulds are found to possess stronger enzyme producing ability than bacteria and yeasts. Among these potential microbes, a strain of mould labeled C-3-3-orange produced the largest clear zone of hydrolyzing tannin acid, with a diameter of 4.99 cm length, which demonstrates that this mould may have a strong capacity of tannin-degrading enzyme producing. Results of the present study also showed that microbes capable of degrading phytic acid have a wider distribution in the environment than tannin-degrading microbes.

Key words: Tannin; Phytic acid; Tannase; Phytase; Biodegradation; Screening
Effect of soybean meal replacement by cottonseed meal on growth, hematology, expression and activity of antioxidant enzymes of grass carp (Ctenopharyngodon idellus)

Qingmei Zheng1,2, Xiaobo Wen1*, Chunyan Han1,2
1 College of Animal Science, South China Agricultural University, Guangzhou, China, 510642
2 College of Life Science, Jiaying University, Meizhou, China, 514011

Abstract
The present study was designed to evaluate replacing solvent-extracted soybean meal (SBM) with solvent-extracted cottonseed meal (CSM), affecting fish growth and health of juvenile grass carp in an 8-week trial. To evaluate the health impact, selected stress- and immune-response biomarkers were quantified at enzyme activity along with gene transcriptional (mRNA) abundance. To determine growth response, hematology, expression and activities of antioxidant enzymes, four diets containing 0%, 16.6%, 32.7% and 48.9% CSM as replacements of 0%, 35%, 68% and 100% of SBM on an equal nitrogen basis (containing ~35% crude protein) were fed to grass carp (initial body weight 7.14±0.75 g fish⁻¹) in triplicate aquaria twice daily for apparent satiation. The results indicated that CSM could replace 35% SBM in diets for juvenile grass carp. Fish which were fed diet containing 16.6% CSM as a replacement of 35% of SBM had no affected weight gain (WG), feed efficiency ratio (FER) or feed conversion ratio (FCR) (P>0.05), but had significant decrease in WG, and PER and increased FCR(P<0.05) as a result of the replacement up to 68%. Values for red blood cell count (RBC), hematocrit (Ht) and hemoglobin (Hb) were significantly affected by dietary levels of CSM. Fish which were fed diet containing 16.6% CSM as a replacement of 35% of SBM had significantly improved Ht and Hb (P<0.05), but total replacement of SBM by 48.9% CSM significantly decreased RBC, Ht, Hb (P<0.05). There were diet-related differences in expression and activities of antioxidant enzymes. The enzyme activities of CAT, GSH-Px and total SOD were significantly increased (P<0.05) when dietary level of CSM was improved from 16.6% to 48.9%. The activities of enzymes of fish fed the highest levels of dietary CSM were similar to that of the control. The content of MDA was also significantly increased as the replacement of SBM with CSM was increased from 35 to 100%. Total replacement of SBM by 48.9% CSM, an increase in GPx and CAT mRNA parallels the increase in the activities of the enzymes compared with low CSM groups (CSM35). In contrast however, an increase in Cu–Zn SOD gene expression (in group CSM35) lead to a decrease in enzyme activity which suggests a role for post-translational modification in altering the activity of Cu–Zn SOD. The results indicate that juvenile grass carp was sensitive to either gossypol toxicity in CSM or nutrition factor in SBM. Gossypol or other compounds present in high proportions of either CSM or SBM in diets may result in fish with high oxidative stress. This status likely due to alteration of activity or changing expression of genes with antioxidant-properties enzymes. In conclusion, juvenile grass carp fed diets containing high level of CSM (32.7.0% and 48.9%) as replacement of SBM showed changes in growth, hematology, gene expression and activities of antioxidant enzymes.

Keywords: grass carp; Ctenopharyngodon idellus; soybean meal; cottonseed meal; growth; hematology; gene expression; antioxidant enzymes

E-mail: address:wenxbo@scau.edu.cn
Effect of replacement of fish meal by soybean meal in the diet on growth of red-claw crayfish (*Cherax quadricarinatus*)

Ye Jinyun¹, Pan Qian², Chen Jianming², Shen Binqian², Wang Youhui², Zhang Xueshu³

¹Huzhou Normal University, Huzhou, Zhejiang 313000, China
²Zhejiang Institute of Freshwater Fisheries, Huzhou 313001, China
³Zhejiang Ocean University, Zhoushan 316004, China

Abstract:

A 60-day laboratory growth trial was conducted with red-claw crayfish (*Cherax quadricarinatus*) to determine the amount of fish meal protein that could be substituted by soybean meal protein in formulated crayfish diets without reducing growth. Crayfish received 34% crude protein (as fed), isocaloric diets in which protein was supplied by soybean meal, fish meal or an isonitrogenous mixture of soybean and fish meal calculated to provide graded levels of each protein source. Dietary protein was provided as: T1) 100% fish protein; T2) 20% soy protein: 80% fish protein; T3) 50% soy protein: 50% fish protein; T4) 80% soy protein: 20% fish protein; T5) 100% soy protein. Results of this study indicate that a dietary protein ratio of 20% soybean protein to 80% fish protein was optimum for red-claw crayfish *Cherax quadricarinatus* fed a 34% crude protein diet under laboratory-culture conditions. Survival and body composition (MOI, CP, EE, ASH) of crayfish were not significantly different among all the treatments.

Keywords: *Cherax quadricarinatus*, Fish meal, Soybean meal, Weight gain, SGR
**P-006**

**Effect of oregano essential oil (**Origanum heracleoticum L.)** on intestinal bacterial flora of channel catfish (**Ictalurus punctatus**)

Zonglin Zheng 1, 2*, Lizhi Jin 3, Kaiyu Wang 1, Xinghua Zhou 2, Xiao Xiang 2

1School of Veterinary Medicine of Sichuan Agriculture University, Sichuan 625014, P. R. China
2Fisheries Breeding and Healthy Cultivation Research Centre, South-west University, Chongqing 402460, P. R. China
3Meriden (Guangzhou) Biotech Co. Ltd., Guangzhou 510080, P. R. China

**Abstract:**
Oregano is a member of the Labiatae family of plants, indigenous to the Mediterranean region. The bacteriostatic effects found in oregano are due to its high content of phenolic compounds, particularly carvacrol. Carvacrol and thymol are the two main active components of oregano essential oil (OEO). In this study, the effect of carvacrol and thymol were evaluated in combination and in their natural composition as OEO. A total of 3 treatments, i.e., negative control group, combination of carvacrol and thymol and Orego-Stim® (OS, commercial product containing natural OEO from *Origanum heracleoticum L.*) were added to the diets of channel catfish (**Ictalurus punctatus**) to investigate the effects of the respective treatments on the growth performance and intestinal bacterial flora. Fishes were fed for eight weeks. Results showed that the addition of Car+Thy or OS in fish diets significantly decreased the numbers of intestinal aerobic and anaerobic bacteria, but a remarkable phenomenon was the significant decrease in numbers of harmful intestinal bacteria such as *Enterobacteriaceae, Aeromonas* and *Bacteroides*; and a significant increase in beneficial intestinal bacteria such as *Bifidobacterium, Lactobacillus* and *Bacillus*.

**Keywords:** Channel catfish (**Ictalurus punctatus**); *Origanum heracleoticum L.* extract; intestinal bacterial flora

*E-mail address: zhengzonglin@126.com*
P-007

Effects of Raffinose on Growth Performance and Physiological Effects of Grass Carp (*Ctenopharyngodon idellus*)

Qiu Yan¹, Ye Yuan-tu¹*, Cai Chun-fang¹, Ma Hong¹, Xiao shun-ying¹, Dai Xiao-fang¹, Gu Dong²

¹ Medical College of Soochow University, Suzhou, 215123, China
² Beijing China Cotton-unis Bioscience, LTD, Beijing, 100044, China

Abstract:

(Objective) The aim of this experiment was to investigate the effects of raffinose on growth performance and physiological effects for grass carp. (Methods) The 288 grass carp with an average body weight of 69.51 ± 6.29 g were randomly allocated into 6 groups with 3 replicates of 16 fish. The control group was fed with the basal diet, while trial groups were fed with the basal diet added with 200 mg/kg, 400 mg/kg, 800 mg/kg, 1200 mg/kg, 2000 mg/kg raffinose respectively. The experiment lasted for 70 days. (Results) The results showed that: compared with the control group, (1) Specific growth rate (SGR) and feed conversion ratio (FCR) were improved by adding different quantities of raffinose, and the group of 1200 mg/kg was increased significantly (P<0.05), and the fat deposition rate (FDR) was also increased significantly (P<0.05), except the group of 400 mg/kg. Protein deposition rate (PDR) and feed conversion ratio (FDR) of the group 1200 mg/kg were the highest. (2) Triglyceride (TG) was significantly improved (P<0.05), and high-density lipoprotein (HDL), low-density lipoprotein (LDL) were decreased significantly (P<0.05). (Conclusion) In conclusion, ① Raffinose can improve the growth performance by promoting absorption and utilization of grass carp and promote the absorption of the fat through economizing the protein. ② Raffinose can play a significant role in grass carp by regulating lipid metabolism.

Key words: Raffinose; Growth performance; Blood biochemical indices; Grass Carp

*E-mail address: yeyuant@pub.sz.jsinfo.net
Apple seed and pumpkin seed on growth performance and part of the physiological, biochemical indices in *Megalobrama amblycephala*

Dai Xiao-fang¹, Ye Yuan-tu¹*, Cai Chun-fang¹, Jin Su-ya¹, Xiao Shun-ying¹, Xiang Chao-lin¹, Hu Xian-qiong¹

¹Preclinical Medicine and Biological Science College of Soochow University, Suzhou 215123, China.

Abstract:

(Abjective) This experiment was conducted to investigate the feasible of apple seed and pumpkin seed as lipid materims for feed. (Method) Triplicate groups of fish (initial weight, 14.2±1.5 g) were hand fed visual satiety one of ten diets containing 1.5% soybean oil, 5% apple seed, 10% apple seed, 15% apple seed, 4% white pumpkin seed, 8% white pumpkin seed, 12% white pumpkin seed, 2.8% black pumpkin seed, 5.5% pumpkin seed, and 20% pumpkin seed for 60 days in flush-out aquarium system. The results showed that: (Results) (1) There was no significant difference in growth performance compared with the 1.5% soybean oil group, but additive amount have a certain influence on the growth performance. The result showed that with the increase of dietary apple seed levels, the specific growth rates (SGR) decreased, feed conversion ratios (FCR) increased. Compared with the group of 1.5% soybean oil, the group of 15% apple seed SGR decreased by 12.94% (P<0.05), FCR increased by 18.99% (P<0.05). With the increase of dietary white pumpkin seed levels, the specific growth rates (SGR) increased, feed conversion ratios (FCR) decreased. With the increase of dietary black pumpkin seed levels, the specific growth rates (SGR) decreased, feed conversion ratios (FCR) increased. (2) The serum glutamic - pyruvic transaminase (GPT) and glutamic - oxaloacetic transaminase (GOT) activities were influenced but within normal limits, the liver is healthy. (3) The three kinds of oilseeds can increase mucus, serum total superoxide dismutase (T-SOD), lysozyme (LSZ) activities. (4) Compared with soybean oil, the oilseeds can increase the total cholesterol (CHO) level, the ratio of high-density lipoprotein cholesterol (HDL-C) and serum low-density lipoprotein cholesterol (LDL-C) (HDL / LDL) (P>0.05). White pumpkin seeds and black pumpkin seeds can reduce the level of LDL-C (P>0.05). (Conclusion) These results indicated that: (1) Apple seed, white pumpkin seed, black pumpkin seed as lipid materims for feed is feasible. With the supplement of apple seed at 5% level, white pumpkin seed at 12% level, black pumpkin seed at 2.8% level the growth performance of *Megalobrama amblycephala* were better than 1.5% soybean oil group. (2) Apple seed, white pumpkin seed, black pumpkin seed can increase the non-specific immunity in fish to a certain extent. (3) White pumpkin seeds, black pumpkin seeds can regulate fat metabolism to a certain extent.

Keywords: Apple seed; Pumpkin seed; Growth performance; Physiological and biochemical indexes; *Megalobrama amblycephala*

*E-mail address: yeyuant@pub.sz.jsinfo.net*
Utilization of different dietary lipid source in in yellow croacker (Pseudosciaena crocea R.): effects on growth performance, tissue fatty acid composition, histological changes and PPARγ expression

Xinxia Wang*, Yongjin Li, Chonglin Hou, Yang Gao, Yizhen Wang*
Institute of Feed Science, Zhejiang University, No. 164 Qiutao North Road, HangZhou, Zhejiang Province, China

Abstract
A 9-week feeding trial was conducted to investigate the impact of dietary lipid sources on lipid mechanisms of large yellow croaker by feeding three isonitrogenous and isoenergetic diets containing fish oil (FO), soybean oil (SO) and beef tallow (BT), respectively, as the only added lipid source. Triplicate groups of 50 fish (initial weight 243.52±5.40g) were fed to apparent satiation by hand twice daily (05:00 and 17:00) for 9 weeks in floating sea cages. The water temperature ranged from 25.5 to 29.5°C, the salinity from 25 to 28‰ and dissolved oxygen content was approximately 7 mg L⁻¹ during the experimental period. The effects of the diets on growth performance, somatic indices, tissue fatty acid composition, histological changes and PPARγ expression were evaluated. Experimental diets were all well accepted by fish and no significant (P>0.05) difference were found in weight gain (WG), specific growth rate (SGR) and feed conversion rate (FCR). The fatty acid profile of the fish fillet and liver reflected the fatty acid composition of the diets. However, specific fatty acids were selectively retained in the flesh of the fish, particularly DHA and ARA concentrations were higher than the dietary concentrations. When FO was replaced by SO or BT diets, the reduction of EPA in fish tissue was more pronounced suggesting a preferred utilization of this fatty acid. Consumption of soybean oils displayed intense lipid accumulation in liver of the fish. PPARγ played a vital role in the control lipid metabolic and storage functions in mammal and fish. The expression of PPARγ significantly increased in the SO group compared with FO and BT groups (P<0.05), which in agreement with the significant increase in liver lipid storage of fish fed SO diet. In summary, dietary fat type affects lipid metabolism in large yellow croaker. In addition, the effects of SO and BT on yellow croaker for longer periods of time, and the interaction effects of different fatty acids on lipid metabolism need further studied.

E-mail address: yzwang@zju.edu.cn.
Novel protein sources for aquaculture: potential use of solid state fermentation to improve nutritional value of plant feedstuffs

Aliro Bórquez¹, ³, Carolina Shenne², ³, Adrián Hernández¹, ³*, Mónica Rubilar², ³, Patricio Dantagnan¹, ³

¹ Escuela de Acuicultura, Universidad Católica de Temuco, Casilla 15-D, Temuco, Chile
² Departamento de Ingeniería Química, Universidad de la Frontera, Av. F. Salazar 01145, Temuco, Chile
³ Centro de Genómica Nutricional Agroacuícola (CGNA), Casilla 58-D, Temuco, Chile

Abstract

Global fishmeal supplies are becoming insufficient to sustain aquaculture production using fishmeal based feeds. In response to environmental concerns, fishmeal production sustainability and increased costs, substantial efforts have been focused on research aiming to reduce or eliminate this component from aquafeeds. In this sense, it is imperative to evaluate the feasibility of new alternative protein sources for use in aquaculture feeds. The main limiting factors in the selection and use of alternative protein ingredients for commercial diets in aquaculture are: sufficient amounts of essential nutrients for optimal growth, digestibility and bioavailability of the nutrients, commercial availability and reasonable cost, and presence of antinutritional factors (ANF). Enhancement of the nutritional quality of alternative ingredients is one of the main strategies for the sustainable development of aquaculture. Efficiency and optimum utilization of alternative plant raw materials could be maximized by means of biotechnological processes that improve these limiting factors. In this sense, the biotechnological innovations aimed to concentrate the protein content, to improve the essential amino acid profile, to reduce the level of ANF and to increase nutrient digestibility and availability could optimize the use of plant raw materials. Among the biotechnological processes, solid state fermentation (SSF) has various applications and potential advantages for the bioconversion of plant feedstuffs and production of new products with added value. The positive effects of SSF on the nutritional composition of plant raw materials is essentially based on the capacity of the microorganisms, that participate in this bioprocess, to synthesize necessary nutrients for their growth from the substrate in which they are reproduced. The process of SSF can increase, for example, the amount of available protein in the raw materials and reduce some of the anti-nutritional factors. Here, we present some preliminary results of the SSF process in plant protein feedstuffs (soy, raps, lupine and peas) for the enhancement of their nutritional profile.

Keywords: Solid state fermentation; Aquafeed ingredients; Improvement of nutritional value; Alternative protein feedstuffs

*E-mail address: ajhernandez@uct.cl (A. Hernández).
Effects of partial replacement of fish meal with soybean protein concentrate on growth performance, body composition and nutrition digestibility of juvenile black sea bream, *Sparus macrocephalus*

**Bergo Owari Ngandzali***, Fan Zhou¹, Qing Jun Shao¹, Wen Xiong¹, Yuan Jian Xu² and Jun Zhuo Xu²

¹College of Animal Sciences, Zhejiang University, Hangzhou 310029, P.R. China
²Marine Fisheries Research Institute of Zhejiang Province, Zhoushan 316100, P.R. China

**Abstract**

The 8-week experiment was conducted to evaluate the effects of partial replacement of fish meal (FM) with soybean protein concentrate (SPC) on growth performance, feed utilization and body composition of juvenile black sea bream, *Sparus macrocephalus*. Experimental fish (10.70 ± 0.04 g) were fed six isonitrogenous and isoenergetic diets which were formulated to replace FM protein by SPC at 0, 8, 16, 24, 32 and 40% (designated as T0, T1, T2, T3, T4, and T5, respectively) for 8 weeks. Diets except T0 were supplemented with the phytase at 2000 phytase activity units kg⁻¹. Each diet was randomly assigned to triplicate groups of 25 fish per tank in eighteen 600-L indoors fiberglass tanks. Tanks were supplied with flowing seawater (3 L min⁻¹) at 28 ± 1°C and fish were fed twice daily (0800 h and 1600 h) to apparent satiation. The results showed that survival rate, growth performance, feed utilization and the values of phosphorus discharge were not significantly affected by increasing dietary SPC; however, feed intake (FI) was higher for fish in T3 than those of fish in T0, T1 and T4. Apparent digestibility coefficients (ADCs) of dry matter was higher in fish fed T5 diet than those of fish fed T0 and T1 diets, ADCs of phosphorus increased significantly from 71.53% to 88.14% with dietary SPC level up to T3 and then decreased significantly, however, ADCs of crude protein and lipid were not significantly different among groups. Whole body compositions of black seabream were significantly influenced by SPC replacing FM except for ash content. Total essential amino acids (∑EAA) showed a decreasing tendency as SPC replacing level increasing except for that of fish in T3, while the highest content of total non-essential amino acids (∑NEAA) was in T4 group. Regarding morphometrical parameters, condition factor (CF) of fish was significantly lower in T2 than that of in T3, but hepatosomatic index (HSI) and intraperitoneal fat (IPF) were unaffected. The results obtained in the present study indicates that FM protein could be effectively replaced by SPC protein up to 40% with phytase of black seabream.

**Keywords:** Black sea bream juvenile *Sparus macrocephalus*; Soybean protein concentrate; Fish meal replacement; Growth performance

*E-mail address: ow_ari@hotmail.com (Bergo Owari Ngandzali)*
Application of some carbohydrate waste food in grower diet of rainbow trout, *Oncorhynchus mykiss*

Dadgar, sh.1,., M.Alizadeh2
1 Department of aquaculture, Iranian Fisheries Research Organization, POBOX 14155-6116, Tehran, Iran.
2 Saltwater Fishes Research Station, Iranian Fisheries Research Organization, POBOX 89715-1123, Bafgh, Iran.

Abstract

Growth performance of rainbow trout studied by use of bread and macaroni industries wastes (as carbohydrate source) in Grower diet as replacements of wheat and corn. Six experimental diets that were prepared according to amount of replacing waste materials with wheat and corn, included: 1) 100% bread wastes 2) 50% bread wastes 3) 100% macaroni wastes 4) 50% macaroni wastes 5) 100% equal mix of bread and macaroni wastes and 6) 50% equal mix of bread and macaroni wastes. Control diet prepared as same as commercial common diet. 21 polyethylene net cages (1×1×1 m) located in a raceway (30×3×1.2 m) with 10 l/s continuous flow trough, were used randomly for fish holding in culture period. Each cage was stoked by 20 fish with initial average weight of 85±5 g. Experiment period was 75 days and in this period water temperature, pH and dissolved oxygen were 14.5±2°c, 7.5-8.8 and 6.8-8.5 mg/l, respectively. The best growth performance belonged to diet 4, although the obtained results from diets 2, 4, 6, and control had no significant different (p<0.05). In term of economic value of used diets in Iran condition, diets 2 had the best result. Carcass analysis in the end of experiment showed that carcass protein and ash contents in all of treatment were the same but lipid content in treatments 1, 3, and 5 were lower than other treatments (p<0.05).

Key words: Rainbow trout; Carbohydrate waste food; Bread and macaroni wastes; Growth performance.

*E-mail: shdadgar@yahoo.com (Sh.Dadgar)*
Different utilization of plant sources by the omnivores jundiá catfish (*Rhamdia quelen*) and Nile tilapia (*Oreochromis niloticus*)

Ana Paula O. Rodrigues¹, Maria do Carmo G. Rosa¹, Eduardo Cargnin-Ferreira², Alicia de Francisco³, Débora M. Fracalossi¹*

¹Aquaculture Department, Federal University of Santa Catarina (UFSC), Rodovia Admar Gonzaga 1346, 88034-001 Florianópolis, SC – Brazil.
²Department of Cell Biology, Embryology, and Genetic, UFSC, Brazil
³Department of Food Science and Technology, UFSC, Brazil

Abstract

The intestinal morphology of Nile tilapia and jundiá - a catfish native to Latin America - is quite distinct despite their same omnivorous feeding habit. Jundiá has short intestines, predominantly straight with quite complex mucosal folds, unlike tilapia, whose intestine is long and meandering, with simple mucosal folds. This difference suggests a distinct use of nutrients from plant sources between the species. We evaluated the apparent digestibility coefficients (ADC) of energy, protein, and dry matter of the following sources (% total fiber-% soluble-% insoluble fiber): broken rice (BR 1.98-0.84-1.14), ground corn (GC 11.23-0.52-10.71), citrus pulp (CP 46.25-10.18-36.07), wheat bran (WB 46.96-2.80-44.16) and soybean hulls (SH 76.63-5.06-71.57). Three groups of 25 jundiá (93.90 ± 34.03 g) and 31 tilapia (93.67 ± 51.57 g) were fed the experimental diets twice a day at approximately 3.0% body weight. Experimental diets contained 30% test-ingredient, 69.5% semipurified reference diet, and 0.5% chromic oxide. Faecal samples were collected daily after settlement into collection devices. As expected, the starchy vegetable sources, BR and GC, were more digestible for both species than the fiber-rich ones. However, tilapia presented higher energy and dry matter digestibility for the starchy sources in comparison to jundiá. The plant source with the highest content of insoluble fiber (SH) provided the lowest protein digestibility for tilapia whereas for jundiá the lowest protein digestibility was observed with the source with the highest content of soluble fiber (CP). A growth trial was also carried out for 30 days, where three groups of 30 fish (jundiá 10.79 ± 2.37 g and tilapia 7.20 ± 1.73 g) were fed a semipurified reference diet with 20% inclusion of each plant source. Tilapia showed a linear decrease in weight gain, feed efficiency and protein retention as the dietary soluble fiber content increased. However, it was not possible to detect such growth differences for jundiá due to its slower growth. The deposition of body fat decreased linearly with increasing dietary insoluble fiber content in jundiá, but for tilapia fat deposition was similar between fish fed the plant source with the highest content of insoluble fiber (SH) or starch (BR). Our results indicate a significant difference in the use of energy and protein from plant sources among omnivores, which is related to the source contents of starch and soluble fiber.

Keywords: Digestibility; Growth; Starch; Soluble fiber; Insoluble fiber

*E-mail address: deboraf@cca.ufsc.br*
Protein concentrates from wheat distillers’ grains as feed source for rainbow trout

Felipe E. Reveco1* and Murray D. Drew1
1 Department of Animal and Poultry Science, University of Saskatchewan, 51 Campus Drive, Saskatoon, Saskatchewan, S7N 5A8, Canada.

Abstract

Wheat wet distillers’ grains (WWDG) are a potential protein source for aqua feed. However, its use in salmonid diets is restricted by low protein content and high level of fiber. The aims of this study were to produce a high-protein low-fiber fraction from WWDG using aqueous fractionation and examine the effect of this ingredient on nutrient digestibility and growth performance of rainbow trout. A digestibility trial was performed using 105 rainbow trout randomly divided into 15 tanks (n = 7/tank). They were fed with a reference diet or one of the four WWDG products in diets (two WWDG and their corresponding protein concentrates) containing 0.7 of the reference diet and 0.3 of the WWDG products. Feces were collected using a modified Guelph system and the data was analyzed as a 2 × 2 factorial design with 2 ethanol plants (1 and 2) and 2 levels of ingredient processing (unprocessed and fractionated). Plant 2 had significantly higher levels of dry matter, gross energy, fat and ash digestibility (P<0.05). The fractionation process significantly increased DM, GE and fat digestibility but reduced ash digestibility. In contrast, crude protein digestibility was not influenced by the plant or the processing method. A significant interaction between plant and processing was observed on GE digestibility. In a 56 d growth trial rainbow trout (n = 22/tank; body weight 136 g and 3 tanks/treatment) were fed diets containing 0, 75, 150, 225 and 300 g/kg of WWDG protein concentrate from plant 2. This product contained 20.7 MJ/kg DE, 595.3 g/kg digestible CP and 166.5 g/kg of neutral detergent fiber. All diets contained 17.58 MJ/kg DE, 386.2 g/kg digestible CP and were balanced for essential amino acids. Linear and quadratic regression models were fitted to the data. There were no significant linear or quadratic relationships between inclusion rate and specific growth rate, average daily gain or feed:gain ratios. However, there was a significant negative linear relationship between inclusion rate and average daily feed intake (ADFI) (ADFI = 0.866 -0.005 (inclusion rate); P<0.05). ADFI was reduced to 95% of the controls at 8.7% inclusion rate and 90% of the controls at 15.8% inclusion rate. The reduction in ADFI may have been due to residual levels of soluble fiber in the WWDG protein concentrate. The use of xylanase and β-glucanase may reduce this effect and allow the use of higher inclusion levels of this product.

Keywords: Distillers’ grains; Aqueous fractionation; Digestibility; Growth; Rainbow trout

* E-mail address: felipe.reveco@usask.ca (F.E. Reveco).
Fractionation of stickwater (SW) by micro-, ultra- and nano-filtration. Effect of different SW fractions and supplemented hydroxyproline and taurine on Atlantic salmon (Salmo salar L.) performance fed very low fish meal diets

Katerina Kousoulaki1, Hanne Jorun Olsen*2, Sissel Albrektsen1, Eyolf Langmyhr1, Paddy Campbell2, Anders Aksnes1
1 Nofima Ingredients AS, Kjerreidvik 16, N-5141 Fyllingsdalen, Norway
2 BioMar AS

Abstract

Stick water (SW) separated during fishmeal production from frozen herring was fractionated following successive micro- ultra- and nano-filtration processes. The presscake (PC) produced during separation of the SW and the different SW fractions were combined again in order to produce 4 fish meals containing different molecular size soluble protein mixes. The SW fractions used for the production of the experimental fish meals were 1) the micro-filtration (MF) retentate, 2) the ultra-filtration (UF) retentate, 3) the nano-filtration (NF) retentate and 4) the nano-filtration permeate. The experimental diets contained 25 g kg\(^{-1}\) commercial fish meal and 50 g kg\(^{-1}\) PC or 1 out of 4 SW fraction fish meals. The remaining dietary protein in the diet was 235 g kg\(^{-1}\) soy protein concentrate, 235 g kg\(^{-1}\) corn gluten, 180 g kg\(^{-1}\) field beans and 53.5 g kg\(^{-1}\) wheat gluten. A control diet was prepared containing 300 g kg\(^{-1}\) diet commercial fish meal. Two more very low fish meal experimental diets were prepared with PC supplemented either with crystalline taurine or crystalline hydroxyproline up to the level present in the high fishmeal control diet. The experimental diets and the control were iso-nitrogenous, iso-lipidic and iso-energetic. The experimental diets contained similar amounts of total water soluble protein, but the control diet was higher. The diets were fed to 8 triplicate groups of salmon (80 fish/group of initial body weight 133 g) for 69 days. The control group had a significant higher growth rate (SGR) compared to the other groups, except for the PC+UF retentate group. Feed intake (FI) was higher in the control group compared to all groups, but not significantly compared to the PC diet. Hepatosomatic index (HSI) was lower while dress out percent (D%) and apparent digestibility coefficient (ADC) of energy was higher in the control group compared to the other groups, except for the PC+MF retentate group were no significant difference was found. There was also a lower feed intake and growth in the group with taurine and hydroxyproline alone supplemented to the low fish meal PC diet. In summary, we did not find any component or fraction of fish meal that promoted feed intake and growth.

Keywords: Salmon; Low fish meal; Stickwater; Marine water solubles; Fractionation

*E-mail address: hanne.olsen@biomar.no (H.J. Olsen)
The influence of varying dietary lipid resources on growth, survival and fatty acid composition of brown trout, *Salmo trutta*.

Murat Arslan¹*, A. Necdet Sirkecioglu², Abdulkadir Bayir³, Harun Arslan²

¹Department of Fisheries and Aquaculture, Ispir Hamza Polat Vocational School, Ataturk University, Ispir, Erzurum 25900, Turkey
²Department of Fisheries and Aquaculture, College of Agriculture, Ataturk University, Erzurum 25640, Turkey

Abstract

Brown trout (initial average weight, 1.098 ± 0.01 g) were fed four casein-gelatin based diets with different lipid resources for 6 weeks. The sources of dietary lipid were menhaden oil (MO diet, rich in highly unsaturated fatty acids), hazelnut oil (HO diet, predominantly oleic acid), a blend of linseed oil and soybean oil (LO + SO diet, linoleic and linolenic acids), and soybean lecithin (LE diet, phospholipids; mostly linoleic acid). Fish were fed at a restricted-readjusted feeding rate in a recirculating water system at 13 ± 0.2°C. At the end of the experiment, fish fed LO + SO diet demonstrated significantly higher growth performance than those of the three other groups (P<0.05). HO diet fed fish had the lowest growth rate. Survival was not affected by the dietary treatments averaging 86%. Whole body lipid content was not significantly influenced by the dietary lipid resources with the numerically lowest lipid level occurred in fish fed LE diet. The fatty acid profiles of neutral and polar fractions of whole body lipids reflected the fatty acid composition of the diets. High level of 22:6n-3 (docosahexaenoic acid, DHA) in whole body lipids of fish fed LO + SO diet corresponded with the high level of 18:3n-3 (linolenic acid, LNA) in the lipids of this diet. Our results also showed that fish fed LE diet which was rich in 18:2n-6 (linolenic acid, LA) had the highest level of 20:4n-6 (arachidonic acid, ARA) in their whole body lipids. The results suggest that the efficiency of elongation and desaturation of C18 fatty acids depend on the dietary lipid source and brown trout has considerable capacity to transform LA and LNA to ARA and DHA, respectively.

Keywords: Brown trout; Growth; Lipids; Fatty acids

* E-mail address: arslan.7@gmail.com, muratars@atauni.edu.tr (M. Arslan).
Partial replacement of fish meal by wheat gluten meal in practical diets for juvenile cobia (*Rachycentron canadum*)

Jun Yang*1, Qi-Cun Zhou1, Hua-lang Wang2, Hai-Tao Zhang2, Xian-Jun Cui1

1 Laboratory of Aquatic Economic Animal Nutrition and Feed, College of Fisheries, Guangdong Ocean University, Zhanjiang 524025, People’s Republic of China
2 Aquatic Animal Feed Research and Development Center, Guangdong Evergreen Group Corp. Zhanjiang 524094, People’s Republic of China

Abstract

An 8-week feeding experiment was conducted to evaluate the effects of replacing fish meal with wheat gluten meal on the growth, feed utilization, and hematological index of juvenile cobia. Six isonitrogenous and isoenergetic diets (crude protein, 42.5%; crude lipid, 9%) containing graded levels, in which 0%, 10%, 20%, 30%, 40% and 60% of proteins from fish meal were replaced with wheat gluten meal, were fed to triplicate groups of fish (initial body weight of 10.50±0.03g). The results revealed that up to 30% of fish meal could be replaced by wheat gluten meal without causing significant reduction in growth. Fish fed the diets which replacement levels of fish meal with wheat gluten meal were above 30% had a significantly lower weight gain than those fed the other diets. High survival was observed in all dietary treatments, and no significant difference among treatments was observed. Feed conversion ratio (FCR) showed an increased trend even though there was no significant difference could be founded. The hepatosomatic index and viscerasomatic index were significantly affected by the replacement of fish meal with wheat gluten meal. No significant differences were detected in the moisture and ash content in dorsal muscle, but protein and lipid in dorsal muscle were significantly affected by the wheat gluten meal levels. Blood hemoglobin was significantly affected by the dietary wheat gluten meal incorporation. The broken-line regression analysis between weight gain and replacement level indicated that a growth optimum occurring at 34.05% replacement of fish meal by wheat gluten meal.

Keywords: Cobia (*Rachycentron canadum*); Fish meal, Wheat gluten meal; Growth performance; Hematology indexes

*E-mail addresses: qicunzhou@21cn.com (Q. C. Zhou)
Partial replacement of fish meal by corn gluten meal in practical diets for juvenile cobia (*Rachycentron canadum*)

Jun Yang 1*, Qi-Cun Zhou1, Hua-lang Wang2, Hai-Tao Zhang2, Xian-Jun Cui 1

1 Laboratory of Aquatic Economic Animal Nutrition and Feed, College of Fisheries, Guangdong Ocean University, Zhanjiang 524025, People’s Republic of China
2 Aquatic Animal Feed Research and Development Center, Guangdong Evergreen Group Corp. Zhanjiang 524094, People’s Republic of China

Abstract

An 8-week feeding experiment was conducted to determine the potential use of corn gluten meal as a partial replacement of fishmeal in the isonitrogenous (approximately 42.5% crude protein) diet for juvenile cobia with an initial average weight of about 11.50±0.05g. Diets were formulated to include 0, 10, 20, 30, 40 and 60% of fishmeal protein being substituted by corn gluten meal without methionine and lysine supplementation. The results showed that weight gain rate decreased significantly when the replacement level of fishmeal protein was increased from 20% to 30%, and fish fed the diet which 60% replacement of fish meal with corn gluten meal had lower weight gain than the other diets. These results indicate that up to 20% of fishmeal protein can be replaced by corn gluten meal without causing significant reduction in growth. High survival was observed in all dietary treatments, and no significant difference among treatments was observed. Feed conversion ratio (FCR) and protein efficiency ratio (PER) were significantly affected by the replacement of fish meal with corn gluten meal. There was an increased trend in fat deposition in dorsal muscles and livers of fish fed diets with plant protein sources. Biological indices were also affected by replacement of fish meal with corn gluten meal. Hepatosomatic index and viscerosomatic index increased significantly with inclusion level of CGM increased. The incorporation of CGM in diets significantly affected white blood cell (WBC), hematocrit (HCT) and plasma glucose (GLU), while no effect was observed on serum triglyceride (TG), total protein (TP), red blood cell counts (RBC) and hemoglobin (HGB). Serum glucose and white blood cell values significantly increased with the increase of replacement level. The broken-line regression analysis indicated that 16.91% corn gluten meal in diet of juvenile cobia could be acceptable.

Keywords: Cobia (*Rachycentron canadum*); Fish meal, Corn gluten meal; Growth performance; Hematology indexes

*E-mail addresses: qicunzhou@21cn.com (Q. C. Zhou)
Partial replacement of fish meal with a mixture of canola meal and cottonseed meal in practical diets for Litopenaeus vannamei

Tuo Wang*, Qi-Cun Zhou
Laboratory of Aquatic Economic Animal Nutrition and Feed, College of Fisheries, Guangdong Ocean University, Zhanjiang 524025, People’s Republic of China

Abstract

An 8-week feeding trial was undertaken to evaluate the effects of replacement of fish meal with a mixture of canola meal and cottonseed meal (1:1 on a crude protein basis) with or without essential amino acids on growth performance, feed utilization, apparent digestibility coefficients and non-specific immune for Litopenaeus vannamei. Six isonitrogenous and isoenergetic diets (crude protein, 43.0%; crude lipid, 8.5%) containing graded levels, in which 0%, 10%, 20% and 30% of proteins from fish meal were replaced with mixture of canola meal and cottonseed meal with or without essential amino acids, were fed to triplicate groups of shrimp (initial body weight of 0.66g). The results indicated that weight gain, specific growth, feed conversion ratio and protein efficiency ratio were not significantly affected by the replacement of fish meal with mixture of canola meal and cottonseed meal. However no significant effects on growth performance and feed utilization were found between with or without essential amino acids supplementation. Apparent digestibility coefficients for dry matter, protein and lipid were not significantly affected by the dietary replacement of fish meal with the mixture of canola meal and cottonseed meal. Shrimp fed the control diet had higher catalase, superoxide dismutas, glutathione peroxidase, glutathione S-transferases activities than shrimp those fed the other diets. It is concluded that replacement of fish meal with RM-CM was up to 30% without negative growth, feed intake and protein utilization for Litopenaeus vannamei.

Keywords: Litopenaeus vannamei; Fish meal, Canola meal an; Cottonseed meal; Growth performance; Non-specific immune

*E-mail addresses: qicunzhou@21cn.com (Q. C. Zhou)
Cage culture of the GIFT strain of Nile tilapia (*Oreochromis niloticus*) fed with aquafeed prepared using local fishmeal and Ipil ipil leaves

M.H.S. Ariyaratne

Abstract

The per capita fish consumption of the rural community in the dry zone of Sri Lanka is below the WHO recommended amounts in terms of nutrition. Accordingly, promotion of inland fish production through aquaculture practices in the dry zone should be given priority. Since feed cost is the highest operating cost in aquaculture, it is necessary to develop low-cost aquafeed, to be used in inland aquaculture to raise the per capita fish consumption. The study was carried out with the objective of evaluating the growth performance of *O. niloticus* (GIFT strain) with aquafeeds (Feed-A and Feed-B) in cage culture. Nine cages (10 m$^3$ each) were installed in Mahawewa, a reservoir (80 ha) in the dry zone of Sri Lanka. Cages were stocked with advanced fingerlings of *Oreochromis niloticus* (GIFT strain) (mean weight 18.7±12.07 g and mean total length 9.7±2.08 cm) according to the stocking density of 45 fingerlings m$^{-3}$. Two aquafeed i.e. Feed-A and Feed-B were tested while commercial feed (Feed-C) was used as control. Each treatment was carried out in three replicates. The locally available ingredients ricebran (Rb), coconut residue (CR) and local fishmeal (Fm) were used in both feed types. Part of the Fm in Feed-B was replaced through plant protein; fresh ipil-ipil (*Leucaena leucocephala*) leaves (Ip). The % protein in Feed-A and Feed-B was adjusted to 29%. Cassava forage was used as binder in both feed types. During the 167 days experimental period, fish were fed twice daily at 5% of body weight at the beginning up to 3.6%, 3% and 2.5% onward. The amount of feed was adjusted according to the total biomass in respective cages assuming there was no mortality. Protein % content in Feed-A and Feed-B were 17.20-23.97 and 19.6-25.5 respectively. Data were analyzed with One-way ANOVA. The mean % survival, Condition Factor (K) and Specific Growth Rate in weight (SGR-W) of the fish fed on Feed-A, Feed-B and Feed-C were not significantly different (p>0.05) but the Average Daily Growth (ADG) of the fish was significantly different (P<0.05). The total fish production in cages through Feed-A, Feed-B and Feed-C were 4.3, 3.85 and 5.33 kg m$^{-3}$, respectively. Feed-A could be recommended and Feed-B could be considered with improvements for *O. niloticus* (GIFT strain) cage culture in perennial reservoirs. The financial support received from the Spanish Agency for International Cooperation for Development (AECID) is gratefully acknowledged.

Key words: GIFT strain, cage culture, food fish culture, plant protein, aquafeed

E-mail address: soma_ariyaratne@hotmail.com
Effect of dietary inclusion rate of canola products on the growth performance of rainbow trout, *Oncorhynchus mykiss*

Stephanie A. Nilson*, Andrew G. Van Kessel, Janet E. Hill, Murray D. Drew

1College of Agriculture and Bioresources, Department of Animal and Poultry Science, University of Saskatchewan, 51 Campus Drive, Saskatoon SK Canada S7N 5A8

2Western College of Veterinary Medicine, Department of Veterinary Microbiology, University of Saskatchewan, 52 Campus Drive, Saskatoon SK Canada S7N 5B4

Abstract

Nutritional replacements for fishmeal in salmonid diets must maintain growth performance and have a minimal impact on the environment. Canola meal (CM) and an aqueous-extracted canola protein concentrate (CPC; MCN Bioproducts, Inc., Saskatoon, Canada) were compared to determine the effect of increasing inclusion rates of these two products in a commercial-type diet on the growth performance of rainbow trout (*Oncorhynchus mykiss*). The total tract digestibility of all ingredients was measured prior to diet formulation. The reference diet was fishmeal-based and contained Celite, high-purity flux-calcined diatomaceous earth (Celite®, 545, Celite Co., World Minerals Co., Lompoe, CA, USA) at an inclusion ration of 1% as an indigestible marker. Test diet compositions were 70% reference mash and 30% test ingredient and the diets were triplicate tanks of rainbow trout. Following a 6-day acclimation period, feces were collected for three weeks using a settling column, centrifuged, freeze dried, ground and analyzed for digestible energy, amino acids, phosphorus and for levels of antinutrients. Dry matter (0.81) and gross energy (0.84) apparent digestibility coefficients (ADC) for fish fed CPC were significantly (P<0.05) higher than those in fish fed CM (0.68 and 0.77, respectively). Fish fed CPC also had a significantly higher phosphorus ADC (0.78) than fish fed CM (0.28), which is likely due to the CPC being completely devoid of phytate. In contrast, canola meal had a phytate content of 4.88% on a dry matter basis. Using information from the digestibility trial, diets were formulated for two 56-day growth trials (one per test ingredient). All diets contained 17.6 MJ/kg digestible energy, 386.2 g/kg digestible crude protein and were balanced for essential amino acids. Each growth trial had five diets containing the test ingredients at levels of 0, 7.5, 15, 22.5 or 30%. There was a significant negative linear relationship between the inclusion rate of CM and the specific growth rate of the fish (P<0.05). In CM fed-fish, growth rate was reduced to 95% of the controls at a 4.5% inclusion rate and 90% of controls at a 15.6% inclusion rate. There was no significant linear or quadratic relationship between the inclusion rate of CPC and specific growth rate of the fish (P>0.05). This indicates that for inclusion rates up to 30%, CPC has no effect on fish growth. Thus, CPC is nutritionally superior to CM in its phosphorus digestibility and effects on the growth performance of rainbow trout.

Keywords: Canola meal; Canola protein concentrate; Phytate; Rainbow trout

*E-mail address: stephanie.nilson@usask.ca (S.A. Nilson).*
Partial and total replacement of fish oil either canola or cotton seed oils in diets for European sea bass (Dicentrarchus labrax): effects on flesh and whole body fatty acid composition

Hatice A. Yılmaz 1, Tufan O. Eroldoğan 1*, Kenan Engin 2, Abdüllatif Ölçülü 1, Oguz Taşbozan 1, Serhat Türkmenc 1
1 Department of Aquaculture, Faculty of Fisheries, Cukurova University, Adana 01330, Turkey
2 Department of Aquaculture, Faculty of Fisheries, Mersin University, 33169 Mersin, Turkey

Abstract

Substantial research on fish oil substitution has indicated that fish oil can be replaced by a range of plant seed oils including linseed, rapeseed and olive oils in carnivore marine Teleost European sea bass. There is little information on cotton seed oil (CSO) as alternative vegetable oil for marine fish diets. It was our intent to assess the suitability of CSO and canola oil (CO) as alternatives to fish oil for European sea bass. Triplicate groups of 20 European sea bass (38.2 g) were fed four cold press-pelleted diets in which the added lipid was 100% fish oil (FO), 100% canola oil (CO), 100% cotton seed oil (CSO) and 50% CO + 50% CSO (CO/CSO), for a period of 130 days. There were no effects of diet on growth and feed conversion, except fish fed CSO which was lowest among the groups. While the greatest accumulation of whole body lipid was in fish fed FO and CSO, muscle lipid accumulation in fish fed CO and CO/CSO was highest, which corresponded to significantly lower muscle protein in these groups. Whole body and flesh fatty acid composition mirrored those of diet treatments. Total mono unsaturated fatty acids (primarily 18:1n-9) were highest in flesh of fish fed CO, CSO and CO/CSO and lowest in fish fed FO. Linoleic acid (LA) was preferentially deposited in whole body and flesh lipid relative to dietary lipid in fish fed CO/CSO. 18:1n-9 and LA were all significantly increased in fish fed the CSO. While the concentration of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in whole body and flesh were significantly reduced in fish fed CSO and CO/CSO. However, there were no differences in whole body and flesh EPA levels in fish fed CO and FO. Finally, a blend of CSO and CO might be used as a substitute for fish oil in the culture of European sea bass.

Keywords: Vegetable oil; European sea bass; Fatty acid; Cotton seed oil; Canola oil

* E-mail address: mtufan@cu.edu.tr
Feed sustainability of high-energy feeds with graded fishmeal inclusion for Atlantic salmon (Salmo salar L.)

Vidar Gundersen1*, Kjell A. Måsøval1 and Eldar Åsgard Bendiksen1
1 BioMar AS, Trondheim, Norway

Abstract

Feed sustainability of salmon farming has been questioned because of the high inputs of marine raw materials into compound feeds. A commercial scale feeding trial was designed to document the longer-term effects on fish performance of using high-energy feeds with reduced fishmeal inclusions, and quantify the effects on feed sustainability. Three triplicate groups of salmon were fed either high fishmeal diet (High FM; 20% of the total feed ingredients), a medium fishmeal diet (Medium FM; 15% of the total feed ingredients) or a low fishmeal diet (Low FM; 10% of the total feed ingredients). Different legumes and oil seed meals were used to substitute the fishmeal and a blend of marine fish oils (FO) and crude rapeseed oil (~ 50% of added oil) were used as dietary lipid sources. Fish-In:Fish-Out ratio (FIFO) of each treatment was assessed from kg of raw fish materials required to produce 1kg salmon, using a conversion factors for raw fish yield, 225g fishmeal and 50g fish oil kg ww fish-1, combined with feed recipe inclusions and feed:gain ratio (g ww feed provided/g ww salmon gained). In addition a more comprehensive assessment of feed sustainability of the three different feed treatments was conducted by life cycle assessment (LCA) and eco-efficiency analysis (EEA). EEA of the feed regimes was conducted using BASF’s methodology adapted to fish feed including life cycle cost and LCA of land use, energy use, resource consumption, emissions, risk and toxicity potential. The cage means were used to examine the effect of feed treatment on growth and estimated feed:gain ratio. The fish weight increased from 1216±35 g (overall cage mean ±S.D.) to 4625±234g over the nine months feeding period. The overall mean TGC3 and feed:gain ratio were 3.28±0.17 and 1.12±0.07, respectively. Based on cage means there was no significant difference in growth or feed:gain ratio between the feed treatments. FIFO was estimated to 0.9 and 1.5kg for fishmeal and fish oil usage, respectively, per kg salmon produced in High FM treatment. Less fishmeal inclusion and use of specific oil raw materials resulted in decreased feed fish consumption, and net production of both marine protein and oil. EEA modelling seemed to be a useful tool for future optimisation of feed sustainability in salmon farming, and modelled intriguing outputs suited to nuance the debate around optimal use of limited marine resources.

Keywords: Sustainability, marine raw material, life-cycle assessment, eco-efficiency analysis.

* E-mail address: vidar.gundersen@biomar.no (V. Gundersen)
Demonstration of salmon farming as a net producer of fish protein and oil.

Vivian O. Crampton1*, Dominic A. Nanton1, Kari Ruohonen1, Adel El-Mowafi1

1 EWOS Innovation AS, Dirdal, N-4335, Norway

Abstract

To date aquaculture’s reliance on dietary marine sources has been calculated on a fish weight-to-weight basis without considering the absolute amounts of nutrients but this approach neglects the often considerable differences in the nutritional value of fish. We propose simple nutrient-to-nutrient based dependency measures that take into account these nutritional differences. Based on these measures it is evident that during the last ten years salmon farming has reduced by approximately half its reliance on both marine proteins and oils and that the dependency ratio is currently only just above one, that is salmon farming is now close to being marine protein and oil neutral.

We describe two studies in which the aim was to reduce the inclusion of marine ingredients so that the amount of salmon protein and lipid produced through growth exceeded the amount of marine protein and lipid consumed. The first used individually tagged Atlantic salmon (Salmo salar) initial body weight 350g reared in seawater supplied tanks fitted with feed collection facilities using four replicate tanks per treatment. In the second, commercial net pens with six replicates per treatment were used to grow over 200,000 fish, initial body weight 1,200g. In both studies a low marine ingredient feed containing approx 165 g kg⁻¹ fishmeal was compared to a control feed (approx 300 g kg⁻¹ fishmeal) whilst fish oil inclusion was less markedly reduced. The growth, feed efficiency and body composition including fatty acid profile were measured. In both studies the low marine feed supported similar growth and feed efficiency compared to the control feed. The amount of n-3 long chain polyunsaturated fatty acids deposited was sufficient to meet current recommendations from human health organisations. This demonstrates that it is possible for salmon farming to be a net producer of fish protein and oil whilst still achieving high concentrations of fatty acids in the fillet that are beneficial to human health, and without a measurable decrease in growth rate or feed efficiency of the salmon.

Keywords: Atlantic salmon, marine dependency, fishmeal, fish oil, PUFA

* E-mail address: viv.crampton@ewos.com
Replacement of fish meal by skate meal and black cod viscera meal in diets for Pacific threadfin (*Polydactylus sexfilis*)

Z.Y. Ju1*, W.G. Dominy1, I.P. Forster1, P. Bechtel1, S. Smiley3
1Aquatic Feeds and Nutrition Department, Oceanic Institute, 41-202 Kalaniana’ole Highway, Waimanalo, Hawaii 96795, USA
2USDA-ARS, Subarctic Agricultural Research Unit, University of Alaska, Fairbanks, Alaska 99775, USA
3Fishery Industrial Technology Center, School of Fisheries & Ocean Sciences, University of Alaska, Fairbanks, Alaska 99615, USA

Abstract

Pacific threadfin, known in Hawaii as moi, were first cultured in large quantity by the Oceanic Institute, and are now a popular marine seafood derived from fish ponds and sea cages off Hawaii. The commercial production of moi relies on fishmeal based dietary formulations. Fishmeals are generally expensive, while fisheries by-catch and byproducts in Alaska are relatively plentiful and underutilized. The objective of this study was to ascertain the suitability of skate meal and black cod viscera meal as replacements for commercial fishmeal. Composition of skate meal (87.6% crude protein = CP; 1.8% crude lipid = CL) and black cod viscera meal (36.6% CP; 19.8% CL) from Alaska was determined and test diets were processed by replacing 50% or 100% of the pollock fishmeal protein in control diet (45% CP) with either the skate or black cod meals. Five diets (including a commercial feed, Skretting MG 3.0) were randomly allocated to 4 replicate tanks with 8 juvenile fish (9.70±0.23 g) per tank (115L seawater). An indoor culture system was supplied with well seawater: 26.0 ±1.0 °C temperature, 32‰ salinity, and 4L/min flow through water exchange. The fish were fed by hand three times daily to apparent satiation for 6 weeks, at which time all fish were counted and weighed. Results showed that fish survival rates were 100% for all tested diets. Fish fed the skate meal replacing both 50% and 100% of the pollock fishmeal protein in control diet (45% CP) with either the skate or black cod meals. Five diets (including a commercial feed, Skretting MG 3.0) were randomly allocated to 4 replicate tanks with 8 juvenile fish (9.70±0.23 g) per tank (115L seawater). An indoor culture system was supplied with well seawater: 26.0 ±1.0 °C temperature, 32‰ salinity, and 4L/min flow through water exchange. The fish were fed by hand three times daily to apparent satiation for 6 weeks, at which time all fish were counted and weighed. Results showed that fish survival rates were 100% for all tested diets. Fish fed the skate meal replacing both 50% and 100% of the pollock fishmeal used in the control diet exhibited similar growth rates (6.05±0.55 g/wk; 5.95±0.29 g/wk) and similar feed conversion ratios (FCR; 1.29±0.07; 1.27±0.04) as those (5.58±0.74 g/wk; 1.30±0.11) fed the control diet. These results suggest that skate meal could completely replace fishmeal in moi diets without affecting their growth. In contrast, feeding moi with the black cod viscera meal replacing 50% of the pollock fishmeal in the control diet resulted in a considerably lower growth rate (1.84±0.05 g/wk) and higher FCR (2.16±0.12) than the control diets (P<0.01). The fish fed the control diet and skate meal substituted diets achieved significantly higher (P<0.05) growth rates than the fish fed the Skretting commercial fish feed (4.61±0.66 g/wk). The moi muscle products contained 17.18 to 17.39% CP, 1.69 to 2.25% CL and 1.37 to 1.45% ash in the five groups of fish products, and were rich in n-3 fatty acids. In conclusion, the skate meal can replace the commercial fishmeal in moi diet without adverse effect on its growth performance.

Keywords: Replacement; Fish meal; Skate meal; Black cod viscera meal, Pacific threadfin

*E-mail* address: zyju@oceanicinstitute.org (Z. Y. Ju)
Effect of fermented grain protein on growth and feed efficiency of *Carassius auratus gibelio*

Lu Cheng*, Feng Huang
School of Animal Science and Nutrition Engineering, Wuhan Polytechnic University, Wuhan 430023, P. R. China

Abstract

A 90d growth trial was conducted to evaluate the dietary fermented grain protein (FGP) on the growth performance and feed efficiency for *Carassius auratus gibelio*. The control contained 5% fish meal, whereas in the other seven diets, Group I -Ⅶ, were fish meal free diets, directly contained 0%, 2%, 4%, 6%, 8%, 10%, 12% of the FGP, respectively. All the experimental diets were made to be isonitrogenous and isocaloric. The weight gain of fish fed group I was slightly lower than fish fed the control diet ($P>0.05$). An increase in weight gain and specific growth rate of fish were observed in all fish fed FGP diets with the increase of FGP content in diets. The fish fed Group Ⅶ had the highest weight gain and the specific growth rate, but no significant difference in weight gain or specific growth rate was observed between the fish fed Group Ⅶ and the control ($P>0.05$). On the contrary, the feed coefficient of fish fed group I showed highest in all diets, and a decrease in feed coefficient of fish were observed in all fish fed FGP diets with the increase of dietary FGP level. The feed coefficient of fish fed Ⅰ and Ⅶ were significantly lower than the fish fed the control diet ($P<0.05$), but they were significant lower than the fish fed group I ($P<0.01$).

These results indicated that FGP could improve the growth performance of gibel carp and FGP can be partially used in diets for gibel carp. Results of the present study suggested that FGP can be used at 10%~12%.

**Keywords:** *Carassius auratus gibelio*; weight gain; specific growth rate; feed coefficient

*E-mail address: cheng2006lu@126.com*
Optimal replacement of fish meal by soybean protein in diets for Siganus guttatus

Rongbin Gao1,2*, Longzhen Zhang1, Ping Zhuang1, Guangpeng Feng1, Jianyi Liu1
1 East China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Shanghai, China
2 East China University of Science and Technology, Shanghai, China

Abstract

Rabbitfish, family Siganidae, hold particular promise for marine aquaculture development due to their omnivorous feeding habits and consequent ability to feed low in the aquatic food chain. Siganus guttatus is expected to become one of the new omnivorous fishes cultured artificially not only because it has high nutrition values, such as tastes well, rich in unsaturated fatty acid and protein and some other nutritional components but also because it has some excellent aquaculture features, such as omnivorous, easy availability of feed resources, strong environment adaptability, etc. The low capture rate is also one of the reasons why S. guttatus is favored by researchers in China. A 49-day feeding experiment was conducted to evaluate the optimal replacement fish meal by soybean meal of S. guttatus (with the initial weight 104.5 ± 1.5 g). Five isonitrogenous diets were formulated which contain 31 % protein and 10 % lipid. The protein, which was provided by Fish meal was replaced by soybean meal at five levels, all fish meal (diet 1), soybean meal : fish meal = 1 : 2 (diet 2), soybean meal : fish meal = 1 : 1 (diet 3), soybean meal : fish meal = 2 : 1 (diet 4), all soybean meal (diet 5). S. guttatus which were fed to satiate twice daily, were cultured in the conical rearing tank (0.5 m3) with each group 25 ind and three replications per group. Weight Gain (WG) and Special Growth Ratio (SGR) were maximized when fish were fed with pure fish meal, 24.18 % and 0.44 % d−1, respectively. Significant differences were found in WG between each group. The lowest feed conversion ratio (FCR) was found in diet 1, but there was no significant difference in diet 2. There are also no obvious difference between diet 2 and diet 3, it is also the case of protein efficiency ratio (PER) between diet 2 and diet 3. The replacement had no significant effect on hepatopancreasomatic index (HSI) and mesenteric fat index (MFI). As to the condition facto (CF), the replacement also had no significant difference among diet 2, diet 3 and diet 4. Based on broken-line regression analysis of SGR, it is recommended that the optimal protein source replacement in the feed of S. guttatus should be controlled between 1:1 and 2:1.

Key words: S. guttatus; Protein replacement; Fish meal; Soybean meal

* E-mail address: sawyerkingfisher@gmail.com (Rongbin Gao).
Effects of replacements of soybean meal with dried or fermented brewers’ grain on growth and body composition in juvenile tilapia Oreochromis niloticus×O.aureus

Bin-Chong Qiu, Qing Pan*, Bi-wei Liu, Ying-Zuo Bi, Jinghua Fu, Yuan Sun
Department of Aquaculture, School of Animal Science, South China Agricultural University, Guangzhou 510642, China

Abstract
With increasing price of soybean meal, great efforts are made in seeking alternative protein for fish feed. The yield of beer in China was estimated to reach 4.4 million tons in 2008. And brewers’ grain, beer by-product, is a good potential source of dietary protein. Therefore, two feeding trials were conducted to investigate the alternative effects of dried and fermented brewers’ grain on growth, feed utilization and body composition of juvenile tilapia Oreochromis niloticus×O. aureus (with initial body weight of 3.0 g). Soybean meal (20%) diet were made for control diet and test diets were formulated with dried or fermented brewers’ grain replacing 20%, 40%, 60%, 80% and 100% of soybean meal, respectively. All diets had the same lysine and methionine levels added with supplementation of coated lysine and methionine. Each test diet was fed to three replicate tanks of fish. 30 fish were randomly stocked into each tank and reared in recycle system for eight weeks. Feeding rate was 5-6% of body weight to apparent satiation. The results showed that with increasing levels of replacement of dried brewer’s grain, weight gain, feed efficiency, protein efficiency rate decreased gradually. And significant difference (P<0.05) were observed when replacing level exceeded 60%. Survival rate had no markedly difference (P>0.05) among groups. Viserosomatic index and hepatosomatic index decreased significantly when replacing level reached 100% and exceeded 80%, respectively. Whole body crude lipid content decreased significantly when replacing level exceeded 80% and ash content increased significantly when replacing level exceeded 60%. Muscle crude lipid content decreased significantly when replacing level reached 100%. When dried brewers’ grain was replaced by fermented brewers’ grain, growth and protein utilization was improved significantly. Weight gain, feed efficiency and protein efficiency decreased significantly when replacing level reached 100%, exceeded 60% and 80%, respectively. Viserosomatic index decreased significantly when replacing level reached 100%. Whole body and muscle composition had no significant difference among groups. According to WG and PER, the results suggested that dried brewers’ grain and fermented brewers’ grain could replace 40% and 80% dietary soybean meal, respectively without negative effects on growth and body composition in juvenile tilapia.

Keywords: Dried brewer’s grain; Fermented brewer’s grain; Tilapia; Growth, Body composition

* E-mail address: qpan@scau.edu.cn (Qing Pan).
P-029

Effect of dietary fish oil replacement by rapeseed oil on the growth, fatty acid composition and serum non-specific immunity response of fingerling black carp, *Mylopharyngodon piceus*

Shengming Sun¹,², Jinyun Ye²*, Jianming Chen³, Youhui Wang³, Liqiao Chen¹*

¹ Life Science College, East China Normal University, Shanghai 200062, China
² Huzhou Normal University, Huzhou, Zhejiang 313000, China
³ Zhejiang Institute of Freshwater Fisheries, Huzhou, Zhejiang 313001, China

Abstracts

An eight-week experiment on fingerling black carp was conducted to evaluate the effects of dietary replacement of fish oil (FO) by rapeseed oil (RO) on growth, fatty acid composition and non-specific immune responses. Five triplicate fingerling groups (initial weight=2.72 ±0.35 g) were fed isocaloric (~16.6kJ•g⁻¹ feed energy) and isonitrogenous (~38% crude protein) diets in which the dietary FO was replaced with RO in graded increments of 25% (0–100%). No significant effects were observed on specific growth ratios, survival rates, and feed conversion ratios, but there were significant differences in whole body moisture and liver lipid contents (P<0.05), and the 100% RO replacement diet significantly enhanced hepatosomatic indexes compared to control group (P<0.05). Other approximate whole body constituents, viscerasomatic ratios and condition factors were not influenced. Fatty acid composition of muscle and liver generally reflected that of the diets, Linoleic acid and linolenic acid were significantly increased with increasing RO, but eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and the n−3/n−6 ratio were significantly reduced (P<0.05). Alternative complement pathway, lysozyme and superoxide dismutase activities were not significantly influenced. The results indicated that 100% replacement of fish oil by rapeseed oil was recommended in diet formulation for black carp under our conditions of this study.

Keywords: *Mylopharyngodon piceus*; Fish oil replacement; Rapeseed oil; Growth; Fatty acids; Non-specific immunity

*E-mail address: ziff2006@163.com (J. Ye), E-mail address: lqchen@bio.ecnu.edu.cn (L. Chen)
P-030

Effects of dietary cottonseed meal protein on growth, body composition and some blood indices of juvenile *Mylopharyngodon piceus*

Xiao Tiaoyi*, Hu Yi, Huang Yun
College of Animal Technology, Hunan Agriculture, ChangSha 410128

Abstract

A 8-week feeding experiment was conducted to evaluate the effects of dietary cottonseed meal (CM) protein level on growth, body composition and some blood indices of juvenile *Mylopharyngodon piceus* (initial average weight 5.80 ± 0.03g). The basal diet was used as control, the added levels of cottonseed meal were 0, 10, 20, 30 and 40%, in which soy protein (SM) protein was replaced by CM at 0, 25, 50, 75 and 100%, respectively. Each diet was randomly fed to triplicate groups of 25 fishs per tank (300L). Fish were fed three times a day by hand at a rate of 3% of body weight during eight weeks. The results showed that no significant differences in specific growth rates (SGR), feed efficiency (FE) and survival rate were observed among dietary treatments in the previous four weeks. However, fishes fed the diets containing 40% CM showed lower the SGR and survival rate than those fed the control diet in the whole eight weeks (\(P<0.05\)). Viscerosomatic index were not significantly affected by dietary cottonseed meal levels, while hepatosomatic index were significantly affected by dietary cottonseed meal levels (\(P<0.05\)), hepatosomatic index increased with increasing of dietary CM levels. Glucose (GLU), Total Protein (TP) and Total Cholesterol (TC) in hemolymph were not significantly affected by dietary CM levels. The number of RBC and WBC in blood was significantly affected by dietary CM level, which decreases with increasing of dietary CM levels. When the dietary CM levels more than 30%, the activities of glutamic-pyruvic transaminse (GPT) and glutamic-oxalacetic transaminase (GOT) in serum were significantly higher than control group (\(P<0.05\)); When the dietary CM levels more than 40%, the activities of Alkaline phosphate (ALP) in serum were significantly higher than the control group (\(P<0.05\)); Body moisture, crude protein, crude fat were not significantly affect by dietary cottonseed meal levels, but body ash contents significantly increased with increasing of dietary cottonseed meal (\(P<0.05\)). These results suggested that there is no negative impact on the growth when the dietary CM levels reaches 30%, but there is a negative influence on liver function, the dietary CM levels of *Mylopharyngodon piceus* should be not more than 30%.

Keyword: *Mylopharyngodon piceus*; cottonseed meal; growth; body composition; some blood indices

Email: tyx1128@yahoo.com.cn,
Effect of fish-meal replacement by fermented soybean meal on growth, flesh quality, nitrogenous and phosphorus metabolism of Japanese seabass, *Lateolabrax japonicus* under digestible amino acid profile

Liang HU, Min XUE*, Xiu-feng WU, Ying-hua ZHENG, Lan-mei WANG, Hong-yun GE
Feed Research Institute, the Chinese Academy of Agricultural Sciences, Beijing 100081, P. R. China

Abstract:
A 16-week growth trial was conducted to study the effects of replacing dietary low temperature steam dried fish meal (LTFM) by fermented soybean meal (FSM) under digestible ideal amino acid (DIAA) profile on growth performance, nitrogen and phosphorus digestibility, retention and excretion, and flesh quality of Japanese sea bass (*Lateolabrax japonicus*) (initial body weight = 13.3g ± 0.1g). Four isoenergetic and isonitrogenous at digestible level diets were formulated. A control diet contained 36% LTFM was formulated according to the DIAA profile of Japanese seabass and named as LTFM. In the other 3 diets, 25%, 50% or 75% of LTFM were replaced by FSM, in which lysine (Lys), methionine (Met), and threonine (Thr) were balanced as control diet with crystallized amino acid, and named as FSM25, FSM50, FSM75 respectively. Fish were sampled at 8w and 16w, respectively. Flesh qualities were determined by textural analyzer and near-infrared spectrum (NIR) by the end of 16w.

The results showed that feed intake (FI), weight gain rate (WGR), specific growth rate (SGR), feed conversion ratio (FCR), productive protein value (PPV), productive energy value (PEV), body composition and hematological parameters were not different (P>0.05) between LTFM and FSM25 group, but both of them showed higher growth performance than other groups (P<0.05). Fish fed diet FSM75 showed too low growth performance in first 8w and did not continue the last 8w growth trial for this group. Fish fed FSM50 diet showed a compensatory growth in the latter 8w, which showed significant higher FI, SGR and phosphorus retention than those of LTFM and FSM25 groups during 8-16w, but still showed lower PPV and PEV and higher FCR and nitrogen excretion than those of fish fed diets LTFM and FSM25. Fish of FSM50 group showed lowest hardness, gumminess and chewiness of flesh. Flesh of each group were clearly classified as 3 groups under NIR.

Keywords: Japanese sea bass, fermented soybean meal, fish meal, growth performance, flesh quality, nitrogen and phosphorus metabolism, digestible ideal amino acid pattern

*E-mail address: xuemin@caas.net.cn (Min XUE)*
Growth, nutrient utilization, gut and liver histology of rainbow trout (Oncorhynchus mykiss) fed extruded diets including medium and high levels of lupin and pea concentrate

Zhang, Yuexing1, Øverland, Margareth1, Shearer, Karl D.1, Penn, M.2, Sørensen, Mette1,3, Denstadli, Vegard1, Romarheim, Odd Helge1, Storebakken, Trond.1,*
1Aquaculture Protein Centre, Centre of Excellence, Norwegian University of Life Sciences, Department of Animal and Aquacultural Sciences, P.O. Box 5003, N-1432 Ås, Norway.
2Aquaculture Protein Centre, Centre of Excellence, Norwegian School of Veterinary Science, Department of Basic Sciences and Aquatic Medicine, P.O. Box 8146 Dep, N-0033 Oslo, Norway.
3Nofima Marine, P. O. Box 5003, N-1432 Ås, Norway.

Abstract

Plant proteins represent significant alternatives to fish meal in feed for carnivorous fish. There are, however, still challenges connected with replacing fish protein with plants, such as imbalanced essential amino acid composition, low nutrient concentration, poor palatability, and the existence of anti-nutritional factors (ANF), causing problems in fish digestive tract health and nutrient metabolism. Those problems seem particularly serious in carnivorous fish when plant protein with low degree of processing is used. Some strategies to overcome these problems are: 1) using the supplementation of limiting amino acids to balance the protein; 2) using semi-concentrated or concentrated plant ingredients by removing the undesirable components in the low ingredients by the processes such as dehulling, air-classification, and liquid extraction; 3) using the combination of different ingredients to eliminate negative effects of each single material. A 62-day feeding trial and a followed 20-day digestibility experiment were carried out to evaluate nutritional effects of dietary inclusion of four different plant protein sources (yellow lupin protein concentrate (LPC), pea protein concentrate (PPC), and two combinations of LPC and PPC with the mixing ratio of 1:2 and 2:1) at two different levels of inclusion (30% and 50%) in extruded diets for 58g rainbow trout (Oncorhynchus mykiss). Eight isonitrogenous (47 % crude protein) and isolipidic (23 % crude lipid) diets were made based on four plant proteins (LPC or PPC or combinations) with two inclusion levels, and a high-quality fish meal based control diet (FM control) using high-quality fish meal as the only dietary protein source was also used, in which an elevated dietary protein (53 %) and lipid (25 %) level were adopted to keep the same dietary protein to lipid ratio with an average carbohydrate level as other experimental diets. Each diet was fed to duplicate tanks (40 fish per tank). There were no significant differences among dietary treatments for feed intake. For weight gain (WG), only the 50% LPC group showed a lower value than the other dietary treatments (P < 0.05. Fish fed 30% PPC diet gave a feed conversion ratio (FCR) of 0.70 which was not significantly different from with fish fed FM control diet (FCR=0.68) (P > 0.05). The digestibility of dry matter, protein, fat, starch and most essential amino acids were significantly affected by the plant protein source and their inclusion levels. Under both inclusion level dry matter and starch were increased with decreasing of LPC and increasing of PPC in the diet (P < 0.05). The groups fed with 50%PPC and 50%LPC diet showed the best ADC of protein and lipid individually, and both of these values were significantly higher (P < 0.05) than that of FM control group. There’s no effect on body composition, while the 50%LPC diet reduced (P < 0.05) the content of ash compared with other diets. The retention efficiency of protein and phosphorus were both increased by the inclusion of plant protein concentrate, 30%PPC group and 30% LPC group showed the best protein and phosphorus retention individually, but the energy retention was not affected. In conclusion, pea protein concentrate could provide 50% protein in diet for rainbow trout without showing any adverse effect on growth, digestibility, nutrient retention, and body composition. Using combination has a great potential in elevating the inclusion of the plant protein sources in trout.

Keywords: Lupin protein concentrate; Pea protein concentrate; Combination; Growth; Nutrient utilization; Gut histology
* E-mail address: trond.storebakken@umb.no (Zhang, Y.).
Effects of Fish Meal Partly Replaced with Corn Gluten Meal on Digestive Enzyme Activities in *Fugu Obscurus*

Zhong Guo-fang*, Hua Xue-ming, Han Bin, Zhou Hong-qi
College of Fisheries and Life Science, Shanghai Ocean University, Shanghai, 201306

Abstract

To examine the effects of fish meal partly replaced with corn gluten meal on digestive enzyme activities in *Fugu obscurus*, the fish were fed with diets containing 0%, 5%, 10%, 15% and 20% corn gluten meal in replacement of 0%, 7.4%, 14.8%, 22.2% and 29.6% of conventional fish meal for 60 days, respectively, protease, amylase and lipase activities were examined. The results show that:(1) There was not significant different on trypsin, chymotrypsin and Carboxypeptidase A activities when the corn gluten meal was less than 15% in the feed (P>0.05). The Carboxypeptidase A activity in the 5%, 10% trial group were significantly higher than that of the control group (P<0.05). The leucine aminopeptidase activity of the trial groups were significantly lower than that of the control group. The pepsin activities of the 5%, 10% trial groups significantly higher than that of the control group, but the 20% trial group was lower than that of the control group. (2) Compared with the control group, the lipase activities were significantly improved in the 5%, 10% trial groups but there were not significantly different in 15%, 20% trial groups. (3) The amylase activities of the trial groups were significantly higher than that of the control group (P<0.05). In a word, the appropriate amount of corn gluten meal replaced fish meal can improve digestive enzyme activities, and the optimal digestive enzyme activities in *Fugu Obscurus* was the feed using 10% corn gluten meal, 14.8% of fishmeal protein of the control diet could be replaced by corn gluten meal.

Keywords: *Fugu Obscurus*, protease, lipase, amylase, corn gluten meal

*E-mail address: gfzhong@shou.edu.cn (Zhong Guo-fang)*
Growth performance and feed efficiency of rainbow trout (Oncorhynchus mykiss) juveniles fed on extruded diets with varying levels of Lupine (Lupinus albus), Peas (Pisum sativum) and Raps (Brassica napus)

Adrián Hernández1, 2*, David Román1, 3, Aliro Bórquez1, 2, Patricio Dantagnan1, 2, Javier Alcaino1, Jamie Hooft4

1 Unidad de Nutrición Acuícola, Escuela de Acuicultura, Universidad Católica de Temuco, Casilla 15-D, Temuco, Chile
2 Centro de Genómica Nutricional Agroacuícola (CGNA), Casilla 58-D, Temuco, Chile
3 Dpto. de Producción Animal, Facultad de Ciencias Agronómicas, Universidad de Chile, Santiago, Chile
4 Fish Nutrition Research Laboratory, Department of Animal and Poultry Science, University of Guelph, Guelph, Canada

Abstract

The use of suitable alternative protein sources, such as plant proteins, is crucial for sustainable growth of the aquaculture sector. Plant protein sources are among the best candidates to replace fishmeal in aquafeeds for carnivorous species. However, most of these plant protein ingredients have certain limitations such as lower protein content, low palatability, unbalanced amino acid profile and presence of antinutrient factors. A laboratory growth trial was conducted to evaluate the influence of various plant raw materials as dietary protein sources for rainbow trout juveniles (37.08±3.58 g) on the basis of feed acceptability, survival, growth, feed conversion, protein utilisation, protein digestibility, body composition and histopathological changes. Ten experimental extruded diets were formulated containing different levels of lupine (15, 25, and 35%), raps (10, 20, and 30%) and pea (5, 15, and 25%) meals. The control diet was prepared with fish meal as the sole source of protein. All diets were isonitrogenous (45% crude protein) and isolipidic (17% crude lipid). Triplicate groups were assigned to each experimental diet. The feeding experiment was conducted for 9 weeks, with a continuous water supply at a rate of 0.6-1.0 L/min and water temperature of 14.3±0.4°C. The fish were hand fed three times per day, 6 days a week to apparent satiation level. Daily food consumption was variable. The mean consumption did not vary significantly between plant protein substituted diets. The results of the study showed that growth and feed performance were significantly affected by the type of plant protein as well as level of inclusion. Based on these results, the possibility of using plant ingredients as a partial replacement for fish meal in diets for rainbow trout juveniles is discussed.

Keywords: Growth performance; Rainbow trout; Extruded diets; Lupine; Raps; Peas

* E-mail address: ajhernandez@uct.cl (A. Hernández).
Replacement of fishmeal by plant protein in turbot (*Psetta maxima*): performance, nitrogen budget and histological evaluation

Alessio Bonaldo1*, Luca Parma1, Luciana Mandrioli2, Rubina Sirri2 Ramon Fontanillas3, Wolfgang Koppe3, Pier Paolo Gatta1

1 Dipartimento di Morfofisiologia Veterinaria e Produzioni Animali, Via Tolara di Sopra 50, 40064 Bologna, Italy
2 Dipartimento di Sanità Pubblica Veterinaria e Patologia Animale, Via Tolara di Sopra 50, 40064 Bologna, Italy
3 Skretting Aquaculture Research Centre, Box 48, N-4001 Stavanger, Norway

Abstract

The aim of the present study is to determine the effects of fish meal substitution by plant protein (soybean meal, wheat gluten and corn gluten) in turbot *Psetta maxima* juveniles’ diet in terms of growth, nitrogen budget and gut histology. Four isoproteic and isolipidic diets (protein: 53%, lipid: 22%) were manufactured using extrusion technology and common feed ingredients. Diets were formulated containing 55 (FM55), 45 (FM45), 35 (FM35) and 25% (FM25) fishmeal (FM). 480 juveniles (initial body weight: 23-24g) were equally distributed into twelve 450 liters tanks connected with a recirculation system (temperature 18°C±1, photoperiod 12hrs of light, dissolved oxygen above 6.5 ppm). Animals have been hand-fed twice a day to apparent satiation during the week and once on Sunday. Diets were tested in triplicate for 77 days. At the beginning and at the end of the trial, fish were weighed to determine performances and pools of animals were taken for carcass proximate composition analysis, nutrient retention calculation and histopathological gut examinations. At the end of the growth trial, ammonia excretion has been also measured during a 24 hrs-cycle. One-way ANOVA with Newmann – Keuls’ post test were used to analyze data (P≤0.05). No differences in terms of palatability were observed and feed intake was similar among groups (from 1.14 to 1.19 % day\(^{-1}\)). At the end of the experiment, final weight ranged from 126.6 (FM55) to 99.5 g (FM45). A significant decreasing of specific growth rate (SGR) was observed from fish fed diet FM55 (2.14 % day\(^{-1}\)) to those fed diets FM35 and FM25 (2.01 and 1.82 % day\(^{-1}\), respectively) together with an increasing of feed conversion rate (FCR) (0.65 vs. 0.70-074). Fish fed diet FM45 showed intermediate values for both indices (SGR: 2.09 % day\(^{-1}\); FCR: 0.67). No statistical differences were found in terms of PER or GPE between fish fed diet FM55 and FM45. Fish fed diet FM55 presented a lower ammonia excretion in comparison with those fed diet FM 25 (2.61 vs. 3.41 g N-NH\(_4\) 100g protein intake\(^{-1}\)). Experimental diets influenced condition factor and viscerosomatic index but not hepatosomatic index. Carcass proximate composition was similar among groups. Histological evaluation of the three intestinal segments showed in all groups examined scattered cells identified as lymphocytes and macrophages present in the lamina propria, that was rich in capillary network and loose connective tissue. The histological evidence of gut segments that was similar in all groups cannot support the finding of the growth reduction observed in groups fed diets FM35 and FM25. The results of this trial suggest that a minimum level of 45% of FM is optimal for growth and protein utilization of turbot. Further studies are needed, most probably on aminoacid availability and antinutritional factors, to define efficient diets with an higher content of plant ingredients.

Keywords: Turbot; *Psetta maxima*; Fish meal substitution; Growth; Gut histology

\* E-mail address: alessio.bonaldo@unibo.it (A. Bonaldo).
Organic vegetable proteins and oil in feed for organic rainbow trout
(*Oncorhynchus mykiss*)

Ivar Lund1, Johanne Dalsgaard1*, Alfred Jokumsen1, Bodil K. Larsen1
1 Technical University of Denmark, DTU Aqua, Section for Aquaculture, The North Sea Research Centre, P.O. Box 101, DK-9850 Hirtshals, Denmark

Abstract

The demand for organic trout is increasing, stressing the need for organic, vegetable feed ingredients as replacement for fish meal, as the principles of organic aquaculture encourage the development of feed that do not deplete global fish stocks. In addition, the organic code of practice does not allow addition of artificial amino acids to the feed, and optimization of the amino acid profile of organically based diets must therefore derive from the protein sources alone.

The aim of this study was to evaluate the digestibility and growth performance of organic vegetable dietary ingredients as replacement for fish meal and fish oil in feed for organic rainbow trout (*Oncorhynchus mykiss*).

Six iso-energetic and iso-nitrogenous diets were prepared, comprising a fish meal and fish oil based control diet and three diets in which the inclusion of fish meal was gradually reduced from 59 to 35 % and replaced by a matrix of organic horse bean, pea and rape in the proportion of 1:1:0.7. In the last two diets, the inclusion of fish oil was reduced by 50 and 100 %, respectively and replaced by flax seed oil high in omega-3 fatty acids.

Digestibility was measured directly using a modified, flow-through Guelph System consisting of 18 tanks, and feeding each diet in triplicate. Growth performance was measured using a recirculation system consisting of 12 square formed fibreglass tanks. The fish were reared in duplicate for 9 weeks, from an initial individual weight of about 60 g to a final weight of about 200 g.

The fish showed good growth performance with a specific growth rate (SGR) of 1.8 % d⁻¹, and a feed conversion ratio (FCR) of 0.75, and there were no significant differences between the groups. Likewise, there were no significant differences in nutrient digestibility between the diets.

The results indicate that a matrix of organic horse bean, pea and rape may partially replace fish meal, and flax seed oil may replace fish oil in feed for organic rainbow trout without compromising growth performance and feed utilization.

Keywords: Rainbow trout; Organic protein; Organic oil; Horse bean; pea; Rape; Flax seed oil; Digestibility; Growth; Feed utilization.

*E-mail address: jtd@aqua.dtu.dk*
P-037

Applications of apple snail meal as alternative protein source in white shrimp (Penaeus vannamei) feed

Anut Kiriratnikom1*, Suphada Kiriratnikom2, Punthasit Choksawatdikorn2 and Kritsana Ruengklay2

1 Division of Environmental Science, Faculty of Science, Thaksin University, Phatthalung Campus, 93110 Thailand.
2 Aquacultural Biotechnology Research Unit, Faculty of Science, Thaksin University, Phatthalung Campus, 93110 Thailand.

Abstract

The feeding trials were undertaken to determine the effects of substitution of shrimp head meal, squid meal and fish meal with golden apple snail meal in white shrimp (Penaeus vannamei) feed. In trial I, Six test diets with 40% protein were formulated by substitution each sources of dietary protein with golden apple snail meal. Diet I was the control without golden apple snail meal supplementation, diet II-V was substituted of squid meal, shrimp head meal, either squid meal and shrimp head and fish meal with golden apple snail meal. Diet VI using golden apple snail meal as single protein source. The shrimp (average weight 1.80-1.90 g) were rearing in 100 l glass aquaria (15 shrimp per aquaria, 3 replications per treatment) feeding the shrimp with each test diet 3 times daily for 8 weeks. At termination of feeding trial, average body weight, growth rate, survival, FCR, and chemical body composition of the shrimp fed diet 1-5 were not significantly different among treatment (P>0.05). Moreover, PER, ANPU of the shrimp fed diet IV and diet V were higher than the group fed control diet. Whereas the average body weight of the shrimp fed test diet replaced all protein sources with golden apple snail meal (diet VI) was decreased and lower than other treatments (P<0.05). The result indicated the possibility of partial replacement of protein sources with golden apple snail meal in the diet for white shrimp. Trial II was conducted to determine the effects of golden apple snail meal levels in white shrimp diet. Five test diets were prepared with different levels of golden apple snail meal. Diet I was the control without golden apple snail meal supplementation, diet II-V were substitution of fish meal with 25,50,75 and 100% golden apple snail meal. Average body weight, growth rate, survival, FCR, PER, ANPU and chemical body composition of the shrimp fed diet contained 0-75 % substitution of fish meal with golden apple snail meal (diet I-IV) were not significantly different among treatment (P>0.05). Whereas the average body weight of the shrimp fed diet V (substitution of fish meal with 100% golden apple snail meal) was decreased and lower than other treatments (P<0.05). In conclusion, golden apple snail meal shown high possibility for applied as alternative protein source in white shrimp diet. The optimum level was 50.61 % golden apple snail meal and 11.25 % fish meal in the practical diet.

Keywords: White shrimp; Alternative protein source; Golden apple snail

* E-mail address: ksuphada@yahoo.com
The effects of feeding black or yellow seeded canola as a partial replacement for fishmeal in practical diets fed to rainbow trout

C. Zhang1*, D. M. Anderson2, M. D. Drew1

1 Department of Animal and Poultry Science, 51 Campus Drive, University of Saskatchewan, Saskatoon, Canada
2 Department of Plant and Animal Science, Nova Scotia Agricultural College, Truro, Canada

Abstract

Yellow seeded varieties of canola have lower amounts of NDF (19% vs. 26%) than black seeded varieties and may be a better ingredient for use in rainbow trout diets. The nutrient digestibilities of black and yellow seeded canola were measured. Rainbow trout (initial body weigh 44 g) were randomly assigned to 12 x 360 l tanks (N = 30 per tank). A reference diet containing 1% celite as an indigestible marker and test diets composed of 70% of the reference diet and 30% of the test ingredients were fed. Feces were collected using a settling column and feed and feces analyzed for digestible dry matter, energy, and crude protein. The chemical analysis of the ingredients indicated that the black seeded canola (BSC) was lower in gross energy (29.5 vs. 30.2 MJ/kg) and lipid (439.9 vs. 508.1 g/kg) than the yellow seeded canola (YSC), but higher in crude protein (226.4 vs. 221.6). The apparent digestibility coefficients of dry matter (0.66 vs 0.71), energy (0.74 vs 0.77) and crude protein (0.88 vs 0.90) of BSC were significantly lower than those of YSC. Subsequently, an eight-week feeding trial was conducted to examine the impact of feeding black seeded canola meal (BSCM) or yellow seeded canola meal (YSCM) on the growth performance of rainbow trout. Five isonitrogenous and isocaloric diets were formulated. The control diet (FM) contained 35% fishmeal and the test diets consisted of BSCM included at 20% and YSCM included at 15, 20 and 25% as partial substitutes for fishmeal. Each diet was fed to four tanks of rainbow trout (average initial weight of 3.6g; N = 30/tank) and the fish were fed to apparent satiation 3 times daily. At the end of the growth trial, fish fed the 20% BSCM had lower mean weight gain (62.0) than fish fed FM (75.2g) or 25% YSCM (75.2g) (P < 0.05). The weight gains of fish fed the YSCM diets were not significantly different than those fed the FM diet. Fish fed the FM (2.87%/d) and the 25%YSCM (2.84%/d) had the highest specific growth rates; significantly greater than fish fed the 20% BSCM diet (2.65%). Fish fed the 25% YSCM (0.99) or FM (0.99) had feed conversion ratios significantly better than fish fed the 20% BSCM (1.18). In conclusion, YSC has higher levels of digestible nutrients than BSC and supports significantly better growth performance when used to replace FM in rainbow trout diets.

Keywords: Black seeded canola; Yellow seeded canola; Digestibility, Growth performance; Rainbow trout

* E-mail address: chz556@mail.usask.ca (C. Zhang).
Influence of dietary vegetable oils on energy storage and gonad development in gilthead seabream, *Sparus aurata*

Wahbi¹, O.M; Shalaby², S.H. and Wassef⁎², E.A.
¹Fish Reproduction Laboratory, National Institute of Oceanography and Fisheries (NIOF), Kayet-Bey St., Anfousy, Alexandria, Egypt
²Fish Nutrition Laboratory, National Institute of Oceanography and Fisheries (NIOF), Kayet-Bey St., Anfousy, Alexandria, Egypt

Abstract
This study was conducted to investigate the influence of two dietary vegetable oils (VO) mix on the lipogenesis process, tissue lipid uptake, lipid composition and gonad development in gilthead seabream, *Sparus aurata* (mean I.W ~ 131g) during the grow-out phase. Fish were fed 3 experimental diets: diet A (control, CTR) contained fish oil as the sole lipid source (100%FO), whereas in diets B&C 60% of FO was substituted by a VO-mix of: cottonseed oil, CO; sunflower oil, SO & either linseed oil, LO (1:1:1, w/w) or soybean oil, BO respectively. Fish were fed in triplicate groups for each dietary treatment. The feeding trial had lasted 20 weeks in seawater tanks at a mean ambient temperature about 27°C, in triplicate groups for each dietary treatment. Results have shown that partial replacement of FO by VO-mix with the BO only had an effect on lipogenesis process in liver. Activities of Glucose-6-phosphate dehydrogenase (G6PDH), and Malic enzyme (ME) were significantly (P<0.05) higher in fish fed diet C than in CTR fish, whereas activity of fatty acid synthetaze (FAS) hasn't changed among treatments. Hepatosomatic and gonadosomatic indices (HSI & GSI) were significantly higher in fish fed VO-diets. Tissue (liver & gonad) lipid uptake modification and a hypocholesterolemic effect were suggested. Lipid content of both liver and gonads in fish fed VO had increased significantly (P<0.05) as compared to those of CTR fish and fish fed diet C had the highest values among all dietary treatments. Fatty acid profiles of gonads of fish fed VO-diets showed relatively lower highly unsaturated fatty acids (HUFA) content, as compared to that of CTR fish, leading to imbalanced ratios of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and arachidonic acid (ARA). Fish fed either diet B or C had a reduced serum steroids level as compared to that of fish fed diet A. Fish fed diet C recorded the significantly lowest serum estradiol level among all treatments. Histological examination of gonads has indicated a relative maturity-retardation stage in fish fed VO-diets compared to that fed CTR diet. Results suggested that diet B (with LO) is relatively better than diet C (with BO) in terms of gonad development, suggesting a 100% FO finishing diet for gilthead seabream broodstock after long term feeding with VO's mix.

Keywords: *Sparus aurata*; Vegetable oils; Liver lipogenic-enzymes; Sex-hormones; Gonad histology

⁎E-mail : elamalywa2007@yahoo.com (E.A.Wassef)
Effect of replacement of dietary fish meal by soybean meal on growth performance and enzyme activities in gibel carp, *Carassius auratus gibelio*

HaoKun LIU *,1,2, Xiaoming ZHU1, Yunxia YANG1, Dong. HAN1, Shouqi. XIE1

1 State Key Laboratory of Freshwater Ecology and Biotechnology; Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China
2 Graduate School of the Chinese Academy of Sciences, Beijing 100039, China

Abstract

Ten weeks experiment was conducted to determine the effects of soybean meal (SBM) based diet on growth performance, feed utilization, nitrogen budget, blood physiology and digestive enzymes of gibel carp (*Carassius auratus gibelio*). Seven isonitrogenous and isoenergetic (37.82%, 16.02 kJ/g DM) diet were formulated to contain different SBM protein (0, 20, 40, 60, 80 and 100% of diet protein) (Control, SB20, SB40, SB60, SB80, SB100). In order to test the effect of dietary amino acid composition, crystalline amino acids (CAA) coated with carboxymethyl cellulose were added to 100% SBM diet and make a new diet to meet the composition of fish muscle amino acid (SBAA).

The results showed that, during first 30 days, feeding rate decreased with increased dietary SBM. Additive amino acids increased feeding rate. During later 40 days, feeding rate slightly increased with dietary SBM. Specific growth rate decreased with dietary SBM while the fish fed with 20% SBM protein (20SB) obtained the highest feed conversion efficiency and protein retention efficiency. Apparent digestibility coefficients of dry matter decreased while those of protein increased with dietary SBM. Hepatosomatic index and plasma kaline phosphatase activity increased while blood plasma total cholesterol, glutamic-oxaloacetic transaminase activity, glutamic-pyruvic transaminase activity decreased with increased dietary SBM. Plasma triglycerides showed highest value in the fish fed with 40% SBM protein. Midintestine trypsin, amylase and gltamytranspeptidase activity decreased. Supplemental amino acids increased glutamic-oxaloacetic transaminase activity and gltamytranspeptidase activity.

Keywords: Soybean meal; Gibel carp; Protein replacement

* E-mail address: lhk315@gmail.com (H. Liu).
Dietary hempseed meal and hempseed oil enhanced growth performance in juvenile turbot (Scophthalmus maximus)

Paulo Rema¹, Kátia Pedrosa¹, Giampaolo Grassi², Jorge Dias³*
¹ CIIMAR-CIMAR L.A., Centro Interdisciplinar de Investigação Marinha e Ambiental and Universidade de Trás-os-Montes e Alto Douro, Quinta dos Prados, P.O. Box 1013, 5001-801 Vila Real, Portugal.
² Centro di Ricerca per le Colture Industriali (CRA-CIN), Sede Distaccata di Rovigo, Via Amendola 82, 45100 Rovigo, Italy.
³ SPAROS Lda. & CCMAR-CIMAR L.A., Centro de Ciências do Mar, University of Algarve – Campus Gambelas, 8005-139 Faro, Portugal.

Abstract

A trial was undertaken to evaluate the effect of incorporating moderate levels of hempseed meal and hempseed oil as alternative ingredients to fishmeal and fish oil in diets for juvenile turbot, in terms of growth performance, feed intake, protein digestibility and body composition. Four isoproteic (crude protein, 50% DM) and isolipidic (15% DM) experimental diets were formulated to fulfil the nutritional requirements of juvenile turbot. A fishmeal and fish oil based diet was used as control (FMFO). In comparison to this control formulation, in the other three diets, fishmeal was replaced at a 40% level by dehulled hempseed meal (HMFO), fish oil was replaced at a 44% level by hempseed oil (FMHO) and both fishmeal and fish oil were concomitantly replaced at 40 and 44% levels by hempseed meal and hempseed oil (HMHO). Homogenous groups of 15 turbot (IBW: 27.5 g) were stocked in rectangular tanks (volume: 60 L; water-flow rate: 2.5 L·min⁻¹), supplied with recirculated seawater (temperature: 18±1°C; salinity: 33-34 g·L⁻¹, dissolved oxygen above 7 mg·L⁻¹). Each dietary treatment was tested in duplicate tanks over 77 days. Fish were fed to apparent satiety, by hand, twice a day (09.30 and 15.30) and feed intake was recorded on a weekly basis. In comparison to the fishmeal and fish oil control diet (FMFO), the dietary incorporation of hempseed meal and oil, either alone or in combination significantly enhanced (P<0.05) the daily weight gain and SGR values. No differences regarding these criteria were found among the treatments containing hempseed products. Fish fed diets with hempseed meal and/or oil showed significantly higher feed efficiency values (P<0.05) than those fed the control diet. Apparent digestibility of protein was not affected by dietary treatments. In terms of the whole-body composition of fish at the end of the trial, protein content was little affected by dietary treatments, while fat levels were significantly reduced in turbot fed the diets containing hemp oil (FMHO, HMHO). The fatty acid profile of turbot muscle at the end of the trial reflected that of diets, but muscle TBARS values were similar among the various treatments. Additionally, levels of δ-9-tetrahydrocannabinol (THC) were measured by HPLC in muscle samples from all treatments, and values were below the detection limit of 1 mg/kg.

Keywords: Turbot; Vegetable ingredients; Hempseed products; Growth performance

* E-mail address: jorgedias@ualg.pt (J. Dias).
Effects of dietary Makgeolli by-products on growth performance and body composition of juvenile olive flounder, *Paralichthys olivaceus*

Ki-Min Bae¹*, Sang-Min Lee¹, Suhee Hong¹, Kyoung-Duck Kim²

¹Faculty of Marine Bioscience and Technology, Gangneung-Wonju National University, Gangneung 210-702, South Korea
²Aquafeed Research Center, National Fisheries Research and Development Institute, Pohang 791-923, South Korea

Abstract

Nutritional quality of ingredients can be improved by fermentation process due to partial or complete elimination of anti-nutrient factors. Makgeolli by-product is a solid residue obtained by filtration of an aqueous mixture of fermented rice with *Aspergillus oryzae* and yeasts in production of Makgeolli which is traditional Korean wine made from fermented rice. Makgeolli by-product has great potential for animal feed because of its high protein content and well-balanced amino acid profile. Although, Makgeolli by-product is used as a feedstuff for domestic animals including pig and chicken, but there is no available information on the utilization of Makgeolli by-product for fish. Six isonitrogenous (50% crude protein) and isocaloric (4.3 kcal/g) diets (designated as M0, M7, M14, M21, M28 and M35) were formulated to contain Makgeolli by-products (0%, 7%, 14%, 21%, 28% and 35%). Juvenile flounder (averaging weight 9.6 ± 0.2g) were randomly distributed in eighteen 300L tanks in a flow-through system. Three replicate groups (30 fish/group) of fish were fed one of the six experimental diets to visual satiety, twice a day for 8 weeks. No significant differences were observed in weight gain, specific growth rate, daily feed intake and feed efficiency were observed in fish fed the M0, M7, M14 and M21 diets. The values of fish fed the M28 and M35 diets were lower than those of fish fed the control diet. Body composition of fish was not affected by dietary Makgeolli by-product level. The results of this experiment suggest that dietary supplementation of 14-21% Makgeolli by-products in the formulated diet do not affect growth performance and body composition of juvenile flounder.

Keywords: Flounder; *Paralichthys olivaceus*; Feed ingredient; By-product

*E-mail address: smlee@gwnu.ac.kr (S.M. Lee)
Lipid digestibility in feeds for Atlantic salmon (Salmo salar L.) containing different fish oil sources

Kjell A. Måsøval1*, Patrick C. Campbell2
1BioMar AS, 7011 Trondheim, Norway
2BioMar Ltd, Grangemouth Docks, Grangemouth FK3 8UL, Scotland

Abstract

Lipids coming from fish oils or vegetable oils are major sources of energy in high energy feeds in the salmon aquaculture industry. Both fatty acid profile and temperature are known to affect lipid digestibility in salmon. The objective of this study was to study the influence of both of these parameters and how they interact on lipid digestibility in order to maximise the energy available to the fish from different fish oils at different temperatures. Three different fish oils were used in this experiment, a Scandinavian fish oil (NH FO), a South American fish oil (SA FO) and a Menhaden fish oil (MH FO). These fish oils differ markedly in content of saturated fatty acids (SAFAs) and in other fatty acids, and were chosen because these differences are shown to have an effect on lipid digestibility. The feeds differed only in oil source used and were designed to make regressions where rape oil substituted NH FO, SA FO or MH FO resulting in 3 regressions with 0, 4.8, 9.6 and 14.4% dietary inclusions of rape oil in feeds with a total of 24% added oil. The content of SAFAs for the 4 dietary regressions varied from 13 to 18% (as percentage of total fatty acids), 15 to 25% and 17 to 29% for the NA FO, SA FO and MH FO regressions, respectively. The experiment was conducted with Atlantic salmon (1.8 kg start weight and 3.5 kg end weight) in cages in seawater and the temperature varied from ~4°C to ~11°C. In the trial the mean thermal growth coefficient (TGC) and feed conversion ratio (FCR) for the different treatments varied from 2.9 to 3.5 and 0.94 to 1.04, respectively. Lipid digestibility was analysed at two different temperatures (~5 and 11°C) in the experiment. There was a negative correlation between lipid digestibility and content of saturated fatty acids at both temperatures. At 5°C the lipid digestibilities varied from 85.4% to 97.0% and at 11°C the lipid digestibilities varied from 93.1% to 98.2%, increasing most in diets with highest levels of SAFAs when temperature increased. The results will be further discussed in relation to how the fatty acids profile affects the lipid digestibility and how the results can be used to optimise the use of different oils at different temperatures.

Keywords: Atlantic salmon; Fish oils; Fatty acid profile; Lipid digestibility

E-mail address: kjell.maasoval@biomar.no (K.A. Måsøval)
Effects on growth of adding yeast (*Saccharomyces cerevisiae*) to diets with high contents of soybean meal to fed juvenile rainbow trout *Oncorhynchus mykiss*

Laboratorio de Producción Acuícola, UNAM FES Iztacala, Av. de los Barrios 1, Los Reyes Iztacala, Tlalnepantla, Estado de México., C.P. 54090, México

**Abstract**

Use of soybean meal as substitute of fish meal on aquafeeds is common. However, levels of substitution are still low (less than 25%), because of its certain nutritional characteristics (low levels of some indispensable amino acids and high content of carbohydrates) and the presence of some antinutritional factors, such as phytic acid. To improve the use of higher levels of soybean meal on aquafeeds, the aim of this research was to use the yeast *Saccharomyces cerevisiae* in diets with high content of soybean meal for juveniles of rainbow trout *Oncorhynchus mykiss*. Three experimental diets (40% protein, 10% lipid, 10% moisture) with a 75% of substitution of fish meal with soybean meal and adding 1.5% of yeast (diet 1); 1.5% of yeast + 0.8% of phytase (diet 2) and 0.8% phytase (diet 3). A diet with 100% of fishmeal and a commercial diet were used as controls. Each diet was fed to triplicate groups of 15 juveniles of rainbow trout with an initial weight of 1.2 ± 0.02 g (mean ± S.D.) for a period of 60 days. Growth data were analyzed with a one-way ANOVA and statistical differences were evaluated with a Tukey-Kramer test. Data on weight gain (WG) and specific growth rate (SGR) showed a significant lower value of the fish fed with diet 1 (687%) when compared with diets 2 (801%), 3 (843%) and the control with 100% fishmeal (1081%). The higher WG among the treatments was observed on diet 3, but no significant differences were observed with respect the diet with yeast (diet 2). Survival rate of all groups showed a minimum percentage of 90%. Growth data indicate that use of yeast on diets with high supplementations of plant meals, might be helpful in the utilization of soybean meal in rainbow trout.

**Keywords:** rainbow trout; soybean meal; yeast; growth; phytase

*E-mail address:* hernandezh@campus.iztacala.unam.mx
Use of earthworm meal as substitute of fish meal: effects on the growth of juveniles of rainbow trout *Oncorhynchus mykiss*

Laboratorio de Producción Acuícola, UNAM FES Iztacala, Av. de los Barrios 1, Los Reyes Iztacala, Tlalnepantla, Estado de México., C.P. 54090, México

Abstract
Fish meal is an important ingredient of aquafeeds, but recently it has becoming an expensive and limited resource as its demand is rising. Several ingredients have been proposed as substitutes of fish meal and among them, the Californian red earthworm (*Eisenia foetida*) meal has a high protein content and a reasonable good amino acid profile. For these reasons, the main objective of the present research was to determine the effect of three levels of substitution of fish meal with red earthworm meal on the growth performance of juvenile rainbow trout *Oncorhynchus mykiss*. Diets (40% protein, 10% lipid and 10% moisture) were prepared with substitutions of 25, 50 and 75% of fish meal with earthworm meal. A diet with 100% of fishmeal and a commercial diet were used as controls. Each diet was fed to triplicate groups of 15 juveniles of rainbow trout with an initial weight of 1.2 ± 0.02 g (mean ± S.D.) for a period of 60 days. Growth data were analyzed with a one-way ANOVA and statistical differences were evaluated with the Tukey-Kramer test. The weight gain (WG) did not show significant differences among the diets with 25 (925%), 50 (814%) and 75% (866%) substitution; but a tendency of lower vales was observed as earthworm meal increased on the diet. A similar behavior of data was observed for the specific growth rate. The control with 100% fishmeal was significantly higher than the rest of the groups. Survival rate was above the 90% on all the groups. The data of the present research indicates the possibility of use red earthworm meal as a protein source without affecting significantly the growth performance up to a level of 75%.

Keywords: rainbow trout; earthworm meal; growth; fish meal; *Eisenia foetida*

*E-mail address: hernandezh@campus.iztacala.unam.mx*
P-046

Effect of replacing soybean meal with fish meal on growth performance and body Composition at Red Pacu fish (*piaractus brachypomus*)

M. Saedi1,∗ M. M. Sajjadi2, H. Hosseinzade3, H. Emadi4

1Department of Fisheries, Agriculture Faculty, Hormozgan University of Iran
2Department of Biology, Science Faculty, Hormozgan University of Iran
3Department of Aquaculture, Iranian Fisheries Research Organization
4Department of Fisheries, Marine Science and Technology Faculty, North Tehran Azad University

Abstract

Eight week feeding experiment was conducted to evaluate the effects of replacing Soybean Meal (SBM) with Fish Meal (FM) on growth performance, body composition of Red Pacu (*Piaractus brachypomus*). Five isonitrogenous and isocaloric diets (about 32% crude protein and 3600 kcal/kg energy) were formulated. The control feed was formulated to contain 50% fish meal (P1), whereas in the other four feeds SBM was included at 16.5%, 33.5%, 49.5% and 66.0% to replace 25%, 50%, 75% and 100% with fish meal protein (P2 to P5 respectively). 195 Fingerling (initial body weight of 1.8±0.07g) were equally distributed into fifteen aquariums (60×30×40 cm filled with 60 liter water) with stocking 13 fish at any aquarium. Fishes were fed their respective diets for 8 weeks, twice daily (9.00 and 17.00) until satiation to investigate the growth performance. The fish readily accepted all five experimental diets and any fish did not dye during the experiment. We saw a significant difference fish Weight Gain (WG), Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER) and Specially Growth Rate (SGR) factors in treatments; When the replacement level soybean meal protein with fish meal protein.

Diet of P2 and P3 had significantly differences in feed intake, WG, SGR and PER to other diets that P2 and P3 had high amounts. FCR factor in P5 treatment diet had significant differences to other diets and this amount was lower than other treatment diets, and Significant differences were in carcass protein, lipid, ash, and moisture content was found among the treatments, as fish fed the P3 replaced 50% of the fish meal protein (33.5% soybean meal in diet) had lower amount this factors in carcass than those of fish fed the control feed and other diets contain SBM. The results of the present study indicated that soybean meal can replace fish meal at the level of 100% of fish meal protein in diets for Red Pacu with good growth and feed utilization, but using about equal of plant protein (soybean meal) and animal protein (fish meal) is better in Red pacu diets.

Keywords: Red Pacu (*piaractus brachypomus*); diet replacement; soybean meal; growth performance; protein sources

∗E-mail address: saedi.majid@yahoo.com (M. Saedi)
Study Replacement of soybean meal by canola meal on growth performance and body composition of rainbow trout (Oncorhynchus mykiss) in pre-growing stage

Alizadeh Morteza¹, R.Mahmoudi²
¹Inland Brackishwater Fishes Research Station, POBOX 89715-1123, Bafgh, Iran
²Cold water Fishes Research Center, Yasuj, Iran

Abstract

This research was carried out in coldwater fish research center in Yasuj. In this study, 5 dietary treatments including A, B, C, D, E and F were formulated on base of replacing 0, 25, 50, 75, and 100 % of soybean meal by canola meal respectively. All of the experimental diets were iso-caloric and iso-nitrogenous. 450 rainbow trout with average initial weight of 10 grams were stocked randomly in fifteen 100lit flow trough fiberglass tanks, so each experimental diet was fed to triplicate groups of 30 fish. Fishes were fed for 9 weeks at a rate between 3.5-5% of body weight per day in three equal amounts, adjusted weekly. Values of water temperature, dissolved oxygen and pH were between 12.5±1°C, 8.9±0.5 mg/L, 7.8±0.3, respectively. At the end study, growth performance by determination of weight gain, total feed intake, feed conversion ratio, protein efficiency ratio, condition factor and survival rate were evaluated. Comparison of measured factors by Duncan test demonstrated the better result in WG, PER and DGR through replacing 75% soybean meal by canola meal (treatment D), although there were no significant differences about growth factors between treatments (p>0.05). According to results replacement of soybean meal by canola meal had no significant difference on body moisture and crude fat among treatments, while crude protein and crud fiber were affected through this replacement. It was also cleared that by increasing canola meal in diets, the cost of final food is reduced. This study showed that canola meal has potential to replacing with soybean meal in diet of juvenile rainbow trout without special negative effect on growth and body composition.

Keywords: canola meal; soybean meal; replacement; Rainbow trout; Oncorhynchus mykiss; body composition; Growth.

*E-mail address: m_alizadeh47@yahoo.com (M.alizadeh).
Effects of dietary cottonseed and/or canola oil inclusion on the growth performance and fatty acid composition of the juvenile rainbow trout, *Oncorhynchus mykiss*

Mustafa Yıldız1*, O.Tufan Eroldoğan2, Kenan Engin3, Muhammed A. Baltacı4

1 Istanbul University, Fisheries Faculty, Department of Aquaculture, Ordu cad. No:200, 34470 Laleli, Istanbul-Turkey.
2 Cukurova University, Fisheries Faculty, Department of Aquaculture, 01330, Balcalı, Adana-Turkey.
3 Mersin University, Fisheries Faculty, Department of Aquaculture, 33169, Yenişehir Kampüsü, Mersin-Turkey.
4 Istanbul University, Fisheries Faculty, Sapanca Inland Waters Research Center, Kurtköy, Sapanca, Adapazari-Turkey.

Abstract

This study was undertaken to evaluate the possibility of replacing fish oil by cottonseed oil (CSO) and/or canola oils (CO) in diets for the juvenile rainbow trout. Five iso-nitrogenous, iso-lipidic and iso-energetic diets (45 % CP, 17 % CL and 20 MJ/kg on a dry matter basis) were formulated to contain cotton seed oil and canola oils replacing fish oil either totally or in different combinations in diets. The control diet contained only anchovy oil (FO) whereas diets initialed as CSO and CO contained only cotton seed oil and canola oil as dietary oil sources respectively. However in the fourth and fifth dietary treatments, fish oil was replaced by 50% (FO50/CSO25/CO25) and 100 % (CSO50/CO50) by cotton seed oil and canola oils in equal amounts. Duplicate groups of 50 rainbow trout (~16 g individual weight) held under similar culture conditions (~13.6 °C water temperature; ~8.4 mg/L dissolved oxygen; 12-h light/12-h dark photoperiod) and were fed two times daily to apparent satiation. The results showed that fish weight gain increments (WGI), specific growth rate (SGR), feed intake, feed efficiency (FER) and protein efficiency ratio (PER), and viscerosomatic index (VSI) were not influenced by dietary treatments. However, hepatosomatic index (HSI) was significantly higher (1.70 and 1.76, respectively) (P<0.05) in fish fed with CSO and CSO50/CO50 diets than in those fed with the other experimental diets. Whole body proximate composition of fish was also influenced by dietary treatments. Fish fed CSO and CO had the highest crude lipid (13.5% and 12.8%, respectively) and the lowest moisture contents (68.5% and 69.2%, respectively) (P<0.05). The replacement of dietary FO by CSO or CO produced fish that had significantly lower levels of n-3 highly unsaturated fatty acids (HUFA) and increased the levels of 18:2n-6 in the whole body fatty acid composition (P<0.05). The n-3:n-6 fatty acid ratio in the whole body fatty acid composition of fish fed the FO diet was 2.5 compared with 0.2-0.8 in fish fed the CSO and CO diets and their combinations (P<0.05). However, liver lipid fatty acid compositions indicated that docosahexaenoic acid (DHA) and arachidonic acid (ARA) were preferentially incorporated and regulated, which in return resulted in relatively small levels of difference among experimental groups compared to what was found in whole body lipid fatty acid compositions. These results suggest that the 50 % replacement of dietary fish oil by cotton seed oil and canola oils in equal amounts could be used in diets for rainbow trout without compromising in growth parameters and whole body and liver fatty acid compositions compared to fish oil only diet.

Keywords: Rainbow trout, Nutrition, Cottonseed oil, Canola Oil, Fatty acids, Growth performance.

*E-mail addresses: mstar@istanbul.edu.tr, musstar@gmail.com (M. Yildiz).
Effects of dietary total replacement of fish oil by vegetable oils on flesh quality of juvenile and growing flounder, *Paralichthys olivaceus*: Recovery of fatty acid profiles by a fish oil finishing diet

Sang-Min Lee1*, Dong-Gyu Kim1, Hee-Guk Byun1, Minh Anh Pham1, Kyoung-Duck Kim2

1Faculty of Marine Bioscience and Technology, Gangneung-Wonju National University, Gangneung 210-702, South Korea
2Aquafeed Research Center, National Fisheries Research and Development Institute, Pohang 791-923, South Korea

Abstract

This study was conducted to evaluate the effects of dietary total replacement of fish oil by vegetable oils on growth performance and flesh quality of flounder (*Paralichthys olivaceus*). Four experimental diets (designated as FO, LO, SO, MIX) were formulated to contain 5% squid liver oil, 5% linseed oil, 5% soybean oil and a mixture of 1% squid liver oil, 2% linseed oil and 2% soybean oil, respectively. Juvenile (average weight, 8.8±1.2 g) and growing (average weight, 121±1.4 g) flounder were randomly distributed into tanks with flow-through system. Three replicate groups of fish were fed one of the experimental diets to visual satiation, twice a day for 15 weeks. At the end of growth trial, fish fed LO or SO diet during feeding trial were fed FO finishing diets and fish fed FO during feeding trial were fed the LO and SO diet for 12 weeks to determine the progressive recovery of the body fatty acid profiles.

At the end of 15-week feeding trial, no significant differences were observed in weight gain and feed efficiency of juvenile and grower flounder fed the experimental diets. Blood total cholesterol was higher in fish fed the FO diet. Higher concentration of C18:2n-6 in muscle was observed in fish fed the SO diets. Fish fed the LO diet had the highest concentration of C18:3n-3. Concentrations of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in fish fed the FO diet were higher than those of fish fed other diets. EPA and DHA concentrations in dorsal muscle were not adequate recovered by feeding of finishing diets for 12 weeks. But EPA and DHA concentrations in liver and visceral were almost comparable to those at the end of the feeding trial.

Keywords: Flounder, *Paralichthys olivaceus*; Lipid source; Fatty acids; Finishing diet

* E-mail address: smlee@gwnu.ac.kr (S.M. Lee)
Evaluation of *Calanus* Copepod and *Euphausia* Krill meal and Oil as the Dietary Protein and Lipid Supplements for Juvenile Atlantic Halibut (*Hippoglossus hippoglossus*)

Stefanie M. Colombo¹², Rolf E. Olsen³ and Santosh P. Lall²*
¹ Department of Biology, Dalhousie University, Halifax, Canada B3H 4J1
² National Research Council, Institute for Marine Biosciences, Halifax, Canada B3H 3Z1
³ Institute of Marine Research, N-5984 Matredal, Norway

Abstract

The *Calanus* copepod (*Calanus finmarchicus*) and Antarctic krill (*Euphausia superba*) are considered potential feed ingredients for marine fish feeds particularly as protein and lipid sources; however, their nutritional value for Atlantic halibut has not been investigated. In addition to nutrients (protein, amino acids, lipid and micronutrients), these invertebrate contain bioactive compounds and feed attractants. However, their nutrient composition shows some variations due to the seasonal changes in the marine environment (temperature, food availability), and harvesting and processing methods. Average protein and lipid content of the Antarctic krill and Norwegian *Calanus* copepod meals tested in this study were 50% protein and 16% lipid, and 60% protein and 20% lipid, respectively. In contrast to krill, lipid stored by *Calanus* contains high amounts of wax esters, a neutral lipid component that is not easily hydrolyzed due to its hydrophobic nature, and potentially more difficult to digest than krill lipid.

Two experiments were conducted to determine the suitability of *Euphausia* krill and copepod *Calanus finmarchicus* meals and oils as a protein and lipid source for juvenile halibut. Weight gain was significantly higher (P<0.05) when 12, 20 and 30% dietary protein was replaced in a diet based on fish meal and plant protein (soybean and corn gluten meal). The FCR and SGR did not vary significantly between diets; however SGR increased (1.42 to 1.56%) with greater inclusion of *Calanus* protein, and was over 14% greater in fish fed the 30% *Calanus* protein diet compared to the plant protein diet. Lipid, protein, and energy digestibility of krill and *Calanus* meals ranged from 90-99%. Protein and energy digestibility significantly decreased with an increased degree of heat treatment to the *Calanus* meals, ranging from 90-99% digestible for both protein and energy. Krill meal had higher lipid digestibility (99%) than *Calanus* meals (90-95%). Heat processing did not significantly affect lipid digestibility of *Calanus* meals; however the freeze-dried meal showed higher digestibility (95%). Krill oil contained lower amounts of both eicosapentaenoic acid (EPA) (6%) and docosahexaenoic acid (DHA) (2%) than *Calanus* oil (10% EPA and 11% DHA). The digestibility of total fatty acid from krill oil was relatively high (>90%), however *Calanus* oil was slightly less digestible, likely due to the presence of wax esters which may not be efficiently digested. Despite overall lower fatty acid digestibility of *Calanus* oil, DHA in particular was highly digested (91%). It appears that Antarctic krill and *Calanus* copepod meal and oil can be effectively utilized as dietary protein and lipid supplements in Atlantic halibut diet.

Keywords: Atlantic halibut; Antarctic krill; *Calanus*; Copepod, Protein; Lipid; Digestibility

*E-mail address: santosh.lall@nrc-cnrc.gc.ca*
Effect of feed supplement with krill meal and vegetative ingredients replacing fish meal on the growth performance of red striped snapper, *Lutjanus erythropterus*

**Liao, W-L.*, Wu, L-C. and Ou, J-N.**  
Institute of Fisheries Science, National Taiwan University, Taipei 10617, Taiwan

**Abstract**

Red striped snapper, *Lutjanus erythropterus* with red-colored of integument that make this species has ideal candidate for main of net cage cultured fish in Taiwan. Fish meal have many advantages, especially be demanded in aquaculture growth performance, let make fish meal to become a major protein source in aquaculture feeds. Due to steady yield and short of demand, the price raises recently years. The research direction is tendency towards reducing the percentage of fish meal in aquaculture feeds. Previous study show lupin meal, rapeseed meal, corn protein, respective formulating in 20%, 10% and 15% of cobia feeds, can decrease usage of fish meal and had no difference in growth performance. For seeking lower fish meal formula, this study was focus the growth performance of red striped snapper in using krill meal combine with lupin meal, rapeseed meal and corn protein for reducing the ratio of fish meal in feed.

Experiment I : The control group was supplied with 60% fish meal, experiment groups which contain lupin meal 20%, rapeseed meal 10% and corn protein 15%, were supplied krill meal 5%, 7.5%, 10% and 12.5% respectively. Experiment groups reduce fish meal from 60% to 22%, 19.5%, 17% and 14.5% to test their growth performance of red striped snapper. After 42 days feeding, the results showed that the optimal replacement ratio of krill meal is 10%.

Experiment II : The control group was supplied with 60% fish meal, experiment groups contain 10% krill meal and arrange percentage of 2%, 5% and 10% rapeseed meal that fish meal were 25%, 20% and 17% respectively. After 42 days feeding, the results showed that experiment groups weight gain and feed efficiency were no significantly differences with control diet

**Keywords**: Red striped snapper, Krill meal, Lupin meal, Rapeseed meal, Corn protein

*E-mail*: williao@ntu.edu.tw
Replacing dietary fish oil with palm fatty acid distillate improves lipid utilization in rainbow trout, *Oncorhynchus mykiss*, raised at two different water temperatures

Wing-Keong Ng1*, Basseer M. Codabaccus2,4, Chris G. Carter3, Peter D. Nichols4

1 Fish Nutrition Laboratory, School of Biological Sciences, Universiti Sains Malaysia, Penang 11800, Malaysia
2 School of Aquaculture, University of Tasmania, Launceston, Tasmania, Australia
3 Tasmanian Aquaculture and Fisheries Institute, University of Tasmania, Hobart, Tasmania, Australia
4 CSIRO Food Futures Flagship and Division of Marine and Atmospheric Research, Hobart, Tasmania, Australia

Abstract

Salmonid fishes such as the Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) account for more than 60% of the total fish oil use in aquafeeds. The rapid increase in global aquaculture production and the resultant anticipated shortfall in fish oil supply have generated much research in evaluating alternatives to dietary fish oil. Previous research by the first author has shown that crude palm oil (CPO) can be successfully incorporated into salmonid diets without affecting growth performance. Nevertheless, the high content of saturated fatty acids (SFA) (about 50%) in CPO has been shown to be a significant factor affecting fatty acid digestibility and subsequent energy availability when used in aquafeeds. The reduction in the apparent digestibility (AD) of saturates is due in part to the increasing resistance of triacylglycerols (TAG) to digestion with increasing CPO content in rainbow trout diets. Therefore, the present study was conducted to evaluate the effects of using palm fatty acid distillate (PFAD), which contains more than 90% of its fatty acids in free form (FFA), on fatty acid digestibility in rainbow trout raised at two different water temperatures.

Three isolipidic diets (20% lipid) with 0, 10 or 15% (w/w) PFAD, at the expense of fish oil, were formulated and fed to triplicate groups of female trout maintained at water temperatures of 15 or 20°C. After six weeks, fecal matter was manually stripped and the AD of the fatty acids measured using yttrium oxide as an inert marker. Increasing dietary PFAD led to significant (P<0.05) increase in the AD of SFA but not that of total monounsaturated (MUFA) or polyunsaturated fatty acids (PUFA). In general, the AD of fatty acids decreased with increasing chain length and increased with increasing unsaturation within each temperature regimen irrespective of PFAD level fed to the fish. n-3 PUFA were preferentially absorbed compared to n-6 PUFA in all diet and temperature treatments. Except for 15:0, no significant (P>0.05) interaction between diet and temperature effects on fatty acid digestibility was found. Increasing water temperature slightly reduced total MUFA and PUFA digestibility of diets regardless of PFAD level. With increasing dietary PFAD level, a significant decrease and increase in trout fecal TAG and FFA content, respectively, was observed. Total lipid content of expelled feces decreased with increasing dietary PFAD indicating that the free SFA of PFAD was better utilized by rainbow trout compared to the same fatty acids in the form of TAG found in fish oil. The potential impact of improved lipid and SFA digestibility in cold-water fish fed diets supplemented with high levels of PFAD on fish growth performance is currently being investigated.

Keywords: Fatty acids; Palm oil; Digestibility; Rainbow trout; Water temperature

* E-mail address: wkng@usm.my
P-053

Effects of partially replacing fish meal with fermented soybean meal and unfermented soybean meal in diets on feed intake and growth performance of juvenile Japanese flounder (*Paralichthys olivaceus*)

Jinghua Chen¹²*, Kangsen Mai¹, Wei Xu¹, Qinghui Ai¹, Wenbing Zhang¹, Zhiguo Liu¹, Hongming Ma¹, Xiaojie Wang¹, Beiping Tan¹

¹ The Key Laboratory of Mariculture, Education Ministry of China, Ocean University of China, Qingdao, 266003, P.R. China
² College of Animal Science and Technology, Qingdao Agricultural University, Qingdao, 266109, P.R. China

Abstract

A study was conducted to examine the effects of partially replacing fish meal in the diets with fermented soybean meal (FSBM) and unfermented soybean meal (USBM) on feed intake and growth performance of juvenile Japanese flounder (*Paralichthys olivaceus*). Four FSBM were obtained by fermenting soybean meal (SBM), respectively, with mixed strains of *Candida utilis*, *Aspergillus ficuum* and *Aspergillus oryzae* (CAA), *C. utilis*, *A. ficuum* and *Bacillus subtilis* (CAB), *C. utilis*, *A. ficuum* and *Rhizopus oligosporus* (CAR), five strains above (CAABR). The contents of phytate, trypsin inhibitor, stachyose and raffinose in FSBM were lower than those in USBM. Six isonitrogenous and isocaloric diets were formulated. The control diet contained 68.0% fish meal (FM) as the main protein source. In five SBM diets, 45% of FM protein was replaced by USBM or FSBM protein. Each diet was randomly fed to triplicate groups of Japanese flounder with average initial weight (2.00 ± 0.02) g/fish. Fish were fed to apparent satiation twice daily (08:00 and 16:00). During the experiment, water temperature fluctuated from 22.5 to 25.5 °C, salinity from 29.5 to 32.0 and dissolved oxygen was not less than 7.0 mg/L. At the end of the 10-week feeding trial, the feed intake (FI), specific growth rate (SGR), feed efficiency ratio (FER) and protein efficiency ratio of the fish fed the diets containing 45% USBM protein were significantly lower than those of the control (*P*<0.05), while replacement of FM protein by CAA, CAR and CAABR protein could improve the FI, growth and retention of nitrogen, phosphorous and calcium (*P*<0.05). The SGR and FER of the fish fed 45% CAABR protein were significantly higher than those of the fish fed USBM and CAB protein (*P*<0.01). The results indicate that fermentation can decrease the contents of some anti-nutritional factors in SBM, and improve the growth performance and feed utilization of juvenile Japanese flounder (*Paralichthys olivaceus*).

Key words: Japanese flounder *Paralichthys olivaceus*; Soybean meal; Fermentation; Growth; Feed utilization

*E-mail address*: Chen_jinghua@163.com (J.H. Chen).
Incorporation of a mixture of plant feedstuffs as substitute for soybean meal in diets of juvenile tilapia, *Oreochromis niloticus*

Yun-Xue Guo¹, Xiao-Hui Dong¹, Bei-Ping Tan¹, Qi-Hui Yang¹, Shu-Yan Chi¹, Gang Chen², Lu Zhang³

¹Laboratory of Aquatic Economic Animal Nutrition and Feed, Fisheries College, Guangdong Ocean University, Zhanjiang 524025, People's Republic of China
²Laboratory of Aquaculture for Aquatic Economic Animal, Fisheries College, Guangdong Ocean University, Zhanjiang 524025, People's Republic of China
³Guangdong Yuehai Feed Group Co.Ltd., Zhanjiang 524025, People's Republic of China

Abstract

An 8-week feeding trial was conducted to determine the effects of replacing soybean meal (SBM) with a mixture of protein sources (corn byproduct: rapeseed meal: cotton seed meal: sesame meal=23.31:21.71:36.48:18.50) in diets of GIFT strain of Nile tilapia juveniles. Seven practical diets (33% crude protein, 19.3MkJ kg⁻¹ dry diet) containing substitution levels of 0, 15%, 30%, 45%, 60%, 75% and 100% mixed protein sources for SBM protein were formulated and fed to GIFT fingerlings (mean initial weight of 5.78±0.02g). Final body weight, weight gain, survival ratio, specific growth rate, protein efficiency rate, feed conversion ratio and viscerosomatic index of GIFT in all treatments were not significantly affected (P>0.05). No significant differences were observed in apparent digestibility of dry matter, ash and phosphorus (P>0.05), while the apparent digestibility of protein and lipid decreased with the increasing level of the mixed protein sources. Fish fed the seven diets had no significant differences in carcass nutrients contents (P>0.05), but liver protein contents decreased with the increasing level of the mixed protein sources. The composition of dry matter, protein and phosphorus for dorsal muscle of tilapia had no significant differences (P<0.05). And the composition of ash and lipid were not significantly lower than those of control diet. The feed cost decreased with the increasing of the mixed protein sources in diets. In summary, 100% of SBM protein could be replaced by the mixed protein sources without causing negative effects on growth performance, body composition, dorsal muscle composition and feed utilization.

Keywords: GIFT strain of Nile tilapia; Soybean meal; Mixed protein sources; Growth performance

*E-mail address: dongxiaohui2003@163.com*
The effect of Antarctic krill (Euphausia superb) meal on the growth and immunity of juvenile Epinephelus coioides

Huang Yan qing*, Gao Lu jiao, Lu Jian xue
Key Laboratory of Marine and Estuarine Fisheries Resources and Ecology, Ministry of Agriculture, Shanghai 200090, China

Abstract
The effects of Antarctic krill (Euphausia superb) meal supplemented to diets were investigated by feeding juvenile Epinephelus coioides in seawater. The level of Antarctic krill meal were 2%, 4% and 6% were utilized and the experimental fish (initial body weight, 3.5±0.2g) were reared in columned floating net cages (r=0.5m, h=1m) for 100 days. Based on calculating weight gain, feed coefficient, the experimental results indicated that the supplemented krill meal at 2%, 4% and 6% level were good at growth for the Epinephelus coioides juvenile, and the optimum level was 4%. There was practically difference between 4% krill group and the control group in their special growth rate and feed coefficient (P<0.05). On the other land, the experimental group, which fed diets with 2% to 6% krill, had more lymphocytes in thymus gland and the mRNA of lysozyme, Tachyplesin I and MYD88 than the control group.

Keywords: Epinephelus coioides; Euphausia superb meal; growth; immunity

* E-mail address: hyqrich@yahoo.com.cn
Effects of dehulled soybean meal replaced fish meal on growth, body composition and biochemical parameters of *clarias lazera*

WU Li-fang*, QIN Gui-xin, ZHANG Dong-ming, WANG Hong-he, SUN Ze-wei, SUN Ling, ZHU Dan

College of Animal Science and Technology, Jinlin Agriculture University, Changchun, Jilin 130118, China

Abstract

A growth trial was conducted on juvenile fish with initial body weight of \(22.40\pm0.30\) g for 8 weeks at controlled temperature \((22\pm3)\) °C in single recirculating system. Fish meal and dehulled soybean meal were used as protein source. Five isonitrogenous (40% protein) and isoenergetic (15.8MJ•kg\(^{-1}\)) diets, with dehulled soybean meal replacing level 0, 15, 30, 45 and 60% of fish meal protein, were formulated to feed juveniles to investigate the effects of dietary soybean protein level growth, body composition and main blood biochemical parameters of *clarias lazera*. The results indicated that there were no significant differences \((P>0.05)\) in specific growth rate among fish fed the diets containing different groups dehulled soybean meal levels ranging from 30% to 60% with control groups; When replaced level reached 60%, protein efficiency rate and productive protein value were significant \((P<0.05)\) lower than that of the control groups. Feed efficiency was no significant differences \((P>0.05)\) by dehulled soybean meal level. when dehulled soybean meal replaced fish meal by 60%, the protein content in muscle was significantly decreased\((P<0.05)\); the contents of fat and ash were no significant difference \((P>0.05)\) by dehulled soybean meal level. Dehulled soybean meal have no effects \((P>0.05)\) on the concentration of blood glucose, urea nitrogen, total cholesterol, triglyceride, total protein, albumin, globulin and the activity of glutamic-oxal(o)acetic transaminase and glutamic-pyruvic transaminase. Therefore, the dietary protein content was 40% in compound feed and the dehulled soybean meal of replacement level was 45% in the diet could be recommended for optimum growth and efficient feed utilization of juvenile *clarias lazer*.

**Keywords:** *clarias lazera*; dehulled soybean meal; growth; blood biochemical parameters

*E-mail address: wulifang2915@yahoo.Com.cn*
Studies on replacement of fish-origin meal and oil with those of vegetable-origin in diets for a marine teleost *Siganus canaliculatus*

Shude Xu*, Liang Zhang, Shuqi Wang, Xuebing Liu, Yuanyou Li
Marine Biology Institute of Shantou University & Guangdong Provincial Key Laboratory of Marine Biology, Shantou, Guangdong 515063, China

Abstract

Rabbitfish *Siganus canaliculatus* (an alias *S. oramin*) is an herbivorous marine teleost, which widely distribute throughout the Indo-Pacific and Eastern Mediterranean region, including in Southeast China. With characters of strong disease resistance, fry available, short culture period and high nutrition value, etc, *S. canaliculatus* has becoming a new intensive cultural species in recent years. In order to promote its culture industry, it is necessary to study their nutrition requirements and develop their formulated feed.

With casein as protein source and fish oil (FO) as lipid source, diets with different protein levels or different lipid levels were prepared, and *S. canaliculatus* were cultured for eight weeks. Evaluation based on growth performance, feed conversion ratio, protein efficient rate and hepatosomatic index, it was obtained that the optimal addition level for protein and lipid in their diets were 29-34% and 6-9%, respectively. Furthermore, the replacement experiments showed that the replacing ratio of soybean oil (SO) to FO may be up to 67%, whereas the best replacing ratio was 45%, on the basis of comparison of indexes as mentioned above between the FO- and SO-replacement dietary groups. While the replacing ratio of fish meal by soybean meal may be up to 40%, i.e., the optimum proportion of animal-plant protein in diet may be 3:2. These achievements will be of important significance for enriching fish nutriology and provide data for developing high-effective and low-cost formulated feed for rabbitfish *S. canaliculatus*.

Keywords: *Siganus canaliculatus*, nutrition requirement, replacement, formulated feed

*E-mail address: sdxu1@stu.edu.cn; †Correspondence: yyli@stu.edu.cn
Effects of replacement of fish meal by soy protein concentrate on growth performance and protein metabolism of *Pelteobagrus vachelli* juvenile

Kangsen Mai, Yinghao Yang*, Qinghui Ai
The Key Laboratory of Mariculture (Ministry Education of China), Ocean University of China, Qingdao 266003, PRChina

Abstract

A growth experiment was conducted to investigate the effects of replacement of fish meal (FM) by soy protein concentrate (SPC) in diets on the growth performance and protein metabolism of *Pelteobagrus vachelli* juvenile. Six isonitrogenous (40% crude protein) and isoenergetic (19.5 KJ g\(^{-1}\)) diets replacing 0, 20, 40, 60, 80 and 100% FM protein by SPC protein were formulated. Triplicate groups of 40 fish (initial weight 1.53±0.012g) were fed to apparent satiation by hand twice daily for 10 weeks in a recirculation system. The water temperature ranged from 22 to 31.5°C, ammonia-N was less than 0.5mg l\(^{-1}\), and dissolved oxygen content was approximately 7mg l\(^{-1}\) during the experimental period. Fish fed diets with SPC protein substitution level from 0% to 100% (diets1–6) all showed high survival (above 95%) and no significant differences were observed. Final weight (FW) and specific growth rate (SGR) increased significantly with decreasing dietary SPC protein substitution level. Fish fed the diet with 20% substitution level showed the highest FW and SGR, while fish fed the diet with 60% substitution level did not show significant differences with control group. Histological structure of both hindgut and liver were obviously damaged, when FM was replaced by higher level (80% and 100%) of SPC in diets of *Pelteobagrus vachelli* larvae. The whole body composition showed no significant differences in all groups. The activities of pepsin, trypsin and AST (GOT) also had the similar trend as SGR. In conclusion, The diets with fish meal replaced by soy protein concentrate inhibit protein metabolism and the largest replacement of fish meal with soy protein concentrate could reach the level of 60% in *Pelteobagrus vachelli* juvenile.

Keywords: Fish meal; Replacement; Soy protein concentrate; Growth performance; protein metabolism; *Pelteobagrus vachelli* juvenile

*E-mail address: yangyinghao_12@126.com*
Replacing dietary fish oil with vegetable oil affect oleic acid uptake and Metabolism in Atlantic salmon (*Salmo salar* L.) hepatocytes

Jishu Zhou1, 2 *, Ingunn Stubhaug2, 3, Anne V. Krøvel2, Pål A. Olsvik2, Bente E. Torstensen2

1 College of Animal Science and Technology, Northwest A&F University, 712100 Yangling, Shaanxi, China,
2. National Institute of Nutrition and Seafood Research (NIFES), P.O.Box 2029, Nordnes, 5817 Bergen, Norway
3. current address : Skretting ARC, Stavanger, Norway.

Abstract

The aim was to investigate how uptake and metabolism of 18:1n-9 (oleic acid; OA) in Atlantic salmon (*Salmo salar* L.) hepatocyte is affected by dietary oil sources and hence membrane fatty acid composition. Atlantic salmon post smolt was fed diets containing either 100% fish oil (FO) or vegetable oil (VO; soybean oil) for 5 months to produce hepatocytes with typically different FO and VO fatty acid composition. Then OA uptake and metabolism in isolated hepatocytes were studied by incubating with 37.5 µM OA for 2 h (0.3 µCi/flask) with and without (control) membrane protein inhibitors; phloretin, sulfo-N-succinimidyl 4-maleimido-oleic acid ester, diisothiocyanodisulfonic acid, and sulfo-N-succinimidyl 4-maleimido-butyric acid ester. OA uptake was not different in control cells between FO and VO fed fish. The effect of adding membrane protein inhibitors, however, was significantly different with OA uptake being inhibited in VO but not in FO hepatocytes. The expression of genes related to stress responses and antioxidant defence; glutathione peroxidase 4, heat shock protein 70, metallothionein and ubiquitin was significantly higher in VO than in FO hepatocytes. The incubation with inhibitors, however, did not affect the expression these genes. Overall, the results indicate that fatty acid uptake in Atlantic salmon hepatocyte synergism combination of membrane protein associated and passive diffusion.

Key words: Vegetable oil; Fish oil; Oleic acid; Fatty acid uptake; Fatty acid metabolism; Atlantic salmon hepatocytes; Membrane bound protein; Inhibitor

*E-mail address :zhoujishu@163.com
Effect of Starvation on the Activities and mRNAs expression of Enzymes about Lipid Metabolism in Hybrid Tilapia (*Oreochromis niloticus*×*O.Aureus*)

Chunyan Han1,2, Xiaobo Wen1*, Qingmei Zheng1,2, Haobo Li1
1 College of Animal Science, South China Agricultural University, Guangzhou, China, 510642
2 Department of Biology, Jiaying University, Meizhou, China, 514011

Abstract

The present research was conducted with Hybrid Tilapia to study the effect of starvation on the activities and mRNAs expression of enzymes about lipid metabolism. Tilapias were under the condition of starvation for 28d. Tissues samples were collected at day 0, 7, 14, 21 and 28 of the experiment for the determination. The compositions of body and muscle were affected in the process of starvation. Crude fat and crude protein in body decreased significantly (p<0.05), moisture and crude ash increased significantly (p<0.05). The crude fat in muscle was decreased significantly (p<0.05) in beginning and then increased, at last kept on the lower level. The changing of Lipoprotein Lipase (LPL), Hormone-sensitive lipase (HSL) activities and mRNAs expression in different tissues was different. The activities of LPL and HSL elevated enormously in muscle at day 7 of starvation by 2.5 times (p<0.05) and 11.8 times (p<0.05) than the value on 0 day respectively, and then descended to the level before starvation at day 14 and finally stabilized at a certain level. During starvation, the mRNAs abundance of LPL and HSL in muscle increased significantly (p<0.05). In liver, LPL activity maintained a significantly increasing trend. While HSL activity lifted dramatically at day 7 of starvation by 2.35 times (p<0.05) and finally stabilized at a certain level. The mRNA abundance of liver LPL increased significantly during the whole process of starvation (p<0.05); yet, the mRNA abundance of liver HSL decreased significantly at day 7 of starvation and elevated significantly afterwards (p<0.05).

**Key words:** Hybrid Tilapia, LPL, HSL, enzyme activity, mRNA expression

E-mail: address:wexbo@scau.edu.cn
Dietary methylmercury and n-3/n-6 ratio affects lipid metabolism in Atlantic salmon

Bente E. Torstensen1*, Lise Andreasen1,2, Pål A. Olsvik1 & Heidi Amlund1
1 National Institute of Nutrition and Seafood research (NIFES), P.O. Box 2020, Nordnes, 5817, Bergen, Norway. E-mail: bente.torstensen@nifes.no
2Current address: EWOS, Bergen, Norway.

Abstract
Fish farming has expanded dramatically during the last few decades, and the increasing demand for fish oil for feed production has forced the industry to search for alternative lipid sources, among them vegetable oils. Further, focus on seafood safety has increased in recent years. Among the contaminants, is methylmercury (MeHg) of great concern, since it is highly toxic and damages the nervous system. Seafood consumption on the other hand, has positive effects on both coronary heart diseases and mental health, due to the high content of n-3 highly unsaturated fatty acids (HUFA). Thus, the interaction between MeHg and fish oil in both fish and humans is of great interest. The objective of the present study was to assess the effects of replacing fish oil (high dietary n-3/n-6 ratio) by soybean oil (low dietary n-3/n-6 ratio) in combination with MeHg in Atlantic salmon (Salmo salar L.) on lipid metabolism and MeHg accumulation.

Atlantic salmon was fed five different diets based on either fish oil or soybean oil, with graded levels of MeHg, for three months. Replacing dietary fish oil by soybean oil had greater effect on the fatty acid composition in white muscle compared to brain. In brain, the relative levels of 18:2n-6 and 20:4n-6 increased in all lipid classes in fish fed vegetable oil (VO) based diets, in addition, TAG from VO fed fish had increased relative levels of 18:1n-9 and decreased levels of 14:0 and 20:1n-9. In muscle, the levels of 18:1n-9, 18:2n-6 and 18:3n-3 decreased, whereas the relative level of 20:1n-9, 20:5n-3, 22:1n-11 and 22:6n-3 decreased. Different dietary lipid sources did not affect the accumulation of mercury in brain nor muscle. However, addition of MeHg resulted in decreased levels of arachidonic acid in phosphatidylinositol in brain from fish fed diets based on VO. The decreased amounts of arachidonic acid in VO fish is suggested to be caused by interaction between high n-6 level and MeHg, leading to an increased cPLA2 activity. Furthermore, data on expression of genes encoding for lipid metabolism and MeHg-toxicity proteins will be presented and discussed.

Keywords: Interaction fatty acids and mercury; Atlantic salmon; gene expression; Lipid metabolism; Methylmercury toxicity; Kinetics.

* E-mail address: bente.torstensen@nifes.no
P-062

Renal threshold for urinary lysine excretion by tilapia in response to orally administered and injected lysine

Chyng-Hwa Liou1*, Yen-Yu Chen1, Shuenn-Der Yang1,2, Fu-Guang Liu2
1Department of Aquaculture, National Taiwan Ocean University, Keelung 202, Taiwan
2Chupei Station, Freshwater Aquaculture Research Center, Fisheries Research Institute, Chupei 302, Taiwan

Abstract

Two experiments, oral lysine administration (Experiment 1) and vein lysine (Lys) injection (Experiment 2) were conducted to gain more insight into the ability of tilapia (Oreochromis niloticus) to utilize crystalline lysine and to establish the kidney threshold for urinary lysine excretion. In Experiment 1, after 24 hours fasting, tilapias were force-fed 0.3% body weight (920-1090 g) of control (fish meal protein) diet, basal (soybean protein) diet and crystalline amino acid diet (Lys×1, Lys×5, Lys×10: Lys×1 means the amount of supplemented Lys in basal diet to simulate the Lys in control diet). Urine and plasma were sampled from the fish at selected time intervals from 1 through 24 hours. The urinary lysine concentrations of tilapia fed Control, Basal, Lys×1 diet were very low (~4.8 nmole/ml). The maximum concentration of plasma lysine in fish fed control and Lys×1 diet were at 4h and 2h post administration. The urinary and plasma lysine concentrations were higher in fish fed high level lysine diets following the increase of lysine uptake. Maximum urinary concentrations of Lys×5 and Lys×10 were 7.3 and 30.6 nmole/ml; and plasma lysine concentrations were 866.0 and 2360.3 nmole/ml respectively. Total lysine amount lost in urine for 24h collected increased with increasing amount of dietary crystalline lysine within 1-4h after force-feeding. In Experiment 2, eight concentrations (0%; low: 0.1%, 0.3%, 0.6%; medium: 1%, 3.6%; high: 6%, 8%) of lysine solution were injected at 0.22% of fish body weight (750-1000 g) into caudal vein of tilapia after fish were fasted for 24h. Urine and blood samples were collected at selected time intervals from 1 to 24h after lysine injection. The urinary lysine concentration had significantly increasing tendency after 1h injection in all treatments. Total lysine amount lost in urine for 24h collected increased with increasing amount of dietary crystalline lysine within 1-4h after force-feeding. In Experiment 2, eight concentrations (0%; low: 0.1%, 0.3%, 0.6%; medium: 1%, 3.6%; high: 6%, 8%) of lysine solution were injected at 0.22% of fish body weight (750-1000 g) into caudal vein of tilapia after fish were fasted for 24h. Urine and blood samples were collected at selected time intervals from 1 to 24h after lysine injection. The urinary lysine concentration had significantly increasing tendency after 1h injection in all treatments. Total lysine amount lost in urine for 24h collected increased with the concentration of lysine solution, and the highest excreted amount was at 1-4h after injection. When the urinary lysine concentration in fish injected with the various lysine concentrations were plotted against the plasma lysine concentrations of fish 1h after injection, the kidney threshold for urinary lysine concentration in tilapia appeared to be about 609.6 nmole/ml. This study showed the absorbable rate of crystalline lysine was faster than intact protein source. The lysine lost in urine of fish fed Lys×1 was only about 0.037% in feed, so we conclude that urine was not the major way for excretion of free lysine.

Keywords: Tilapia; Crystalline amino acids; Lysine; Urine; Plasma; Threshold

* E-mail address: chliou@mail.ntou.edu.tw (C.H. Liou).
Whole-body in vivo enzyme activity of fatty acid metabolism in barramundi, *Lates calcarifer*

Ramez Alhazzaa1,3*, Andrew Bridle1, Peter Nichols2 and Chris Carter3

1 NCMCRS, University of Tasmania, Locked Bag 1370, Launceston, TAS 7250, Australia
2 Food Futures Flagship, CSIRO Marine & Atmospheric Research, Castray Esplanade, Hobart, TAS 7000, Australia
3 TAFI, University of Tasmania, Private Bag 49, Hobart, TAS 7001, Australia

Abstract

Fish oil (FO) supply is strongly dependent on the availability and sustainability of wild fisheries and is under pressure from increasing demand of the expanding agricultural and pharmaceutical industries. Some plant oils are rich in polyunsaturated fatty acids (PUFA) and emerging as practical replacements for FO. Feeding fish on different precursors of ω3 long chain (≥C20) PUFA (LC-PUFA) to increase LC-PUFA content in flesh is of potential interest in aquaculture. To test the preferential utilisation of dietary fatty acids and capacity of barramundi to synthesise LC-PUFA from shorter (C18) precursors, three identical diets differing only in lipid source were fed to juvenile fish raised in freshwater and seawater for 8 weeks. Oils from *Echium plantagineum* (EO), rich in SDA (18:4ω3), and from canola (CO), containing ALA (18:3ω3), were compared with a FO diet rich in EPA+DHA (20:5ω3 & 22:6ω3). FA mass balance showed no significant effects for varied salinity on FA metabolism. Key enzymes involved in FA biosynthesis varied in apparent in vivo activity depending on dietary treatment. EO-fed fish, containing SDA to bypass the rate-limiting Δ-6 FA desaturase (FAD6) step within PUFA synthesis pathway, did not show activity for this enzyme, while CO inclusion in diets increased FAD6 and to a less extent when feeding on FO. Higher activity was shown by the downstream Δ5 FA desaturase (FAD5) enzyme needed to synthesise de novo ω-3 LC-PUFA products in EO-fed fish compared to FO and CO. FO-fed fish accumulated oleic acid and showed significantly higher apparent activity for Δ9 FA desaturase (FAD9) compared with the EO and CO treatments. Apparent β-oxidation of total dietary and de novo fatty acids recorded the greatest rates in fish fed on FO compared with plant oils. Barramundi prefer to accumulate most of the dietary SFA, MUFA and utilise PUFA by elongation and desaturation to the more beneficial LC-PUFA. FA biosynthesis did not produce excess amounts of the final products and catabolised LC-PUFA preferentially from the FO-diet, through peroxisomal oxidaition. Using FO as the sole dietary lipid source in barramundi nutrition is a wasteful practice as barramundi is capable of accumulating considerable amounts of the beneficial ALA, SDA although less of DPA, EPA and DHA. Plant oils, especially EO or other oils containing SDA, are possible alternatives for FO in barramundi aquaculture.

Keywords: *Lates calcarifer*, lipid metabolism, fatty acid mass balance

*E-mail address: alhazzaa@utas.edu.au* (R. Alhazzaa).
Fatty acids absorption and lipid metabolism gene expression of Darkbarbel catfish, *Pelteobagrus fulvidraco*, fed different dietary lipid sources

Chuanjie Qin, Erchao Li, Liqiao Chen*
School of Life Science, East China Normal University, Shanghai, 200062 China

Abstract

The effects of dietary lipid sources on fatty acid absorption and expression regulation of genes related to lipid metabolism in fingerlings of Darkbarbel catfish, *Pelteobagrus fulvidraco*, were evaluated. Fish (weighting 1.0±0.2g) were fed five experimental diets with fish oil (FO), soybean oil (SO), pig oil (PO), 75% fish oil and 25% soybean oil (M1), 25% fish oil and 75% soybean oil (M2) as lipid sources respectively for eight weeks. Fish weight gains of SO, M1 and M2 groups were similar to those of FO group treatment, but total replacement of fish oil by PO significantly reduced fish weight gain (P<0.05). There were increasing trends in hepatosomatic index (HSI) and intraperitoneal fat ratio (IPF) of fish feeding SO, M1, M2 and PO diets, but no significant differences were found (P<0.05). Fish of PO group had higher SFA fraction and lower PUFA fraction in feces comparing to those feeding FO diets. Fish of SO, M1 and M2 groups had significantly higher PUFA fraction and lower SFA fraction (P<0.05) in feces, and the PUFA fraction in feces increased with the replacement content of fish oil by soybean oil. Furthermore, replacement of fish oil with SO, PO and 75% SO could significantly increased the fatty acid binding protein (FABP) and fatty acid synthase (FAS) gene expressions in liver and adipose tissue (P<0.05), and significantly reduced the carnitine acyltransferase gene expression in liver (P<0.05). No significant changes were found in lipoprotein lipase (LPL) gene expression in liver and adipose tissue, and hepatic lipase (HL) gene expression in liver (P<0.05). All these findings could suggest that soybean oil could increase lipid biosynthesis and slow down the lipid catabolism of *P. fulvidraco*.

Keywords: Darkbarbel catfish; soybean oil; fatty acid binding protein; fatty acid synthase; lipoprotein lipase; hepatic lipase

*E-mail address: lqchen@bio.ecnu.edu.cn* (Liqiao Chen).
Effect of dietary protein and starch levels on growth performance, feed utilization and hepatic amino acid metabolism of juvenile grass carp (Ctenopharyngodon idella) in practical extruded diets

Yong-Jun Chen*, Yong-Jian Liu, Li-Xia Tian, Hui-Jun Yang, Gui-Ying Liang, Lian Gan, Jian-Jun Liang
Nutrition laboratory, Institute of Aquatic Economical Animals, School of Life Science, Sun Yat-sen University, Guangzhou 510275, P.R. China

Abstract
Six isoenergetic (gross energy: 18.88 KJ g⁻¹) and isolipidic diets with two protein (30% and 34%) and three starch levels (13%, 18% and 22%), were fed to four replicated groups of 30 fish in indoor recirculation system. Fish were fed twice daily at the feeding rate of 5.5% under natural light-dark cycle. Over an 8-week feeding trial, highest final body weight (FBW), weight gain (WG) and specific growth rate (SGR) were obtained with the diet containing 34% protein and 13% starch. FBW, WG, SGR, feed conversion ratio (FCR) and nitrogen retention efficiency (NRE) were dependent on dietary protein level (P<0.05), and protein efficiency ratio (PER) changed with varying starch level (P<0.05), whereas energy retention efficiency (ERE) was affected by both protein and starch level (P<0.05). For the same starch level, condition factor (CF) and hepatosomatic index (HSI) decreased with the increase of protein level (P<0.05), whereas intraperitoneal fat ratio (IPF) of fish fed the diets formulated with the same protein level increased when starch increased from 13% to 22% (P<0.05). Moisture, lipid and protein contents in carcass and liver were significantly affected by dietary protein and starch levels (P<0.05), while muscle composition was dependent on starch level (P<0.05). For the same protein level, fish fed the diets containing higher starch level seemed to have higher plasma total triacylglyceride (TG), cholesterol (CHO) and low density lipoprotein-cholesterol (LDL-C). Fish fed high-protein diets showed higher activities of hepatic glutamic-pyruvic transaminase (GPT) and glutamic-oxalacetic transaminase (GOT) than their low-protein counterparts. These results indicated that there is no evidence of protein-sparing effect of dietary starch for grass carp in practical extruded diets.

Keywords: grass carp, protein, starch, growth, feed utilization, amino acid metabolism

* E-mail address: edls@mail.sysu.edu.cn (Liu Yong Jian).
Effects of dietary n-3 HUFA on growth performance and lipid metabolism in juvenile grass carp, *Ctenopharyngodon idellus*

Hong Ji1,2,3*, Jie Li1, Pin Liu1

1 College of Animal Science and Technology, Northwest A&F University, Yangling 712100, P.R.China
2 Ankang Fisheries Experimental and Demonstration Station, Northwest A & F University, Ankang 725000, P.R.China
3 Fisheries Research Institute, Northwest A&F University, Yangling 712100, P.R.China

Abstract

A 75 days feeding trial was conducted to study the effect of dietary n-3 highly unsaturated fatty acids (n-3 HUFA) on growth performance and lipid metabolism in juvenile grass carp (*Ctenopharyngodon idellus*). Five kinds of purified diet containing graded levels (0, 0.26, 0.52, 0.83 and 1.13%) of n-3 HUFA with efficient basal fatty acid linolenic acid and linoleic acid (1%, respectively) included were fed to satiation to the experimental fish in duplicate [average weight,(5.66±0.21)g; 20 fish/replicate] at 28±1 °C. After the feeding trial, tissue fatty acid composition, growth performance, biological characters, and lipid metabolism related gene expression were determined. Results showed that: (1) HUFA were effectively incorporated into the fish body, showing a correlative relationship between tissue fatty acid content and dietary n-3 HUFA level. ∑PUFA, ∑n-3 PUFA, ∑n-3 HUFA and n-3/n-6 were significantly increased in muscle, intraperitoneal fat (IPF) and brain, while the muscle and IPF C20:1, ∑SAT and ∑MUFA, brain C14:0, C18:0 and C20:1 showed the opposite trend. C16:1n-9 and C18:1n-9 were negatively correlated with dietary n-3 HUFA, while ∑n-3 PUFA and ∑n-3 HUFA were significantly increased among all the treatments in hepatopancreas. (2) The best growth performance, feed utilization and protein sparing effect were observed in fish fed with 0.52% dietary n-3 HUFA. Growth performance and feed utilization increased with the increasing dietary n-3 HUFA levels up to 0.52%. Higher dietary n-3 HUFA level (above 0.52%) made growth performance, feed utilization retarded and no protein sparing effect was found. Quadratic analysis based on weight gain rate (WGR) indicated that dietary n-3 HUFA requirement for juvenile grass carp for best growth performance was 0.524% DM. (3) IPF and hepatopancreas lipid level were lowest in 0.52% dietary n-3 HUFA level group. Hepatosomatic index (HSI) was decreased with the increase of dietary n-3 HUFA level. Spleen weight ratio and muscle lipid content remained steady with the increase of dietary n-3 HUFA levels up to 0.52g/kg while these values in higher dietary n-3 HUFA (above 0.52g/kg) groups were significantly decreased. (4) Gene expression of delta-9 desaturase (SCD) showed the similar trend with growth performance, while the expression of uncoupling proteins 2 (UCP2) was converse to the changes of SCD value. Expression of lipoprotein lipase (LPL) was increased with the increase of dietary n-3 HUFA levels up to 0.83%. In summary, it was implied that n-3 HUFA might be also essential fatty acids for fresh water fish grass carp, and the optimal dietary level is supposed to be 0.524%. On the other hand, n-3 HUFA also have impact on lipid metabolism as they do in marine fish, i.e. the inhibition effect on lipid accumulation, and this might be related to their influence on lipid transportation and energy utilization, as well as the desaturation ability. Furthermore, excess HUFA intake will result in adverse effect.

Keywords: Ctenopharyngodon idellus; n-3 highly unsaturated fatty acids (n-3 HUFA); growth performance; lipid metabolism; gene expression

*E-mail address: jihong@nwsuaf.edu.cn*
P-067

Effects of dietary fat on the growth and energy budget of Hexagrammos otakii

Liu Yang1,2*, Jiang Zhi-qiang1, Wang Fu-qiang1
1 Key Laboratory of Mariculture, Agriculture Ministry, PRC, Dalian FisheriesUniv., Dalian 116023, China
2 Heilongjiang River Fishery Research Institute, Chinese Academy of Fishery Science, Harbin 150070, China

Abstract

Greenling (Hexagrammos otakii) is one of the most important high-value aquaculture species in China. To improve the culturing effectiveness of greenling, dietary fat requirement was studied.

In this paper, 5 semi-purified diets formulated with fish oil as fat sources to contain graded levels of fat ranging from 4.73% to 15.72% were fed to Hexagrammos otakii (initial weight 70.12±1.92g) in triplicate for 7 weeks. The feeding trial was conducted in fiberglass tanks in which the water temperature was controlled at 18±0.5°C during the test period. The results showed that the weight gain, specific growth rate, feed efficiency and protein efficiency ratio of fish fed the test diets with 10.33% crude fat on dry basis were significantly higher (P<0.05) than those of the rest groups. Based on quadratic model regression analysis of SGR it was found that the optimal fat requirements were 10.22% of dry diet respectively. There was no significant effect of dietary fat levels on whole body moisture, protein and ash (P>0.05). However its body lipid of group 1 is significant lower than others (P<0.05) at the end of the feeding trial. The growth energy of group 2 and 3 are significant higher to the others (P<0.05). While metabolism energy are significant lower than others. The fecal energy and excretion energy of group 3 is significant lower than others, too (P<0.05). It is suggested that diet containing 10% fat was helpful to growth of Hexagrammos otakii.

Key words: Hexagrammos otakii; Fat; growth; energy budget

*E-mail address: liuyanghrfri@gmail.com
Hematological responses of southern catfish (Silurus meridionalis) to intraperitoneal injection of free gossypol with determination of LC50s

Lei Pan1,2,5*, Gao D. Liang2,4,5, Ping Xie2,3, Wen S. Zhou3

1 The Institute of Hydroecology, Ministry of Water Resources & Chinese Academy of Sciences,
2 Donghu Experimental Station of Lake Ecosystems, State Key Laboratory of Freshwater Ecology and Biotechnology of China, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, PR China,
3 Fisheries College of Huazhong Agricultural University, Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education, Wuhan 430070, PR China,
4 Graduate school of the Chinese Academy of Sciences, Beijing 100039,
5 These two authors contribute equally to this work.

Abstract

Hematological responses of juvenile southern catfish (Silurus meridionalis) to intraperitoneal injection of free gossypol were studied in a flow-through system. This is the first report of acute toxicity of gossypol in fish. The 24-h, 48-h, 72-h, 96-h LC50s were 225.21 mg Kg⁻¹, 84.62 mg Kg⁻¹, 59.91 mg Kg⁻¹, 55.68 mg Kg⁻¹, respectively. This is the first to determine LC50s of gossypol to fish. In a sub-lethal test, changes in red blood cell count (RBC), hematocrit (Hct) and hemoglobin content (Hb) of the catfish were measured after 24-, 48-, 72- and 96-h of exposure to 0, 20 and 40 mg gossypol Kg⁻¹ BD. Compared with the control, fish in the high-dose group showed a time-dependent decrease in RBC, Hct and Hb (P < 0.05), with statistically significant alterations at 48 h post injection, while fish in the low dose group did not show significant difference in RBC count, Hct and Hb (P > 0.05). Compared with the control, fish in the high-dose group showed abnormal erythrocyte features and hemolysis. The possible mechanism of anemia caused by free gossypol might be hemolysis instead of iron deficiency.

Keywords: Free gossypol; Southern catfish; Hematological responses; LC50

* E-mail address: leipan@mail.ihe.ac.cn (L. Pan)
Heat shock response (Hsp70) in white sturgeon and green sturgeon exposed to different stressors

Weifang Wang*,1,2, Dongfang Deng2, Nicola De Riu2, Silas S. O. Hung2
1The Key Laboratory of Mariculture (Education Ministry of China), Ocean University of China, Qingdao 266003, P.R. China
2Department of Animal Science, University of California, One Shields Ave., Davis, CA 95616-8521, USA

Abstract

The objective of this study was to compare the sensitivity of white sturgeon (Acipenser transmontanus) and green sturgeon (A. medirostris) in response to different stressors commonly occurring in commercial hatchery or natural habitat of the species. One and half years old grow out white sturgeon (2.27 ± 0.08 kg) and green sturgeon (2.34 ± 0.09 kg) were subjected to different stressors: 1) Starvation, 2) Heat shock, 3) Cold shock, 4) Air exposure, and 5) Control (no stress). Five of each species were exposed simultaneously to the same stressor and heat shock proteins 70 (Hsp70) in different tissues (mucus, heart, spleen, gill, liver, the white muscle and gastrointestinal tracts) were measured after stresses. After one week of starvation, the loss of body weight was higher in green sturgeon than in white sturgeon although no statistic difference, and RNA/DNA ratio significantly (P < 0.05) decreased after starvation in two sturgeon respectively. The hematocrit was lower in green sturgeon than in white sturgeon in all treatments. Among different tissues, mucus was more responsive to the stress than the rest of tissues despite of different stressors in both species of sturgeon. Among different stressors, heat shock was the most affective stressor to induce Hsp70 expression in both species. The increased Hsp70 were significantly higher in the mucus of white sturgeon than those in green sturgeon when they were exposed to starvation, heat shock and cold shock. Similarly, the increased Hsp70 were significantly higher in the tissues (except that in liver) of white sturgeon exposed to heat shock stress than those in green sturgeon. These results indicated that white sturgeon have better earlier defense mechanism than green sturgeon in response the stressors under current experimental conditions.

Keywords: Sturgeon; Hsp70; RNA/DNA ratio; Starvation; Temperature; Air exposure

* E-mail address: wangwf2000@hotmail.com (Weifang Wang).
Changes in the liver transcriptome induced by a plant based diet compared to a marine based diet in two lines of rainbow trout selected for muscle fat content.

Ingunn Stubhaug¹, Geneviève Corraze¹, Catherine I. Kolditz¹, Sadasivam J. Kaushik¹, Gregory. Guernec², Stéphane Panserat¹, Françoise Médale¹*
¹. INRA UMR 1067, NUAGE, Nutrition, Aquaculture and Genomics, 64310 St Pee sur Nivelle, France
². INRA, SCRIBE, Campus de Beaulieu, 35042 Rennes Cedex, France

Abstract

The objectives of this study were to analyse the changes in hepatic genes expression induced by the replacement of marine ingredients by plant products in trout diet and to check whether the diet-induced changes were affected by the fish genotype. Two lines of rainbow trout (Oncorhynchus mykiss) selected for high and low lipid content in the muscle, were fed either a control diet (C) containing 70% fish oil and 30% fish meal or a diet totally devoid of marine ingredients (plant-based diet - P) for 7 months. Despite similar feed intake, fish from both lines fed P had a lower growth rate and a lower feed efficiency.

Nylon microarrays containing 9216 trout cDNA spots were used to assess the diet-induced changes in liver transcriptome expression in the two lines. After data treatment and statistical analysis (two-way ANOVA with Limma adjustment, p=0.01), we found that the levels of 81 transcripts were significantly different between the dietary treatments. The genetic selection significantly affected 45 genes and an interaction between lines and diets was observed for 68 transcripts. Among the transcripts that were affected by the dietary treatments, 22% were related to metabolism, 15% to transport, 5% to immune system and 2.5% to oxidative stress. Feeding a diet devoid of marine ingredients changed the expression of genes mainly involved in lipid transport and metabolism. Some genes involved in glycolysis and pentose-phosphate pathway were also up-regulated in the liver of fish fed the plant based diet. Genetic selection for high muscle fat content affected a lower number of genes related to metabolism than did the dietary treatments. Interactions between diets and genotype were observed for some genes involved in lipid metabolism and oxidative stress.

Total replacement of fish meal and fish oil by a blend of plant protein sources and vegetable oils did not strongly disturb hepatic metabolism in the two rainbow trout lines and apparently did not induce stress. Up-regulation of genes that are involved in energy production could be responsible for the lower feed efficiency observed in both lines when fed the diet devoid of marine ingredients.

Keywords: Fish meal and fish oil replacement; Gene expression; Metabolic changes

*E-mail address: medale@st-pee.inra.fr (Dr Françoise Médale)
Effect of dietary salt supplementation on energy partitioning and nutrient digestibility in Nile tilapia

J.W. Schrama*1, M.Y.S. Kallau1, L.T.N. Heinsbroek1, J.A.J. Verreth1
1. Aquaculture and Fisheries Group, Wageningen University, PO Box 338, 6700 AH Wageningen, The Netherlands

Abstract

Osmo-regulation requires energy. An increased effort for osmo-regulation is often suggested to reduce growth by an increased energy requirement for maintenance. Although not consistent, several studies report an increase in growth if salt is supplemented to diets, but it is not clear if this is due to an increased feed intake or an altered maintenance requirement. In contrast, a recent study in Nile tilapia (Oreochromis niloticus) on estimating net-energy from digestible macronutrient intake, found a negative relation between digestible ash intake and energy retention. This suggests an increase in energy requirements for maintenance. Therefore, this study assessed the impact of dietary salt supplementation (4%) on energy balances and nutrient digestibility in Nile tilapia. Ten groups of each 25 fish (initial weight 139g) were assigned to one of two diets: a control diet (96% basal mixture and 4% cellulose) and a test diet supplemented with 4% NaCl (96% basal mixture and 4%NaCl). Fish were fed restrictively at 80% of their assumed satiation feeding level, during a 36-d experimental period. Energy and nitrogen balances were measured by the slaughter method for estimating the energy requirements for maintenance assuming that the efficiency of energy utilization for growth was equal for both diets. Apparent nutrient digestibilities (ADC) were corrected for the supplemented NaCl (assumed 100% digestible) and cellulose (assumed to be indigestible). ADC of ash, energy and fat were similar for both diets. ADC of crude protein and carbohydrates (i.e., NFE) were slightly lower with the NaCl-supplemented diet (P<0.05). Nitrogen retention was similar at both diets (P>0.10) despite the fact the digestible nitrogen intake was higher with the control than with the NaCl diet (P<0.001). Partitioning of energy was affected by NaCl supplementation. The 1.7% lower metabolizable energy intake (P<0.01) together with a 3.9% higher heat production (P<0.05) lead to a 10.3% lower energy retention (P<0.001) at the NaCl-diet compared to the control. Since nitrogen retention was equal at both diets, the differences in energy retention was fully due to a difference in the amount of energy deposited as fat (P<0.001). The estimated energy requirements for maintenance were 15% higher at the NaCl-diet compared to the control diet (P<0.01). In conclusion, dietary NaCl supplementation in Nile tilapia does not improve energy partitioning, since it increases maintenance and reduces digestibility of some nutrients. It is hypothesized that dietary NaCl, disturbs osmo-regulation in the intestine, hampering digestion and increasing maintenance.

Key-words: Energy metabolism; Salt supplementation, digestibility; Tilapia; Oreochromis niloticus

*E-mail address: Johan.Schrama@wur.nl (Johan Schrama)
J.W. Schrama*1, S. Saravanan1, I. Geurden2, K. Coppens1, L.T.N. Heinsbroek1, J.A.J. Verreth1
1. Aquaculture and Fisheries Group, Wageningen University, PO Box 338, 6700 AH Wageningen, The Netherlands
2. INRA, UMR 1067, NUAGE, Nutrition, Aquaculture and Genomics Unit, 64310 St Pée sur Nivelle, France

Abstract
This study on Nile tilapia (Oreochromis niloticus) assessed the effect of the type of dietary non-protein energy source on energy partitioning and on within day variation in oxygen consumption (heat production). Twelve groups each of 34 fish were assigned in a 2x2 factorial design: 2 diets (“Fat” vs. “Starch”) and 2 feeding levels. In the “Fat”-diet 125g fish oil and in the “Starch”-diet 300g gelatinized maize starch were added to 875g of an identical basal ingredient mixture. Fish were fed restrictively one of two ration levels (“Low” 30% or “High” 80% of ad libitum intake) for estimating efficiency of energy utilization for growth (kg). Feed rewards of both diets were aimed to be isoenergetic. Energy and nitrogen balances were measured over a 6-wk period and hourly oxygen consumption once every week during 24h. All balance parameters were affected by the feeding level (P<0.001). Despite a slightly higher digestible nitrogen intake (P<0.01) with the “Fat”-diet, retained nitrogen was similar with both diets. Digestible (DE) and metabolizable (ME) energy intakes were higher with the “Fat”-diet (P<0.10), but did not result in significant differences in heat production between diets; numerically heat production was even higher with the “Starch”-diet. Consequently, energy retention (ER) was lower for “Starch”-diet (P<0.05). The effect of energy source on energy retention as fat depended on the ration levels (P<0.05), being similar at the “Low”-level and being lower for the “Starch”-diet at the “High”-level. For estimating kg by regression of ER on ME intake, data of this study were combined with a previous study using similar diets (i.e., contrast in fat vs. starch) but fed to increase the range in ME-intake. The relationship between ER and ME was: ER = -33(±5.4) + 0.58(±0.027)xME and ER = -42(±4.9) + 0.69(±0.022)xME with the “Starch” and “Fat”-diets, respectively. The kg was 16% lower with the “Starch”-diet than with the “Fat”-diet (P<0.01). Mean daily oxygen consumption was similar with both diets, but daily pattern in hourly oxygen consumption rates strongly differed between diets. Within day variation in oxygen consumption was larger with the “Starch” than with the “Fat”-diet (P<0.001) due to both a higher maximal as well as a lower minimal oxygen consumption with the “Starch”-diet. In conclusion, the type of dietary non-protein energy affects kg of tilapia and also alters the within day patterns in oxygen consumption.

Keywords: Energy metabolism; Oxygen consumption; Carbohydrate; Tilapia; Oreochromis niloticus

*E-mail address: Johan.Schrama@wur.nl (Johan Schrama)
Effect of dietary choline on energy utilization of juvenile gibel carp (Carassius auratus gibelio)

Yuanhui Duan¹,², Xiaoming Zhu¹, Yunxia Yang¹, Dong Han¹, Shouqi Xie¹
¹ State Key Laboratory of Freshwater Ecology and Biotechnology; Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China
² Graduate School of the Chinese Academy of Sciences, Beijing 100039, China

Abstract

A 66 days growth trial was conducted in a flow-through system to investigate the effects of dietary choline on energy utilization in juvenile gibel carp (initial weight: 4.5±0.2g per fish) fed with different energy ingredients. Semi-purified basal diets were formulated using vitamin-free casein as the protein source. In order to limit the endogenous synthesis of choline from methionine, dietary methionine was formulated to be 0.58%, which was less than the requirement (0.69%). Four diets were formulated to contain 35% crude protein and 17 kJ/g gross energy. The control diet contained 8% crude lipid and 37% corn starch and 1000 mg choline/kg diet. Other three diets contained 2% crude lipid, 50% corn starch and different levels of choline (70, 1020, 4000 mg/kg diet).

The results showed that the fish fed with the control diet had significantly lower specific growth rate, feed efficiency, protein retention efficiency, apparent digestibility coefficients of dry matter, significantly higher serum free fatty acids content, whole body dry matter, crude lipid and crude protein than the fish fed with 1020 mg choline/kg diet. Increased dietary choline could significantly decrease feeding rate, hepatic malic enzyme, while significantly increased feed efficiency, protein retention efficiency, apparent digestibility of dry matter, hepatic hexokinase activity and the serum content of free fatty acids. These results suggested that choline is essential for improving the intermediary metabolism of the lipid and carbohydrate when the dietary energy is mainly from carbohydrate. The gibel carp has high ability to use the carbohydrate as the energy source.

Keywords: Choline; Gibel carp; Energy utilization

*E-mail address: joydyh1983@163.com
Energy resource changes in blood of juvenile Chinese sturgeon *Acipenser sinensis* during starvation

Guangpeng Feng1,2*, Ping Zhuang1,2, Longzhen Zhang1, Jianyi Liu1
1 East China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Shanghai, China
2 College of Fisheries and Life Science, Shanghai Ocean University, Shanghai, China

**Abstract**

Chinese sturgeon (*Acipenser sinensis* Gray 1835) is an anadromous species that lives only in the Yangtze River. It has been included in the International Union for Conservation of Nature (IUCN) Red List of Threatened Species, and given Category I State Protection in China. As primitive osteichthyans, sturgeons survived more than 100 million years in the world, and represent an important evolutionary link between the primitive elasmobranchs and more advanced teleosts. Now juvenile Chinese sturgeon faced the threat of scarce food and starvation in the Yangtze River estuary. During starvation, which energy resource was used preferentially depends on the species and duration of the fasting period. The objective of the present study was to assess which energy resource was used preferentially by juvenile Chinese sturgeon during starvation. One hundred juvenile Chinese sturgeons (7 months old) were studied. Blood samples were taken for 8 time every 6 days during the 48-day starvation. On each occasion, 6 fish were randomly selected and immediately anaesthetized. Blood samples of each fish were taken within 2 minutes by puncturing the caudal vein with disposable sterilized syringe. Blood samples were analyzed using BS-200 Auto Chemistry Analyzer. The results showed that albumin (ALB) concentration in the blood of juvenile Chinese sturgeon decline significantly after 31-day starvation. Total protein (TP) concentration increased slighted in the initial period, and then decline significantly after 25-day starvation. Glucose (GLU) concentration was significantly less than initially after 7 days starvation and decreased significantly after 31 days. Triglyceride (TGL) had a similar trend compared to cholesterol (CHOL). The significant reduction in the TGL and CHOL content was found as late as after 25 days. Therefore, GLU concentration of juvenile Chinese sturgeon was significantly less than initially after 7 days starvation, while TP, ALB, CHOL and TGL were significantly less than initially after 25 days starvation. These results implied that during starvation carbohydrates was metabolized preferentially in the blood of juvenile Chinese sturgeon, then proteins and lipid were metabolized.

**Key words:** *Acipenser sinensis*; Nutrition metabolism; Proteins; Carbohydrates; Lipid

* E-mail address: fengguangpeng@gmail.com (Guangpeng Feng).
Partial characterization and activity distribution of proteases, α-amylase and lipase in paddlefish *Polyodon spathula*

Hong Ji1, 2, 3, *, Haitao Sun1, Wei Zhang1
1 College of Animal Science and Technology, Northwest A & F University, Yangling 712100, P.R.China
2 Ankang Fisheries Experimental and Demonstration Station, Northwest A & F University, Ankang 725000, P.R.China
3 Fisheries Research Institute, Northwest A&F University, Yangling 712100, P.R.China

Abstract

In order to obtain the basic nutritional physiological information of paddlefish (*Polyodon spathula*), a series of biochemical assays have been conducted to characterize proteinases, α-amylase and lipase and investigate their activity distribution. Firstly, in comparison with hybrid sturgeon (*Huso dauricus ♀ × Acipenser schrenki Brandt ♂*) and bighead carp (*Aristichthys nobilis*), the enzyme activity and reaction kinetics of proteinases of whole digestive tract in paddlefish were determined under various pH conditions. Then, a comparative study on digestive enzyme activity distribution was conducted between pond-reared (with commercial feed) and cage-reared (with natural diet) paddlefish. Results showed that: (1) Acidic proteinase activity of paddlefish was not significantly different from that of hybrid sturgeon, while significantly higher than that of bighead carp (*p*<0.05); Alkaline proteinase and α-amylase activity of paddlefish was significantly lower than those of hybrid sturgeon and bighead carp(*p*<0.05); lipase activity was not significant different among these three species. On the other hand, the casein digestion assays revealed that the acidic proteinase of paddlefish presented the highest activity at pH 2.5-3.0 and the alkaline proteinases at pH 7.5; the pH values with the highest activities observed were 2.5-3.0, 8.0-9.5 in the hybrid sturgeon and 4.0-5.0, 8.0-8.5 in bighead carp, respectively; (2) Acidic proteinase activity was not detected in small intestine and the activity was higher in esophagus and stomach in both pond- and cage-reared paddlefish; alkaline proteinase activity was detected in small intestine and valvate intestine and the activity of pond-reared paddlefish was significantly lower than that of cage-reared ones (*p*<0.05); α-amylase active was detected in esophagus, stomach, small intestine and valvate intestine and the activity was higher in esophagus and small intestine; lipase activity was only detected in stomach and the activity in pond-reared paddlefish was significantly lower than that in cage-reared ones. It could be concluded that digestive enzyme activity in paddlefish was similar with that of hybrid sturgeon due to their similar digestive system, but different from that of bighead carp in spite of their same food habit as zooplankton filter. The activity and distribution of some digestive enzymes, such as alkaline proteinase and lipase, was influenced by rearing condition (pond or cage) and food (commercial feed or natural diet) in paddlefish.

Keywords: enzyme characterization; activity distribution; paddlefish

* E-mail address: jihong0405@hotmail.com
Effects of dietary carbohydrate on growth performance and metabolism in juvenile yellow catfish, *Pelteokagrus vachlli*

Shiliang Zhang, Hongming Ma*, Kangsen Mai, Qinghui Ai, Wenbing Zhang, Xaojie Wang, Zhiguo Liufu, Wei Xu

The Key Laboratory of Mariculture (Ministry Education of China), Ocean University of China, Qingdao 266003, PR China

Abstract

The effects of dietary carbohydrate on growth performance, body composition and metabolism in juvenile yellow catfish were studied. Six treatments with corn starch levels of 0.0%, 6.5%, 13%, 19.5%, 26% and 32.5% were set, each has three replicates. Juvenile yellow catfish, *Pelteokagrus vachlli* (IBW =1.24±0.07g) were fed for 8 weeks in 150L fiber glass tanks with flow-through water. The water temperature fluctuated from 18 to 25°C, pH 7.6 – 8.1 during the experimental period. Fish fed with 32.5% carbohydrate gained the highest body lipid. Condition factor (CF) and hepatosomatic indices (HSI) of fish did not vary between the test diets. The highest visceral indices (VSI) was obtained in fish fed diets with 26% CHO. After 24 hours of fasting, the plasma glucose levels were the same for all dietary treatments. Data on enzyme activities showed that intestine amylase, hepatic hexokinase (HK), glucokinase (GK), and pyruvate kinase (PK) were significantly affected by carbohydrate level, while phosphofructokinase (PFK-1) did not. The greatest intestinal amylase activity was obtained in diet with 19.5% CHO. The highest plasma insulin level was obtained in fish fed with 32.5% CHO. The number of insulin receptors in liver cell correlated positively with the dietary carbohydrate, while no significant changes were observed in the number of insulin receptors in skeletal muscle. The carbohydrate requirements were estimated to be 28.3% based on growth performance, and protein-sparing effects were observed by adding dietary carbohydrate. These results suggest that the first step of glycolysis is not impaired in yellow catfish, and the insensitivity of the PFK-1 activity and muscle tissue to plasma insulin may be limiting in glucose metabolism in yellow catfish, *Pelteokagrus vachlli*.

Keywords: Yellow catfish; Carbohydrate; Growth performance; Insulin receptors; Enzyme activity

*E-mail address: mahongm@ouc.edu.cn*
Tissues distribution of foreign DNA from genetically modified soybean meal in practical diets for Nile tilapia, *Oreochromis niloticus*.

Indra Suharman\(^a,b\), Shuichi Satoh\(^a,*\), Yutaka Haga\(^a\), Masato Endo\(^a\), Ikuo Hirono\(^a\), and Takashi Aoki\(^a\)

\(^a\)Department of Marine Biosciences, Tokyo University of Marine Science and Technology, Konan, Minato 4-5-7, Tokyo 108-8477, Japan

\(^b\)Department of Aquaculture, Faculty of Fisheries and Marine Sciences, The University of Riau, Pekanbaru 28293, Indonesia

**Abstract**

Interest in the fate of ingested transgenic DNA in fish fed genetically modified (GM) soybean meal (SBM) has arisen because of anxiety for over human consumption of genetically-modified organism. This study investigated the distribution of foreign DNA in Nile tilapia tissues after consumption of GM SBM diet. Two experimental diets were formulated to include either GM or non-GM SBM at 48% (approximately 38% crude protein). The diets were fed to juvenile Nile tilapia (60.5 g average body weight) for 21 days. GM SBM diet was fed to the fish for 12 days. Then, fish were fed the non-GM SBM diet for 9 days. Blood, muscle and liver of fish were taken \((n = 20)\) every three day during that period. During feeding on non-GM SBM diet, 20 fish were sampled for the blood, muscle and liver every three day to determine the residual span of the transferred cauliflower mosaic virus 35S (CaMV 35S) promoter fragment by PCR. The low number of fish muscle samples was positive for the CaMV 35S promoter (205 bp) on the third day after consuming a GM SBM diet. However, it was not detected after that. Similarly, the CaMV 35S promoter fragment was also detected in blood samples of fish fed GM SBM diet. These results demonstrate low possibility of foreign DNA transfer in muscle and blood of Nile tilapia fed GM SBM diet.

**Keywords:** DNA transfer; Foreign DNA; Genetically modified soybean; Nile tilapia.
Inherent Structural Characteristics of Feed at a Cellular Level in Relation to Nutrition Availability: A Novel Approach

Peiqiang Yu*
College of Agriculture and Bioresources, University of Saskatchewan, 51 Campus Drive, Saskatoon, S7N 5A8, Canada

Abstract

Synchrotron radiation-based Fourier transform infrared microspectroscopy (SR-FTIR) as a rapid, direct, non-destructive and bioanalytical technique, taking advantages of synchrotron light brightness (million times brighter than sunlight) and small effective source size, is capable of exploring the molecular chemistry within microstructures of a biological tissue without destruction inherent structures at ultra-spatial resolutions. To date there has been very little application of this advanced technique to the study of feed structures at cellular and molecular levels. The purpose of this article was to illustrate the SR-FTIR as a useful tool to analyze feed molecular-structure characteristics and imaging molecular chemistry of feeds in relation to animal nutrient utilization.

Key Words: Synchrotron, Feeds, Structures, Cellular and Molecular, Feed Chemistry, Animal Nutrition, Imaging

*E-mail: peiqiang.yu@usask.ca
Characterisation of cDNAs of fatty acyl Δ6 desaturase and elovl5 elongase of cobia (Rachycentron canadum)

Xiaozhong Zheng¹, Zhaokun Ding², Youqing Xu², Oscar Monroig¹, Sofia Morais¹, Douglas R. Tocher¹
¹Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland, UK
²Institute for Fisheries Sciences, Guangxi University, Nanning City, Guangxi, 530004 China

Abstract

Omega-3 or n-3 long-chain polyunsaturated fatty acids (LC-PUFA) have beneficial effects in several inflammatory and pathological conditions, including cardiovascular and neurological diseases. The production of LC-PUFA from C18 fatty acids requires fatty acyl desaturases and elongases, but is inefficient in humans, explaining the greater efficacy of n-3 LC-PUFA than 18:3n-3 in intervention studies. Paradoxically, fish, our major dietary source of n-3 LC-PUFA, are also inefficient in LC-PUFA biosynthesis. We hypothesise that characterising the molecular basis of LC-PUFA biosynthesis in fish will enable greater understanding of the mechanisms underpinning the inefficiency of the pathway in many vertebrates including humans. In the present paper, we investigated the expression of fatty acyl desaturase and elongase genes in a marine teleost, cobia. A cDNA was cloned that, when expressed in yeast, was shown to produce desaturation of 18:3n-3 and 18:2n-6, indicating that it coded for a Δ6 desaturase enzyme. Very low desaturation of 20:4n-3 and 20:3n-6 indicated only trace Δ5 activity. Another cloned cDNA enabled elongation of 18:4n-3, 18:3n-6, 20:5n-3 and 20:4n-6 in the yeast expression system, indicating that it had C18-20 and C20-22 activity. Sequence comparison and phylogenetic analysis confirmed that it was homologous to human ELOVL5 elongase. However, the cobia elongase also had activity towards C24 PUFA. The desaturase had a preference for 18:3n-3, but the elongase was equally active with both n-3 and n-6 substrates. Expression of both genes was 1-2 orders of magnitude greater in brain than other tissues suggesting an important role in neural tissues.

Keywords: Desaturase; Elongase; Genes; Activity; Expression; Fish
Molecular and functional characterization of leptin genes in major Chinese cultivated freshwater fishes

Xu-Fang Liang
College of Fisheries, Huazhong Agricultural University, Wuhan 430070, China

Abstract

Leptin is an important hormone for the regulation of food intake, energy expenditure and reproduction in mammals, but information regarding its role in teleosts remains scant. In the present study, the leptin gene structures of grass carp (Ctenopharyngodon idellus), silver carp (Hypophthalmichthys molitrix), Chinese perch (Siniperca chuatsi) and other major Chinese cultivated freshwater fishes were characterized. Recombinant grass carp leptin (rgc-LEP) was expressed in E. coli and purified, and identified by mass spectrometric analysis. A strong anorexic effect on food intake was observed in grass carp on the first day after intraperitoneal (IP) injection of rgc-LEP, but not during the following days. Body weight of the leptin group (LEP group) and the pair-fed group (PF group) showed no difference throughout the experimental period. The acute and chronic effects on the expression of key genes correlating to food intake, energy expenditure, lipid metabolism and digestion were further characterized by real time PCR. Accordingly, the mRNA levels of neuropeptide Y (NPY), Stearoyl-CoA desaturase 1 (SCD1) and lipoprotein lipase (LPL) were significantly reduced whereas the mRNA levels of uncoupling protein 2 (UCP2), bile salt-activated lipase (BSAL) and fatty acid elongase (ELO) were significantly elevated on the first day after injection. No effect on the expression of these genes (except LPL) was observed on day 13. In contrast to the down-regulation by exogenous leptin in mammals, the mRNA level of grass carp leptin was elevated 5.76-fold on the first day after rgc-LEP treatment. Our results suggest that leptin has an acute effect on the regulation of food intake, energy expenditure and lipid metabolism in grass carp, but the effect can be rapidly counteracted through mechanisms that are currently unknown.

Keywords: leptin; gene structure; recombinant expression; food intake; energy expenditure; major Chinese cultivated freshwater fishes

E-mail address: xfliang@mail.hzau.edu.cn
Real-time PCR quantification of the in vitro effects of glutamine on glutaminase and target of rapamycin gene expression in intestinal epithelial cells of Jian carp (Cyprinus carpio var. Jian)

Jun Jiang1,2, Lin Feng1,2, Yang Liu1,2, Wei-Dan Jiang1,2, Kai Hu1,2, Shuhong Li1, Xiaoqiu Zhou1,2

1Animal Nutrition Institute, Sichuan Agricultural University, Sichuan, Ya’an, 625014, China
2Key Laboratory for Animal Disease-Resistance Nutrition of China Ministry of Education, Sichuan Agricultural University, Sichuan, Ya’an, 625014, China

Abstract
The present study explored the effect of glutamine on the mRNA expression of glutaminase and target of rapamycin (TOR) in isolated Jian carp (Cyprinus carpio var. Jian) enterocytes. Fish enterocytes were cultured with two medium containing 0 and 1.0 mg·L⁻¹ glutamine, respectively. The primers of glutaminase and TOR genes in Jian carp enterocytes were designed according to our laboratory cloned DNA sequences. Relative quantification of mRNA transcript abundance required a pair of primers to be designed for each gene of interest (glutaminase and TOR) and the endogenous reference gene (β-act). The results showed glutamine could improve fish enterocytes glutaminase and TOR mRNA expression. Glutaminase and TOR mRNA relative expressions in fish enterocytes incubated with 1.0 mg·L⁻¹ glutamine for 30 minute were significantly higher than glutaminase deficiency group (P < 0.05). Glutamine promoted TOR expression was inhibited by the TOR inhibitor (rapamycin) (P < 0.05), but not by glutaminase inhibitor (diazo-oxonorleucine) (P > 0.05). In the whole, the present results firstly indicated that glutamine could promote fish enterocytes glutaminase and TOR gene expression. These data would be instructive for further study the mechanism that glutamine promote fish enterocyte growth and proliferation in our previous research.

Keywords: Real-time PCR; glutamine; glutaminase; target of rapamycin; Cyprinus carpio var. Jian; Enterocyt

* E-mail address: xqzhouqq@tom.com (X.Q. Zhou).
Effects of threonine on growth, protein synthesis and TOR gene expression in intestinal epithelial cells of Jian carp (Cyprinus carpio var. Jian)

Lin Feng1,2, Weidan Jiang1,2, Jun Jiang1,2, Kai Hu1,2, Yang Liu1,2, Shuhong Li1, Xiaoqiu Zhou1,2*

1Animal Nutrition Institute, Sichuan Agricultural University, Sichuan, Ya’an, 625014, China
2Key Laboratory for Animal Disease-Resistance Nutrition of China Ministry of Education, Sichuan Agricultural University, Sichuan, Ya’an, 625014, China

Abstract

The present study was conducted to explore the effects of threonine (Thr) on growth, protein synthesis and TOR gene expression in isolated Jian carp (Cyprinus carpio var. Jian) enterocytes. The primary objective of this study was to determine the effects of Thr on cell growth and protein synthesis. Jian carp enterocytes were incubated with DMEM containing 0, 135, 170, 205, 240 and 275 mg Thr L\(^{-1}\) medium, respectively, for 96 h. The results totally showed that Thr was significantly improved the growth of Jian carp enterocytes. MTT OD and cell protein content were maximal for enterocytes incubated with 205 mg Thr L\(^{-1}\) medium. The activities of alkaline phosphatase, Na\(^{+}\)-K\(^{+}\) ATPase, aspartate aminotransferase and glutamate pyruvate transaminase were also significantly increased by Thr supplementation (\(P<0.05\)). A secondary objective of this work was to determine whether Thr increased enterocytes growth by up-regulating protein synthesis. Enterocytes were treated with 0 and 205 mg Thr L\(^{-1}\) medium in the presence of Phe-H\(^{3}\) for 30 minute. Results showed that Thr significantly increased protein synthesis rate of carp intestinal epithelial cells (\(P<0.05\)). Finally, TOR gene expression was measured to further determine whether Thr up-regulation the protein synthesis via TOR pathway to increase the growth of fish enterocytes. Cells were treated with 0 and 205 mg Thr L\(^{-1}\) medium for 0-240 minute and detected TOR gene expression. Results showed that Thr completely increased TOR gene expression of Jian carp enterocytes (\(P<0.05\)). Furthermore, cell TOR gene expression in the presence of Thr was increased with increasing treatment time up to 170 min (\(P<0.05\)). In conclusion, Thr promoted the growth of fish enterocytes and promoted amino acid metabolism, and thus finally ensuring the normal structure and function of enterocytes. In addition, Thr elevating the ability of protein synthesis may be via up-regulation TOR gene expression of Jian carp enterocytes.

Keywords: threonine, intestinal epithelial cells, Jian carp, growth, protein synthesis, TOR gene expression
Effects of emodin, Vitamine E on the growth performance, gene expression of heat shock protein 70 (HSP70) and disease resistance of Wuchang bream (*Megalobrama amblycephala*)

Liu Bo1*, Ming Jianhua1, Xie Jun1, Ge Xianping1, Xu Pao1, Liu Wenbin2
1 Freshwater Fishery Research Center, Chinese Academy of Fishery Sciences, Wuxi 214081, China
2 College of Animal Science and Technology, Nanjing Agricultural University, Nanjing 210095, China

Abstract
A total of 1560 Wuchang bream (*Megalobrama amblycephala*) were randomly divided into four groups. The control group was fed with basic diet, the other treated groups were fed with basic diet supplemented with 150 ppm emodin, 1000 ppm Vitamien E and combination of 150 ppm emodin +1000 ppm Vitamien E, respectively. After 10 weeks of feeding, the growth, the lysozyme, alkaline phosphatase, nitrogen monoxide, superoxide dismutase (SOD),catalase (CAT) and malicdialdehyde (MDA) content and the relative level of hepatic HSP70 mRNA were investigated. The results showed that the growth gain rate, the lysozyme, nitrogen monoxide, SOD,CAT and the relative level of hepatic HSP70 mRNA of fish fed with 150 ppm emodin, 1000 ppm Vitamien E were higher than those of the control. However feed conversion ratios and MDA contents in the groups of 150 ppm emodin or 1000 ppm Vitamien E were lower than those of the control. There were slightly differences in the immune parameters between combination group and the control. However the growth gain rate in the combination group was lower than that of the control. The Fish challenged with *A. hydrophi1a* infection and higher temperature caused mortality in all groups of which the control was highest and the treatment group were relatively lower. Therefore 150 ppm emodin or 1000 ppm Vitamien E in the dietary had the potentialities to stimulate immunity, increase resistance of pathogenic infection, and promote the growth of prawns. Their combination group could enhance disease resistance against *A. hydrophi1a* infection and higher temperature stress. However it was not suitable for fish growth.

Keywords: *Megalobrama amblycephala*; Emodin; Vitamien E; Immunity; HSP70; Disease resistance; Growth

* E-mail address: liub@ffrc.cn
Molecular cloning, characterization and mRNA expression of selenium-binding protein in abalone (Haliotis discus hannai Ino): response to dietary selenium, iron and zinc

Chenglong Wu¹, Wenbing Zhang¹*, Kangsen Mai¹, Xufang Liang², Wei Xu¹, Jia Wang¹, Hongming Ma¹
¹The Key Laboratory of Mariculture (Education Ministry of China), Ocean University of China, 5 Yushan Road, Qingdao 266003, P.R. China
²College of Life Science and Technology, Jinan University, Guangzhou 510632, China

Abstract

Selenium-binding protein (SEBP) is believed to play crucial role in controlling the oxidation/reduction in the physiological processes. In this study, the cDNA of selenium-binding protein from abalone Haliotis discus hannai Ino (HdhSEBP) was cloned by homology cloning and rapid amplification of cDNA ends (RACE) technique. The full length of HdhSEBP cDNA was 2071 bp, consisting of a 5' untranslated region (UTR) of 55 bp, a 3' UTR of 522 bp, and an open reading frame (ORF) of 1494 bp. The deduced protein has 497 amino acid residues with a calculated molecular mass of 55.6 kDa and a predicted isoelectric point of 5.47. BLAST analysis reveals that HdhSEBP shares high identities with other known SEBPs from mammal, bird, fish and mollusk, etc. The mRNA expression patterns of HdhSEBP in hepatopancreas and haemocytes were measured by real-time PCR in abalone fed with nine different diets containing graded levels of selenium (0, 1 and 50 mg Kg⁻¹), iron (0, 65 and 1300 mg Kg⁻¹) and zinc (0, 35 and 700 mg Kg⁻¹) for 20 weeks, respectively. The results showed that the expression of the HdhSEBP mRNA increased and reached the maximum at optimal dietary selenium (1 mg Kg⁻¹), iron (65 mg Kg⁻¹) and zinc (35mg Kg⁻¹), respectively. Deficient or excessive level of dietary selenium, iron or zinc, respectively, leaded to significant depression of HdhSEBP mRNA. It is concluded that the expression levels of HdhSEBP are probably involved in the regulation of oxidation/reduction homeostasis affected by dietary selenium, iron or zinc.

Keywords: Haliotis discus hannai; selenium-binding protein; cDNA cloning; mRNA expression; minerals

* Email address: wzhang@ouc.edu.cn
Changes in gene expression in gut tissue from rainbow trout (Onchorhynchus mykiss) fed mycelium biomass from fungi (Rhizopus oryzae)

Liv Torunn Mydland1*, Stanko Skugor2, Thor Landsverk3, Tone S. Martinsen1, Anders Kiessling1, Trond Storebakken1, Lars Edebo4 and Aleksei Krasnov2

1 Aquaculture Protein Centre, CoE, Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, Aas, Norway
2 NOFIMA Marine, Aas, Norway
3 Aquaculture Protein Centre, CoE, Department of Basic Sciences and Aquatic Medicine, Norwegian School of Veterinary Science, Oslo, Norway
4 Department of Clinical Bacteriology, University of Gothenburg, Gothenburg, Sweden

Abstract

Conversion of non-food carbohydrates into valuable feed ingredients is of great importance for a future sustainable aquaculture industry. Spent sulfite liquor (SSL) from the paper pulp industry is a high organic content by-product that contains approximately 50% of the dry weight of wood in a dissolved form. Rhizopus oryzae is a chitin/chitosan-rich, filamentous fungi, which is able to assimilate the hexoses glucose, mannose and galactose, and the pentoses xylose and arabinose, as well as acetic acid, all which are present in SSL. By cultivating R. oryzae in paper pulp SSL we can achieve both a nutrient-rich mycelium biomass (MB) and high ethanol yields. The potential of using MB as an ingredient in diets for carnivorous fish has hitherto not been extensively investigated, thus the present study was conducted to extend the knowledge of MB as an ingredient in diets for rainbow trout (Onchorhynchus mykiss). Rainbow trout were fed three different diets containing 0, 10 and 20% of MB for 15 days before samples were collected (9 fish/diet) for histological examinations and gene expression analyses. The histological examinations revealed some impact on the immune system (lymphoid hyperplasia in the spleen) and also small spots of vacuolization of the epithelium in the mid gut. The salmonid fish microarray (SFA2) enriched in genes involved in functional classes such as immune responses, cell communication, signal transduction, receptor activities, apoptosis, cell cycle, protein catabolism and folding and responses to pathogens, environmental and oxidative stress were used. Pooled samples of RNA isolated from gut tissue from 0, 10 and 20% MB fed fish were used for hybridization; 0% vs. 10% MB and 0% vs. 20% MB, respectively. We observed differential expression in the gut tissue of a number of genes in both 10 and 20% MB fed fish, when compared to fish fed the control diet (>120 up-regulated and >150 down-regulated). Interestingly, many genes involved in intracellular transport and motility were down-regulated (especially actin-binding protein; several myosins and troponins), however the genes for type II keratin E3 (intermediate filaments) and cytoplasmic dynein light chain (tubulin-binding) were up-regulated. Also; many genes involved in cell cycle, apoptosis, the MAPK pathways and calcium-binding proteins were up-regulated, whereas several genes for extracellular matrix proteins were down-regulated. Some of the findings were also verified by real-time qPCR on individual samples using GAPDH as reference gene.

Keywords: Rainbow trout, Single cell protein, Microbial biomass, Microarray, Gene expression

*E-mail: liv.mydland@umb.no
Time response expression profiles of genes, possibly associated with effects of altered intestinal microbiota in Atlantic salmon (Salmo salar L.) fed soybean meal.

Elin C Valen*, Inderjit Singh Marjara, Trond M Kortner, Åshild Krogdahl and Anne Marie Bakke
Aquaculture Protein Centre (a CoE), Department of Basic Sciences and Aquatic Medicine, Norwegian School of Veterinary Science, P.O. Box 8146 Dep, NO-0033 Oslo, Norway

Abstract

Soybean is often used as an alternative protein source in fish feed, although it is well established that it induces mucosal disorder and inflammatory responses in the distal intestine. The exact cause of soybean meal (SBM)-induced enteritis is so far not fully understood. However, SBM contains several potent antinutritional factors that disturb the digestive system and thereby cause both reduced weight gain and reduced feed conversion in Atlantic salmon. Studies have also shown that inclusion of soybean in the feed altered the composition of the intestinal microbiota, both in number and diversity of populations. Inclusion of oxytetracycline in the SBM diets reduced the number of bacteria, but did not affect the severity of the enteritis. Thus a role of intestinal bacteria in the development of the inflammation cannot be excluded and further investigations are merited. Inflammatory bowel disease (IBD) is a chronic relapsing inflammatory disorder in the gastrointestinal tract of humans, caused in part by an unregulated immune response to intestinal bacteria. Structurally conserved molecules derived from microbes can activate Toll-like receptors (TLRs), and in this way disrupt the intestinal homeostasis. Signaling via the TLRs leads to the production of a variety of inflammatory cytokines including interferons and interleukins, which can stimulate an increase in T cell proliferation and differentiation. Results from previous experiments using immunohistochemical and molecular methods indicate an increased presence of T cells during the development of SBM-induced enteritis, possibly linking the TLRs and the altered intestinal microbiota to the inflammatory responses observed after exposure to SBM.

To examine the time response aspect of the SBM-induced enteritis, fish were fed a diet containing extracted soybean meal (20%) for various time intervals from 0 to 21 days. Total RNA was extracted from the distal intestine and quantitative real-time PCR (qPCR) was used to create a time response gene expression profile of immune-related genes. Interestingly the results show significant regulations of several cytokines and molecules involved in the TLR signaling pathway, including interferon alpha (INFα) and interferon-γ-inducible lysosomal thiol reductase (GILT), suggesting that possible changes in gut microbiota can induce an autoimmune response and contribute to the intestinal impairment. Moreover the expression patterns indicate that the development of the enteritis starts after 3 days of exposure to SBM, and subsequently evolves into a more stable and chronic stage after 7 to 10 days of feeding.

Keywords: Atlantic salmon; Soybean meal; Microbiota; Enteritis; Gene expression.

* Email- address: elin.valen@nvh.no (Elin C. Valen)
In vitro study of fish adipocyte differentiation by using a primary culture system

Hiromi Oku*

1National Research Institute of Fisheries Science, Nikko, Tochigi, 321-1661, Japan

Abstract

Adipocyte differentiation is an important factor to determine body lipid deposition in cultured fish. To investigate the molecular mechanisms of fish adipocyte differentiation, we developed a primary culture system of the stromal-vascular cells of red sea bream Pagrus major. The stromal-vascular cells of red sea bream were prepared by enzymatic digestion of visceral adipose tissue and cultured in DME/Ham’s F12 medium containing 65mM NaCl and 10% FBS. Adipocyte differentiation was induced by switching the medium to a serum-free DME/Ham’s F12 containing 65mM NaCl, 5μg/mL bovine insulin and 50ng/mL hydrocortisone. In this condition, adipocyte differentiation, as characterized by cellular lipid accumulation, was observed within 7 days, and bovine insulin enhanced the adipocyte differentiation dose-dependently. The time course study revealed that the expression of various adipogenic genes, e.g. lipoprotein lipase, fatty acid synthetase and stearoyl-CoA (delta-9) desaturase, were activated during the adipocyte differentiation of red sea bream. Furthermore, supplementation of 2-bromopalmitic acid, a non-metabolic fatty acid, modified the gene expression in the differentiating adipocytes of red sea bream: 2-bromopalmitic acid supplementation increased the gene expression level of a stearoyl-CoA desaturase and the relative content of delta-9 fatty acid desaturation product. The results suggest the possibility of control of adipocyte function by external factors. Taken together, our study provides information on the molecular mechanisms of fish adipocyte differentiation.

Keywords: Adipocyte; Differentiation; Red sea bream; In vitro; Primary culture

*E-mail address: hiromi@fra.affrc.go.jp (Oku, H.)
Transcriptomics (oligo microarray) to assess the effects of different diets on gene expression in heart skeletal muscle inflammation virus infection in Atlantic salmon

Laura Martinez¹*, Sofia J. Morais¹, Gordon Bell¹, José L. González Vecino², Simon Wadsworth², Douglas R. Tocher¹

¹ Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, (Scotland) UK.
² EWOS Innovation AS, Dirdal N-4335, Norway.

Abstract

In the last few years there has been a dramatic increase in inflammatory diseases in commercial Atlantic salmon farms in Norway. Heart and Skeletal Muscle Inflammation (HSMI) is currently one of the most prevalent of these with over 600 sites affected since 2003. Although the mortality caused by HSMI is low (less than 4%) there is a significant impact on FCR and growth that is causing considerable financial impact (more than €100 million). Histopathologically, heart and red skeletal muscle appear to be the main organs affected showing severe inflammation, but liver damage is also found. The clinical symptoms are believed to correlate with the intensity of the inflammatory response, so factors modulating inflammation might influence the clinical manifestation of this disease. The important role of dietary fatty acids in modulating immune responses is well recognized in humans and has also been established in cultivated fish. ARA (20:4n-6), EPA (20:5n-3) and DHA (22:6n-3) are the precursors for synthesis of eicosanoids, resolvins and protectins, which have important roles in inflammation. The eicosanoids derived from ARA promote pro-inflammatory responses and those derived from EPA produce a reduced inflammatory response. The aim of this study was to evaluate the use of microarray technology to assess the effects on specific diets, formulated and produced by EWOS Innovation, on fish infected with HSMI disease. It was hypothesised that the response of the genes involved in the inflammatory responses would differ, when alternative marine lipid sources rich in n-3 fatty acids were included to increase cell membrane content.

Triplicate groups of Atlantic salmon (initial weight 110g) reared in tanks at Lønningdal research station (EWOS Innovation AS, Norway) were fed either a standard commercial diet or an experimental functional feed (FF diet). After a 12-week feeding period, all fish were transferred to ILAB Bergen, Norway and challenged by intramuscular injection (0.1 ml on each side close to the lateral line) of virus supernatant collected from HSMI virus culture. Heart samples from six fish per treatment were collected at 8 and 16 weeks post-challenge in order to evaluate changes in gene expression between treatments using a custom-made Atlantic salmon 45K oligo-array (Agilent). Results from microarray analysis, showing considerable differences in gene expression reflecting the immunological response, will be presented and discussed in relation to the potential of the FF diet to reduce the incidence and severity of HSMI.

* E-mail address: laura.martinez@stir.ac.uk
Growth and muscle proteome response to fish protein hydrolysates in the diet of zebrafish

Mahaut de Vareilles1*, Pedro Gomèz-Requeni2, Katerina Kousoulaki3, Odete Cordeiro1, Tomé S. Silva1, Nadège Richard1, Luis E.C. Conceição1, Pedro M. Rodrigues1 and Ivar Rønnestad2
1 Centro de Ciências de Mar do Algarve (CCMAR), Campus de Gambelas, 8005-139 Faro, Portugal
2 Department of Biology, University of Bergen, Bergen, Norway
3 Nofima Ingredients, Fyllingsdalen, Norway

Abstract
The effects of fish protein hydrolysates (FPH) on growth performance and muscle proteome expression in juvenile zebrafish (Danio rerio) were investigated. From 33 to 48 days post-fertilisation (DPF), triplicate groups of 15 fish were fed with one of four iso-nitrogenous, -lipidic, -glucidic, and -energetic diets, in which approximately 30 % of dietary protein was provided either as fish meal (FM, control diet) or as a combination of FM with whole fish protein hydrolysate (FH), retenate after ultrafiltration of FH (to remove lower molecular weight compounds, UF), or nanofiltered permeate of FH (high in free amino acids, NF), at a 30 % level of FM substitution. Fish growth was monitored and the loin muscle was excised at 48 DPF for proteome analysis of the sarcoplasmic fraction, by means of two-dimensional differential in gel electrophoresis (DIGE).

Final mean fork length decreased as FPH size in the diet decreased, being significantly different between FM and NF (p < 0.05), NF also showing the smallest growth rate (expressed as mm/day). Analysis of the muscle partial proteome, using DeCyder 2D Software Version 7.0, enabled the detection and comparative quantification of 480 proteins with a pI between 4 and 7, seventy-two of which were differentially expressed among groups (One-way ANOVA, p < 0.05). According to PCA of significantly differentially expressed proteins (32 proteins, p < 0.01), the spot maps (representing each a biological replicate) were easily divided in four clusters corresponding to dietary treatments, with NF clearly distinguished from all other treatments and the largest difference between NF and UF (78.1 % of variance given by PC1 and PC2). Protein expression patterns were further analysed by pattern analysis using a Hierarchical Clustering algorithm with Euclidean distance measure and average linkage. Again, spot maps were clustered according to dietary treatment. NF was again the most distant from other treatments – especially UF –, with 44 % of proteins under-expressed compared to other groups and 22% over-expressed. Proteins with the highest expression variation among dietary treatments will be identified by MALDI-TOF spectrometry analysis. These results will be discussed in relation to the FPH in the diet of juvenile zebrafish, with emphasis on growth performance and muscle development.

Keywords: Zebrafish; juveniles; fish protein hydrolysates; growth; muscle proteomics

* E-mail address: mahaut13@gmail.com
Expression of genes of long-chain polyunsaturated fatty acid (LC-PUFA) biosynthesis during early embryogenesis in cobia *Rachycentron canadum*

Óscar Monroig 1*, Ken Webb 2, Leonardo Ibarra-Castro 2, G. Joan Holt 2, Douglas R. Tocher 1

1 Institute of Aquaculture, University of Stirling, Stirling, Scotland, UK
2 Marine Science Institute, University of Texas at Austin, Port Aransas, Texas, USA

Abstract

Developing organisms including fish have high requirements for long-chain polyunsaturated fatty acids (LC-PUFA) to fulfil the demands of critical tissues such as brain and retina, where these compounds accumulate in membrane phospholipids. We recently investigated the activation of the LC-PUFA biosynthesis pathway in zebrafish embryos by analysing the expression of genes involved in the pathway. The study revealed that zebrafish embryos express all the enzymatic activities required in the synthesis of LC-PUFA such as docosahexaenoic acid (DHA), and thus can supplement supply of preformed LC-PUFA from the yolk sac. Unlike freshwater species, marine fish have been generally considered unable to biosynthesise LC-PUFA from C18 PUFA precursors. However, little information is available on the biosynthetic capacity of early life stages of marine fish. The present study investigated the expression of genes encoding enzymes of the LC-PUFA biosynthetic pathway in the marine teleost cobia (*Rachycentron canadum*). First, an elongase with sequence similarity to mammalian very long-chain fatty acids 4 (Elovl4) was isolated, and functional characterisation revealed it catalysed conversion of 22:5n-3 to 24:5n-3, which can be further desaturated and chain shortened to DHA, confirming its potential role in DHA synthesis. Further activity of Elovl4 on 22:5n-3 produced pentaenes up to C36, suggesting a role in the synthesis of very long-chain PUFA (VLC-PUFA), which are fatty acids relatively abundant in specific tissues such as retina, testicles and brain. Subsequently, the temporal expression patterns of *elovl4*, together with the formerly characterised elongase *elovl5* and fatty acyl desaturase Δ6fad, were analysed by RT-PCR in cobia embryos collected throughout early ontogeny up to 72 hours post-fertilisation (hpf). Interestingly, *elovl4* exhibited increasing expression levels from 9 to 60 hpf, thus suggesting the biosynthesis of VLC-PUFA is activated early on in developing retina and brain as occurs with zebrafish *elovl4* genes. Although not measured in embryos themselves, *elovl4* spatial expression pattern in cobia juveniles revealed eye (probably retina), brain, gonads and pineal gland, were major tissue sites. In contrast to *elovl4* expression and the results obtained in zebrafish development, no significant expression signals were detected for *elovl5* and Δ6fad in cobia embryos up to 72 hpf.

Keywords: Cobia; early embryogenesis; Elovl4-like elongase; Elovl5-like elongase fatty acid; fatty acyl desaturase; LC-PUFA biosynthesis;

*E-mail address: oscar.monroig@stir.ac.uk* (Ó. Monroig).
Evaluation of candidate reference genes for quantitative real-time PCR (qPCR) assays of distal intestinal tissues after partial soybean meal replacement in diets for Atlantic salmon (*Salmo salar* L.)

Trond M Kortner*, Elin C Valen, Elvis Chikwati and Åshild Krogdahl
Aquaculture Protein Centre (a CoE), Department of Aquatic Medicine and Nutrition, Norwegian School of Veterinary Medicine, P.O. Box 8146 Dep, NO-0033 Oslo, Norway

Abstract

Soybean meal (SBM) has been considered a potential protein substitute in formulated feed for carnivorous fish species such as Atlantic salmon. However, the use of SBM in salmon feed is not trivial, due to the well documented development of an inflammatory like condition (enteritis) in distal intestine after SBM introduction. Regulation of gene expression and subsequent molecular processes are believed to be key mechanisms leading to SBM-induced enteritis in distal intestine, and the most utilized tool for quantification of gene expression is quantitative real-time PCR (qPCR). The accuracy of qPCR assays depends on proper normalization, and the use of reference genes as internal controls is commonly accepted as the most appropriate normalization strategy in qPCR assays. However, there is increasing evidence that expression of many putative reference genes is regulated by the experimental conditions, and there is a growing agreement for the need to evaluate the stability of potential reference genes under different experimental setups, as it has been demonstrated that both false positive and false negative results may be obtained when using inappropriate (i.e. unstable) reference genes. Relatively few studies have evaluated reference genes for use as internal controls in qPCR assays in fish, and previous studies demonstrate that the choice is highly dependent on experimental conditions such as species, tissue, developmental stage and exposure. Therefore, the aim of this study was to quantitatively evaluate the most commonly used reference genes in research for their suitability as normalization factors for gene expression analyses after SBM-induced entereopathy in intestinal tissues of Atlantic salmon. After an initial evaluation of the stability of eight commonly used reference genes, the software applications GeNorm, NormFinder and BestKeeper were used to rank genes according to their stability across 200 samples from three independent feeding trials. Interestingly, beta-actin (actb), elongation factor 1 alpha (ef1a), glycerol 6-phosphate dehydrogenase (g6pdh) and ribosomal protein s20 (rps20) mRNA levels displayed a consistent time dependent induction after SBM introduction. In contrast, hypoxanthine phosphoribosyl transferase 1 (hprt1), RNA polymerase 2 (rnapolII), glyceraldehyde-3-phosphate dehydrogenase (gapdh) and 18s ribosomal RNA (18s) were stably expressed during the experiments. Overall, we recommend a combination of gapdh and rnapolII as an internal normalization factor in Atlantic salmon distal intestine. Furthermore, we evaluated the use of normalization software tools, and we demonstrate that ignoring underlying assumptions made by these softwares may result in an inaccurate or even totally incorrect conclusion when selecting the best reference gene(s).

Keywords: Atlantic salmon, reference genes, nutrition, digestion, gene expression, real-time PCR

* E-mail address: trond.kortner@nvh.no
Construction of the hepatopancreas and kidney cDNA subtractive library of abalone *Haliotis discus hannai* Ino. fed with vitamin C-deficiency diet

**Jia Wang**¹,²*, **Hongming Ma**¹, **Kangsen Mai**¹, **Wenbing Zhang**¹, **Zhiguo Liu**¹

¹The Key Laboratory of Mariculture, Ministry of Education, Ocean University of China, Qingdao 266003, P.R.China

²Feed Research Institute, the Chinese Academy of Agricultural Sciences, Beijing 100081, P.R.China

**Abstract:**

The study was conducted to clone the differentially expressed genes in the hepatopancreas and kidney of abalone (*Haliotis discus hannai* Ino.) under vitamin C deficiency. Abalone, (initial weight: 74.66±1.0 g; initial shell length: 84.36±1.07 mm) were fed 170d with purified diets containing 2 levels of vitamin C: 0 mg/kg and 4967.5 mg/kg. cDNA subtractive libraries of the hepatopancreas and kidney of abalone vitamin C deficiency-treated were constructed using the method of suppression subtractive hybridization (SSH). Subtraction efficiencies were about 2³ and 2³⁻¹₀-fold enrichment in hepatopancreas and kidney cDNA subtractive library respectively. Positive clones were randomly picked and sequenced. Sixty three clones from hepatopancreas cDNA subtractive library were sequenced. The sequences were subjected to GO annotation. According to their physiological function, they could be subdivided into 4 categories: 19.0% of metabolic processes genes, 33.3% of cellular metabolic processes genes, 4.8% of biological regulation genes, 7.9% of other functions genes, and unknown genes. Thirty nine cDNA fragments from kidney cDNA subtractive library were sequenced, and they could be subdivided into 3 categories: 5.1% of metabolic processes genes, 28.2% of cellular metabolic processes genes, 23.1% of biological regulation genes, and unknown genes. In conclusion, the deficiency of vitamin C has significant impact on the metabolic processes of abalone. The genes of ferritin and enzymes related to the energy and electron transport process and the carbohydrate metabolic process had highly expressed in vitamin C deficiency group, which may due to the elevated vitamin C biosynthesis.

**Keywords:** *Haliotis discus hannai* Ino.; hepatopancreas; kidney; vitamin C; suppression subtractive hybridization; subtractive cDNA library.

*E-mail address: wjyt@sina.com.cn*
Genotype by diet interactions in feed utilisation by Black tiger shrimp, *Penaeus monodon*

Brett Glencross\(^1\)*, Simon Tabrett\(^1\), Simon Irvin\(^1\), Nick Wade\(^1\), David Smith\(^1\), Greg Coman\(^1\), Nigel Preston\(^1\)

\(^1\)CSIRO Marine and Atmospheric Research, PO Box 120, Cleveland, QLD 4163, Australia

Abstract

Selected (G8) and wild-type (W) strains of Black tiger shrimp (*Penaeus monodon*) juveniles (initial weight G8 = 9.14 ± 0.36 g/animal and W = 8.44 ± 0.10 g/animal) were fed either of two diet types in a clear-water tank trial to examine the effects of diet type, ration level and genetics on growth and feed utilisation parameters. Animals were fed twice daily at one of five ration levels from starvation to apparent satiety. All feed fed and uneaten was accounted for and all moults removed. Starved animals were measured after three weeks; those fed were measured at both three and six weeks. Diet type varied by protein content, raw material choice and the presence (HSD) or absence (LSD) of bioactive substances. At the end of the study faecal samples were also collected to determine the digestible protein and energy content of each diet by each genotype. Whole animal protein and energy content were also assessed from samples from the initial populations and those from each tank.

Growth after three weeks of those animals fed to satiety showed that those G8 animals fed the HSD diet had grown at a rate of 3.09 g/wk, significantly faster than any other treatment. Those G8 animals fed the LSD diet (2.04 g/wk) had grown significantly faster than the W animals fed the HSD diet (1.33 g/wk), while those W animals fed the LSD diet (0.73 g/wk) grew the slowest. Using the data from the varying ration levels we were able to define that the growth gains of the G8 animals were achieved not only by a greater appetite, but also lower maintenance energy costs and a more efficient energy conversion. Use of a low-specification-diet with the G8 and W prawns limited their growth and impaired their potential as demonstrated by a curvilinear response of growth to intake. By comparison those prawns fed the HSD diet had a linear growth response to intake.

These results demonstrate that genetic effects dominate the impact seen in growth achieved with this species. Although nutritional gains through the use of HSD diets can be large, their effect is eclipsed by the relative effect seen through genetic gains.

Keywords: Prawn; Genetic; Energetics; Bioactives

*E-mail*: Brett.Glencross@csiro.au
The effect of protein growth rate on the optimum dietary indispensable amino acid pattern in growing rainbow trout (*Oncorhynchus mykiss*) fry including requirements for maintenance and for tissue accretion

Xavier Rollin*, Christophe Manssens, Yvan Larondelle
Laboratoire de Pisciculture Huet, Institut des Sciences de la Vie, UCLouvain, route de Bloery, 2, B-1348 Louvain-la-Neuve, Belgium

Abstract

The indispensable (I) amino acid (AA) requirements of a growing fish include two components, a requirement for maintenance and a requirement for tissue protein accretion. To the best of our knowledge, experiments have never been performed to estimate simultaneously the requirements of several IAA for maintenance and for tissue accretion. The estimates of the optimal balance of IAA made by Green & Hardy (2002) and Rollin et al. (2003) in salmonids referred to the sum of these two components, and can only be considered strictly applicable to one particular rate of growth or protein accretion. Therefore, an experiment was made to estimate the requirements of growing rainbow trout fry in five different IAA, separately for maintenance and protein accretion using the methodology of Fuller et al. (1989). The relationship between nitrogen (N) gain and AA intake was estimated for each IAA (or pairing of an IAA and a semi-dispensable AA) by giving, at rates of N intake of 0.01, 0.15, 0.29 and 0.43 g/kg body-weight (W)\(^{0.75}\) per day, diets in which one AA was made specifically deficient. There was no evidence of any departure from linearity in the relationship between daily N gain and daily AA intake for any of the tested AA (0.983< \(r^2\)<0.999; n=8; P<0.001). From the regression coefficients, it was calculated that, for the accretion of 1 g body protein, the dietary AA requirements (mean ± SE) were (mg) threonine 52.9 ± 1.0, lysine 105.4 ± 4.0, arginine 86.4 ± 3.5, methionine + cystine 56.9 ± 0.9 and phenylalanine + tyrosine 119.0 ± 6.7. The daily AA requirements for N equilibrium (mean ± SE) were estimated to be (mg/kg W\(^{0.75}\) per day) threonine 5.4 ± 0.7, lysine 10.6 ± 2.9, arginine 6.6 ± 2.5, methionine + cystine 7.4 ± 0.7 and phenylalanine + tyrosine 7.1 ± 4.3. When expressed in g/16gN, it gave threonine 2.4 ± 0.3, lysine 4.8 ± 1.3, arginine 3.0 ± 1.1, methionine + cystine 3.3 ± 0.3 and phenylalanine + tyrosine 3.2 ± 1.9. In conclusion, our results show that the pattern of AA required for maintenance and protein growth is quite different, and the fish total requirement must, therefore, depend on the relative contributions of maintenance and tissue protein accretion to its total needs. Experiments are in progress for branched-chain AA, histidine and tryptophane.

Keywords: Amino acids requirements; Rainbow trout fry; experimental ideal protein; Diet dilution procedure

*E-mail address: xavier.rollin@uclouvain.be (X. Rollin).
The influence of adiposity of brown trout (Salmo trutta fario) fry on their feeding motivation, agonistic behavior and territorial ability at low density

Xavier Rollin*, Quentin Watthez, Frédéric Dumonceau, Yvan Larondelle
Laboratoire de Pisciculture Huet, Institut des Sciences de la Vie, UCLouvain, route de Blocry, 2, B-1348 Louvain-la-Neuve, Belgium

Abstract

The aim of the present study was to clarify the role of whole-body lipid reserves (adiposity) in social interactions and feeding behavior in brown trout fry at low density. Adiposity decreases the metabolic demands and feeding motivation in juvenile salmonids. As a consequence, adiposity may decrease aggression levels and/or agonistic ability and/or territorial behavior. To test these hypotheses we first produced at similar growth rate two fry groups during a conditioning period with a low fat (12%)-diet (LF) and a high fat (27%)-diet and then we made four experiments with conditioned fry. After the conditioning period, fry had similar mean body weight (P>0.05) and their lipid contents were 54-70% higher in the HF group compared to the LF group (P<0.05). In experiment 1, twelve fry of similar size (1.39-1.41 g) were sampled in each group (LF and HF), marked for group recognition, and then distributed in each of the eight 15-l aquaria at the start of a 7-day trial for assessment of their ability to obtain food items (bloodworms). A significant difference in competitive ability between LF and HF fish was found, where LF fry obtained a significantly higher proportion of the contested food items than did HF fry (72 vs. 28%, P<0.01). In experiment 2, we observed agonistic behavior during a 5-day fasting trial in 21 aquaria of fry consisting of six LF fry (5 aquaria), six HF fry (5 aquaria), or three LF fry and three HF fry (11 aquaria). Aggression was lowest in the HF aquaria, intermediate in the LF/HF aquaria and highest in the LF aquaria, with the difference between the HF group and the LF group being significantly different. This supports the hypothesis that adiposity decreases aggression levels. In the LF/HF aquaria, the number of conflicts won by HF fry was significantly lower compared to LF fry. Thus, social status was decreased in the HF group and adiposity appears to affect directly and negatively fighting ability. These results were confirmed in a similar trial (experiment 3) performed in an artificial river. In experiment 4, we observed territorial behavior in 21 size-matched pairs of fry (one pair/aquarium) consisting of one LF and one HF fry. The surface occupied by LF fry was significantly higher compared to the HF fry. In conclusion, our results indicate that fish adiposity depress feeding motivation, competitive ability to feed, and agonistic and territorial abilities of brown trout fry at low density.

Keywords: Adiposity; Brown trout fry; feeding motivation; agonistic behavior; territorial ability

* E-mail address: xavier.rollin@uclouvain.be (X. Rollin).
P-096

Taking steps toward mass culture of two native Brachionus rotifers from Iran: estimation of temperature preference

Reza Malekzadeh Viayeh and Habib Mohammadi

1Artemia and Aquatic Animals Research Institute, Urmia University, Urmia, Iran
2Department of Fisheries and Aquaculture, Faculty of Natural Resources, Urmia University, Urmia, Iran

Abstract

This study was carried out with the aim of estimating the effects of different culture temperatures on life-table parameters of two native rotifers, Brachionus plicatilis (B. pli) and B. rotundiformis from Iran. Each of the rotifer species were cultured at three temperatures of 10, 15 and 30ºC. The other environmental conditions were constant and the same for all the treatments, that is, salinity was 15ppt, rotifer density was 500 ind./ml and feeding was by same concentration of marine Chlorella vulgaris. The estimated parameters comprised the ratio of unegged females (UF) to egged females (EF), number of mictic females (MF), number of mictic eggs (ME), number of males (M), number of resting eggs (RE) and number of parthenogenetic eggs (PE) all per mL. of culture medium. Maximum average UF/EF, average MF and average ME was for rotifer pli at 30ºC. The highest mean number of male rotifers was for B. rot produced at 30ºC and maximum number of PE was for B. rot at 10ºC. ANOVA showed differences in the values of the estimated parameters among the treatments (P<0.05), except for the number of RE. A Duncan test demonstrated that difference in UF/EF ratio was only significant between B. rot at 10ºC and B. pli at 30ºC, and between the latter treatment and B. pli at 10ºC. For MF, ME and M, there were significant differences among the treatments of same species at different temperatures and between two species. The difference in the number of PE was not significant among rotifers of same species, and number of RE was not different among all 6 treatments. Temperature had notable influence on most of the measured criteria in the studied rotifers, and both the rotifers grew better at 10 ºC.
Effects of inclusion of yeast and pig blood protein hydrolysates in microdiets on larval quality of gilthead sea bream *Sparus aurata*

E. Gisbert¹*, A. Skalli¹, Y. Kotzamanis², Zambonino-Infante, J.L.³

¹IRTA, Centre de Sant Carles de la Ràpita, Unidad de Cultius Experimentals. Crta. del Poble Nou s/n, 43540 Sant Carles de la Ràpita, Tarragona, Spain
²Hellenic Centre for Marine Research, Institute of Aquaculture, Hellinikon 16610, Athens, Greece
³Marine Fish Nutrition Team, Nutrition Aquaculture and Genomics Research Unit, UMR 1067, Ifremer, Technopole Brest-Iroise, Plouzané, France

Abstract

The aquaculture feed industry is continuously seeking for new ingredients to improve the formulation and performance of their diets, and the sector devoted to microdiets for marine fish larvae is not an exception. We evaluated the substitution of fish meal by two commercial protein hydrolysates from yeast (*Saccharomyces cerevisiae*) and pig blood (NORLAN LV⁻²⁰⁻¹ and NORLAN LXTM, respectively; PROALAN SA, Spain) in larval microdiets for gilthead sea bream larvae and compared these results to a control microdiet containing CPSP-90TM (Sopropêche, France). Diets were tested in a dose-response experiment that lasted 45 days, in which live prey was substituted up to 60% with the microdiets during the *Artemia* feeding phase (15-40 dph at 18°C). Dietary treatments consisted of the standard feeding regime (enriched rotifers and *Artemia*), and five co-feeding regimens differing on the protein hydrolysate (CPSP-90TM, NORLAN LXTM and LVTM) and level of fish meal substitution (9-12% for NORLAN; 12% for CPSP-90). NORLAN protein hydrolysates are soluble in water with similar levels of free amino acids (35-36%) and di- and tri-peptides (44%; 300 Da). CPSP-90TM is a fish protein concentrate obtained by enzymatic hydrolysis and dehydration of fish (whole or canning byproducts). The effect of inclusion of commercial protein hydrolysates to inert diets in gilthead sea bream larvae was evaluated in terms of growth, survival and incidence of skeletal deformities.

At the end of the trial, no differences in survival and growth were detected among larvae fed live prey and those fed with microdiets. However, results revealed that diets affected the incidence of deformities in larvae fed different microdiets. Fish fed microdiets containing yeast and pig blood protein hydrolysates showed the same frequency of skeletal anomalies than those fed live prey (10.2%). In particular, the incidence of skeletal deformities in fish fed LV9 and LV12 microdiets was 15.0 and 12.0%, respectively; whereas those observed in fish fed LX9 and LX12 diets were 9.7 and 13.7%. Larvae fed microdiets containing yeast and pig blood hydrolysates at 12% showed ca. 50% less deformities than those fed diets formulated with CPSP-90 (26.4%). Such differences in larval quality between fish fed the above-mentioned diets might be attributed to their different biochemical properties, like the high fraction of water soluble protein, the average weight of the hydrolysate and their high content in free amino acids. The former parameters might have promoted the harmonic morphogenesis of larvae, and consequently, reduced the incidence of skeletal disorders.

*E-mail address: enric.gisbert@irta.es*
Effect of captivity in digestive and metabolic enzymes in the spider crab *Maja brachydactyla* broodstock

G. Rotllant¹*, E. Gisbert¹ and M. Solé²

¹ IRTA. Carretera del Poble Nou km 5.5, 43540 Sant Carles de la Ràpita, Tarragona, Spain
² Institut de Ciències del Mar, CSIC, Passeig marítim de la Barceloneta 37-45, 08003 Barcelona, Spain

**Abstract**

Spider crab *Maja brachydactyla* adults (CL=144±14 mm) were kept in captivity (36‰ and 18°C) for one (10 males, ♂️ C1 and 10 females, ♀️ C1) and two years (10 females, ♀️ C2), and fed with a combination of fresh mussels (*Mytilus sp.*, 5x week) and frozen crab (*Liocarcinus depurator*, 2x week). Ten wild males (♂️ W) and females (♀️ W) obtained from commercial catches were sampled as control group. Digestive enzyme capacities (total protease, trypsin, amylase, lipase, alkaline phosphatase and aminopeptidase N) were compared between wild specimens and those reared under aquaculture facilities for 1 and 2 years in order to evaluate the effects of diet supplied in captivity. In addition, some biomarkers selected as potential indicators of biochemical and oxidative stress were analyzed (acetylcholinesterase, AChE and propionylcholinesterase (PrChE) in muscle; catalase, glutathion reductase and glutathion peroxidase activities and lipid peroxidation, LPO levels in hepatopancreas). Detoxification metabolic enzymes such as glutathion S-transferase and carboxylesterase activities were also quantified in the hepatopancreas but were not significantly affected by captivity. No differences were observed in hepatosomatic indexes (HSI) between males and females, and captive animals presented lower HSI than wild ones (∗️ C1 = 3.8±0.8, ♀️ C2 = 2.8±0.9, ♀️ W = 5.2±0.7; ♂️ C1 = 3.4±1.4, ♂️ W = 5.9±1.1). In females, trypsin and amylase activity remained constant during the two years of captivity (∗️ trypsin = 577±300, ♀️ amylase = 150.2±17.9 UI/g tissue), whereas in males trypsin activity of captive animals was half of that in wild ones (∗️ C1 = 1074±583, ♀️ W = 456±270 UI/g tissue). Lipase activities between wild females and males were different (∗️ W = 138.4±45.9 vs. ♀️ W = 70.1±8.5 UI/g tissue), whereas captivity did not affect them. Nutritional stress biomarkers, AChE and PrChE did not show significant differences between wild and captive animals (6.0±2.0 and 4.7±1.4 mmol/min/mg prot, respectively). Hepatopancreatic LPO levels remained constant in females (17.8±5.7 mmol MDA/g tissue), while it decreased in captive males (15.5±4.4 vs. 21.4±6.1 mmol MDA/g tissue). In both males and females, captivity significantly reduced catalase activity. Digestive physiology biomarkers indicated that wild females and males differed in their feeding habits, females tended to prefer diets with higher lipid content than males, which may be linked to the role of these compounds in egg yolk reserves. Captive animals showed a large plasticity in their digestive capabilities as indicated by the adaptation to food supplied (lower diversity of feed items and monotonous diet) and no signs of nutritional stress.

**Keywords:** Spider crab; Broodstock; Digestive enzymes; Biomarkers

* E-mail address: guiomar.rotllant@irta.cat (G. Rotllant).
Effects of Glutamine Dipeptide on the Growth Performance and Antioxidant of *Hucho Taimen* larvae

Qiyou Xu, Changan Wang, Hong Xu, Jiasheng Yin, Jianzhang Ma

1 Heilongjiang River Fishery Institute of Chinese Academy of Fishery Sciences, Harbin 150070, China
2 College of Wildlife Resource, Northeast Forestry University, Harbin 150040, China

Abstracts

Effects of Ala-Gln on growth performance and antioxidant of *Hucho taimen* larvae were studied. Six experimental diets containing different levels of Ala-Gln (with the levels of 0%, 0.125%, 0.250%, 0.500%, 0.750% and 1.000% respectively) were randomly assigned to triplicate groups of 1 000 fishes for 8 weeks. Compare with the control group, the growth performance were not influenced by adding 0.125% Ala-Gln ($P>0.05$), WGR and SGR increased significantly by adding 0.500%~1.000% Ala-Gln ($P<0.05$), survival rate increased significantly by adding more than 0.125% Ala-Gln ($P<0.05$). Glutamine of fish body tissue fluid increased by adding more than 0.500% Ala-Gln($P<0.05$) and glutamic acid content increased significantly by adding 0.750% and 1.000% Ala-Gln($P<0.05$), Na$^+$, K$^+$-ATPase activity increased by adding 0.500% and 0.750% Ala-Gln($P<0.05$), SOD increased($P<0.05$) and MDA decreased significantly ($P<0.01$), but amino acid content wasn’t influenced by adding Ala-Gln($P>0.05$). Glutamine and glutamic acid content of intestine were increased significantly by adding Ala-Gln($P<0.05$), lipase, Na$^+$, K$^+$-ATPase activity of intestine were increased significantly($P<0.05$), MDA decreased significantly($P<0.05$) or very significantly ($P<0.01$). The experiment showed: The growth performance and antioxidant of *Hucho taimen* larva could improved by adding Ala-Gln, the appropriate adding level of Ala-Gln is 0.750%.

Keywords: Ala-Gln, *Hucho taimen*, larvae, Growth performance, Antioxidant

*E-mail address: xuqiyou@sina.com*
Comparison of the effects of dietary phospholipids on intestinal lipid absorption between rainbow trout fry and juveniles

Franco Daprà, Inge Geurden, Geneviève Corraze, José-Luis Zambonino-Infante, Stéphanie Fontagné-Dicharry

1 INRA, UMR 1067 NuAGe, Pôle d’Hydrobiologie, F-64310 Saint-Pée-sur-Nivelle, France
2 IFREMER, UMR 1067 NuAGe, Technopole Brest-Iroise BP 70, F-29280 Plouzané, France

Abstract

The aim of the study was to compare the effects of dietary phospholipids (PL) on intestinal lipid absorption between fry and juvenile stages of rainbow trout (Oncorhynchus mykiss). Three semi-purified casein-based diets were formulated with 12% fish oil and either 6% soybean lecithin (diet PLS), 6% egg lecithin (diet PLE) or 6% soybean oil (diet PL0) for the PL-free diet. The diets were tested in triplicate on swim-up fry from first-feeding (initial body weight: 66±2 mg) at 17°C over a 5-month growth trial. Trout fry and juvenile were sampled 4 hours after the last meal at days 20 and 146, respectively, for histological analyses (n = 8 fish per dietary treatment). At the fry stage at day 20, the mean body weight of trout fry fed PLS and PLE (0.28±0.06 g and 0.34±0.06 g) was significantly higher than those fed PL0 (0.21±0.02 g). Histological examination revealed intestinal steatosis (accumulation of large lipid droplets in the enterocytes of the anterior intestine) in each of the trout fry fed PL0 associated with a high enterocyte height (51±6 µm). In contrast, trout fry fed PLS and PLE did not show any intestinal steatosis and displayed significantly reduced enterocyte height (39±4 µm and 38±2 µm). At the juvenile stage at day 146, there was no longer significant effect of dietary PL supplementation on growth (final mean weight: 22±2 g). Plasma total cholesterol, triacylglycerol and PL concentrations were not significantly different between dietary groups. No intestinal steatosis and no difference in height of mucosal epithelium were noticed in juvenile rainbow trout (46±3 µm). This study confirms the essentiality of PL in rainbow trout fry diets. The beneficial effect of dietary PL on intestinal lipid absorption is restricted to the fry stages. Results support previous opinions that PL are needed for intestinal triacylglycerol absorption. At early developmental stages, the limiting step seems to be the synthesis of lipoproteins by the enterocytes. Further investigations are in progress to assess the level of expression of genes involved in PL and lipoprotein synthesis between dietary groups.

Keywords: Dietary phospholipids; Intestinal lipid absorption; Fry; Juvenile; Rainbow trout

E-mail address: corraze@st-pee.inra.fr
Effects of different dietary vitamin A levels on the spawning performance and offspring quality of gilthead sea bream Sparus aurata broodstock

I. Fernández¹, N. Duncan¹, A. Estévez¹, F. Hontoria² and E. Gisbert¹.
¹IRTA, Centre de Sant Carles de la Rápita, Unidad de Cultius Experimentals. Crta. Del Poble Nou s/n, 43540 Sant Carles de la Rapita (Tarragona, Spain)
²IATS, Instituto de Acuicultura Torre de la Sal. CSIC. 12595 Ribera de Cabanes (Castellón, Spain)

Abstract

Broodstock and immature fish being on-grown present different metabolic needs, but broodstock diet formulation is often based on juvenile nutritional requirements and the formulation must be improved to enhance spawning performance and offspring quality. Vitamin A (VA) is an essential nutrient that might play a key role in gonad maturation, as well as in embryonic development. Several studies on the dietary VA effects on larval development have shown that the effects were due to excessive or deficient VA levels in the eggs. The present study aimed to evaluate the spawning performance of gilthead sea bream Sparus aurata broodstock fed three different diets (named A, B and C) with increasing content of total vitamin A (15,000, 150,000 and 1,550,000 IU kg⁻¹ respectively). Diets were formulated and manufactured by Skretting ARC, Norway. The following parameters were analysed: number of spawning episodes, total volume and number of spawned eggs, percentage of floating/no floating eggs, egg lipid content and classes, egg retinoid content, egg size, percentage of fertilized and hatched eggs, larval survival rate of hatched larvae at 3, 5 and 7 dph and incidence of deformed larvae. Broodstock fed diet B gave the highest amount of spawned eggs and broodstock fed the highest dietary VA content (diet C) the lowest. Furthermore, broodstock fed diet C presented higher percentage of non floating eggs during the spawning season. The percentage of fertilized eggs decreased, whereas the percentage of hatched increased with the content of VA in the broodstock diet. Total egg lipid content was no significant different during all the spawning season within each broodstock group. Interestingly, while total lipid content in eggs were not different between eggs from broodstock A and B, total lipid was significantly lower in eggs from broodstock C compared to B. No differences were found in larval survival rate at 3, 5 and 7 dph, nor in the percentage of abnormal larvae; however, those parameters have a decreasing trend with the increase of VA content in diet. The present study found that diet B gave the best spawning performance with the highest spawned egg volume or fecundity; while broodstock fed the highest VA content diet presented a significantly higher percentage of non viable eggs.

*E-mail address: ignacio.fernandez@irta.es
Vitamin A and Senegal sole *Solea senegalensis* larvae: the effect of timing and dietary content on larval performance

I. Fernández¹, F. Hontoria² and E. Gisbert¹

¹IRTA, Centre de Sant Carles de la Rápita, Unidad de Cultius Experimentals. Crta. Del Poble Nou s/n, 43540 Sant Carles de la Rapita (Tarragona, Spain)
²IATS, Instituto de Acuicultura Torre de la Sal. CSIC. 12595 Ribera de Cabanes (Castellón, Spain)

Abstract

Vitamin A is an important morphogenetic nutrient, since it plays a key role in morphogenesis, tissue development and larval growth. In this sense, several studies have shown a direct effect of dietary VA content (excess and/or deficiency) in larval performance. Furthermore, it was suggested that the developmental stage at which nutritional VA imbalance is applied is critical for fish development. Therefore, the effects of different doses of dietary VA administered trough live feed (rotifers and *Artemia*) at different developmental periods (3-10, 10-20 and 20-40 days post hatch) were evaluated in Senegal sole *Solea senegalensis* larvae in terms of survival, growth and larval quality (typology and incidence of skeletal deformities). Live preys were enriched with Easy Selco (1666 IU VA g⁻¹, ES, INVE) in the control group Control, or with ES containing 10 and 50 times VA (VA10x and VA50x, respectively; by retinol paltitate addition). Results showed that larvae fed VA10x and VA50x during early developmental phase (3-10 dph) had significantly higher mortality, than in the rest of dietary groups. No differences were found in length whatever the nutritional imbalance was applied during the larval development. VA larval content reflected the dietary VA content of the live prey. Senegal sole larval skeletogenesis was affected by the dietary VA content; at 10 dph larvae fed different dietary VA content showed clearly different stages of ossification in different skeletal structures. For example, VA10x group present higher percentage of larvae with ossified lower and upper jaws than Control group, and that higher than VA50x. In addition, only a hypervitaminosis A during the early ontogeny of Senegal sole increased the number of vertebral bodies. Moreover, even after metamorphosis completion, Senegal sole seems to be sensitive to hipervitaminosis VA, as vertebral deformity incidences still increased. Present results showed how dietary VA imbalance is a potent developmental disruptor, mainly at early phases of larval development, being where the bone cell proliferation and differentiation differentially affected by the nutritional VA imbalance. Moreover, it have been found that the incidence of skeletal deformities was developmental-stage dependent. Therefore, further work is needed to develop a balanced larval nutrition for each larval developmental stage, in order to improve larval performance and produce high quality larvae at intensive Senegal sole aquaculture.

*E-mail address: ignacio.fernandez@irta.es*
Inclusion of protein hydrolysates in the diet of white seabream (*Diplodus sargus*) larvae: impacts on skeleton quality and larvae proteome expression

Nadège Richard1*, Mahaut de Vareilles1, Paulo J. Gavaia1, Tomé S. Silva1, Odete Cordeiro1, Manuel Yúfera2, Pedro M. Rodrigues1 and Luis E.C. Conceição1

1 Centro de Ciências de Mar do Algarve (CCMAR), Campus de Gambelas, 8005-139 Faro, Portugal
2 Instituto de Ciencias Marinas de Andalucia (CSIC), 11510 Puerto Real, Cadiz, Spain

Abstract

In order to investigate the effects of dietary protein hydrolysate content on larval skeleton quality and larval proteome expression, triplicate groups of white seabream (*Diplodus sargus*) larvae were co-fed from first-feeding onwards with live feed and three microencapsulated diets differing only on the molecular weight of their protein hydrolysate fraction. The protein hydrolysate fractions were: commercial hydrolysate (control diet), a high amount in larger molecular weight hydrolysates (3-20 KDa peptides, diet H) and a high amount in smaller molecular weight hydrolysates (<500Da, tri-, di-peptides and free amino acids, diet L). At 15 days after hatching (DAH), proteome expression changes were assessed in entire larvae by two-dimensional gel electrophoresis and the quality of larval skeleton was analysed at 28 DAH through double staining of cartilage and bone. Dietary protein hydrolysate fractions tested did not affect the incidence of deformed larvae, nor the number of deformities per fish. Nevertheless, larvae fed diet L presented a higher proportion of deformities in the pre-haemal region, where the incidence of deformed neural arches was higher compared to the two other groups. Vertebral fusions affecting the cephalic part were only observed in control group larvae. Two-dimensional analysis of larvae proteome, performed with PDQuest 2-D analysis software, allowed the detection and the comparative quantification of a total of 709 protein spots having a pI between 4 and 7. From these spots, 339 showed significant variations (Mann-Whitney U test, p<0.05) depending on the dietary treatment. Among them, 126 were significantly differentially expressed between group control and both groups L and H (100 spots under-expressed and 26 spots over-expressed in group control). A total of 98 spots displayed significant variation between groups H and control (45 spots under-expressed and 53 spots over-expressed in group H) and 84 spots were significantly differentially expressed between groups L and control (15 spots under-expressed and 69 spots over-expressed in group L). Proteome expression of larvae from groups L and H were differentiated by the expression of 93 spots (49 spots under-expressed, 44 spots over-expressed in group H). Some of the spots differentially expressed among the groups were analysed by liquid chromatography-tandem mass spectrometry and identified as proteins involved in cellular contractile system, energetic metabolism, collagen maturation process or chaperoning. These results revealed that the molecular weight of the dietary protein hydrolysate fraction had a strong effect on proteome expression of white seabream larvae. Another set of mass spectrometry analysis will extend the results.

Keywords: Seabream; larvae; protein hydrolysates; skeletal deformities; proteomics

* E-mail address: nrichard@ualg.pt (N. Richard).
P-104

Inclusion of natural carotenoids in diets for gilthead sea bream broodstock (*Sparus aurata* L.) and its effect on spawning

Valeria S. Scabini 1*, H. Fernández-Palacios 2, L. Robaina 2, T. Kalinowski 2 and Marisol Izquierdo 2.

1 Facultad de Ciencias, University of Magellan (UMAG), P.O. 113-D, Punta Arenas, Chile.
2 Grupo de Investigación en Acuicultura (IUSA & ICCM), University of Las Palmas de Gran Canaria, Arucas, Las Palmas de Gran Canaria, Canary Island, Spain

Abstract

It is known that several nutritional factors are associated with reproduction in Teleosts, being the portion size, nutrient levels and type the most important ones. Nowadays the feed pellet industry for aquaculture is in continuous search for alternative sources, based on high quality plants ingredients. The aim of this study was to determine the inclusion effect, of natural carotenoids both animal and plant, in diets for gilthead sea bream broodstock, and its effect on the spawning quality. Thus, four isoproteic diets were formulated, using squid and fish meal, with two lipids levels of (20 and 15%). In addition, two types of natural carotenoids were included: paprika powder and shrimp meal. The denomination and ingredients used in experimental diets is: 20 without carotenoids added; 20 P: with carotenoids, paprika powder (P); 15: commercial diet for broodstock; 15 L: with carotenoids, shrimp meal (L).

Thirty six mature seabream (*Sparus aurata*) of 1.68 kg average body weight for the females and 0.91 kg for the males were divided into 12 groups of 2 males and 1 female in 1 m³ fibre tanks. Fish were fed three times a day to 1.5% body weight/day. Five spawning quality parameters were daily recorded. Samples of eggs were taken along the experimental period and analyzed for proximate and fatty acid composition. After three weeks of feeding, the percentage of live eggs of the broodstock fed with diet 20 P, which contained the highest carotenoids and lipids level, was significantly better than the percentage of live eggs from the spawnings of the broodstock fed diet 20 that contained the same lipid level but without carotenoids. The experimental diets also had an affect on the relative production (kg of female per spawning). This way, the broodstock fed with diet 20P produced a significantly higher number of hatched larvae than the broodstock fed with the diet 15. Similarly, the broodstock fed this diet (20P) produced a significantly higher number of larvae with the yolk-sac reabsorbed than the broodstock fed with diets 20 and 15. The results of this study propose that the spawning quality of gilthead sea bream can be improved by the inclusion of dietary carotenoids. The inclusion of carotenoids, such as paprika powder, of vegetable origin, improved spawning quality than the shrimp meal, of animal origin, even though, the first diet contributed to a higher percentage of total carotenoids. It is also known that several nutritional factors are associated with reproduction in Teleosts, being the portion size, nutrient levels and type the most important ones.

Keywords: Carotenoids, Broodstock, Spawning, Quality, gilthead seabream

*E-mail address: valeria.scabini@umag.cl.
Effects of different dietary phospholipids levels on development of gilthead sea bream (*Sparus aurata*) larvae

Reda Saleh Mohamed Ibrahim, M.B. Betancor, E. Atalah, J. Roo, T. Benítez-Santana and M.S. Izquierdo
Group of Investigation in Aquaculture (GIA), ULPGC & ICCM, P.O. Box 35200 Telde, Canary Island, Spain

Abstract

The aim of the present study was to determine the influence of different levels of dietary phospholipids (PL) on the development of gilthead sea bream (*Sparus aurata*) larvae. Larvae were fed from 17 to 31 day post hatching (dph) five formulated microdiets with different levels of Krill phospholipids (PL) (0, 1, 2, 4 and 6 PL %). Highest growth, survival and resistance to stress was found in larvae fed 6 % dietary PL although without significant difference with larvae fed 4 % PL, (8.68±0.24 & 8.43±0.34 mm in TL and 47.92±1.54 & 47.71±4.34 % survival rate, for 6 and 4 % PL respectively). Biochemical composition of larvae (31 dph) fed 4 and 6 % dietary PL demonstrated significantly higher (P< 0.05) total lipid content, and total n-3HUFA, EPA and n3/n6 both in neutral and polar lipids than larvae fed 0 and 1 % dietary PL. The results of this study suggest a requirement of about 4-6 % PL in diets for early weaning of gilthead sea bream larvae.

Keywords: Krill phospholipids, Larvae development, Gilthead *Sparus aurata*

E.mail address: reda-saleh@hotmail.com (R. S. Mohamed Ibrahim)
Growth response and nitrogen utilization of rainbow trout (*Oncorhynchus mykiss* W.) fry fed graded dietary levels of crystalline amino acids: effects of fish meal substitution level, ontogeny and adaptation

Noélie Bodin¹, Tran Thi Nang Thu¹,², Eric Le Boulengé¹, Yvan Larondelle¹, Xavier Rollin¹*  
¹Laboratoire de Pisciculture Huet, Institut des Sciences de la Vie, UCLouvain, route de Bloery, 2, B-1348 Louvain-la-Neuve, Belgium  
²Faculty of Animal Sciences and Aquaculture, Hanoi University of Agriculture, Trau Quy, Gia Lam, Hanoi, Vietnam

Abstract

Effects of increasing dietary substitutions of cod fish meal by a mixture of crystalline (C) amino acids (AA) of similar composition on growth, voluntary feed intake, nitrogen (N) gain and N utilization efficiency were studied on rainbow trout (*Oncorhynchus mykiss* W.) for two different sizes at the fry stage. Two experiments were carried out in which 1050 rainbow trout fry (0.70 ± 0.01 (SE) mean body weight) were randomly assigned to fifteen 15 l-tanks with 70 fish per tank (3 tanks per dietary treatment) and 450 rainbow trout (2.85 ± 0.02 g mean body weight) were randomly assigned to fifteen 45 l-tanks with 30 fish per tank (3 tanks per dietary treatment). In both experiments, fish were fed twice daily to satiation for 25 days five isoenergetic (18.7 MJ gross energy/kg dry matter (DM)) and isonitrogenous (65 g N/kg DM) diets containing graded levels of coated CAA, replacing from 0% (D0) to 100% (D100) of the cod fish meal on a similar AA composition basis. Compared to D0, mean weight gain (g/fish), the daily growth coefficient (DGC) and voluntary feed intake (g DM/kg metabolic body weight per day) were not significantly reduced for small fry and large fry fed diets D25 and D25-D50, respectively. They were significantly reduced for small fry and large fry fed diets D50-D100 and D75-D100, respectively. Feed efficiency (g/kg DM) and N gain (g N/kg live weight per day) were not significantly reduced for small fry and large fry fed diets D25-D50 though they were significantly reduced for fry fed diets D75-D100. The protein productive value (PPV) (N gain/N intake) was not significantly reduced for small and large fry fed diets D75 though it was significantly reduced for fry fed diets D100. However, the following response criteria were significantly reduced from the control (D0) to the D100 diet for small and large fry: DGC (−58 and −39%), feed intake (−33 and −12%), N gain (−55 and −49%) and PPV (−32 and −43%). In conclusion, this study showed that a maximum CAA level of 65% should still ensure reasonable growth (85% of D0) after an adaptation period of 17 days minimum for both fry sizes and that larger fry react better to CAA-rich diets than smaller fry, indicating ontogeny-related changes in CAA utilization efficiencies.

Keywords: Rainbow trout; Fry; Amino Acids; Fish meal; Crystalline amino acid;

*E-mail address: xavier.rollin@uclouvain.be (X. Rollin).*
The effect of adiposity of brown trout (*Salmo trutta fario*) fry on survival, body composition and energy utilization during long-term fasting at three different temperatures

Xavier Rollin*, Frédéric Dumonceau, Yvan Larondelle
Laboratoire de Pisciculture Huet, Institut des Sciences de la Vie, UCLouvain, route de Blocry, 2, B-1348 Louvain-la-Neuve, Belgium

Abstract

Cultivated brown trout for river restocking have been reported to be fattier than their wild counterparts, due to the lipid-rich commercial diets used by farmers. On the other hand, cultivated fish may encounter difficulties to feed in their new environment after restocking and possibly have to face a long period of starvation. The physiological consequences of such a fasting period have to be evaluated in the context of global warming. The aim of this study was to determine the effect of whole-body lipid reserves (adiposity) of brown trout fry on its survival, body composition and energy utilization during long-term fasting at three different targeted temperatures (12, 16 and 20 °C). Two groups of fry (initial body weight 0.75g) were fed for a 30-day conditioning period at $11 \pm 0.5$ °C on a “low fat” (12%)-diet (LF) or the same diet enriched with 20% fish oil to give a “high fat” (32%)-diet (HF). Ration was adapted so that fry grew at similar rate. After the conditioning period, the two groups were fasted for 90 days at $12.1 \pm 0.3$, $15.6 \pm 0.3$ or $19.6 \pm 0.5$ °C. Fish mortality was recorded daily. Fish body weight and composition were measured after 0, 10, 30 and 60 days of fasting. At the end of the conditioning period, fry had similar weight in both groups (2.1 g/fish, $P>0.05$) but whole-body lipid content was, as expected, much higher in the HF group compared to the LF group (+33%, $P<0.01$). During the fasting period, the LF group showed much lower survival rate compared to the HF group, especially at 16 and 20 °C (+ 47% after 60 days of fasting). Changes in body weight, body composition, and energy, protein and lipid losses largely depended on the fasting time, temperature and diet. During the first 10 days of fasting, the LF group showed higher energy, lipid and protein losses compared to the HF group ($P<0.01$), but weight losses were similar in both groups ($P>0.05$) and the contribution of protein in fish energy losses (%) was not affected by diet (adiposity) ($P>0.05$). Between days 11 and 60 of fasting, while weight and energy losses decreased with time, the contribution of protein in energy losses markedly increased ($P<0.01$); interestingly, this increase occurred earlier in the LF group compared to the HF group ($P<0.01$). In conclusion, our results indicate that fish adiposity strongly affects endogenous energy utilization and losses as well as survival during long-term fasting in fish.

Keywords: Fasting; adiposity; Brown trout; fry; energy losses

*E-mail address: xavier.rollin@uclouvain.be (X. Rollin).
Adiposity depress voluntary feed intake and lipid deposition, but not growth and protein retention efficiency in brown trout (Salmo trutta fario) fry

Xavier Rollin*, Adrien Nemry, Yvan Larondelle
Laboratoire de Pisciculture Huet, Institut des Sciences de la Vie, UCLouvain, route de Bloqry, 2, B-1348 Louvain-la-Neuve, Belgium

Abstract
Exogenous lipids are known to spare dietary protein in growing fish, minimizing their utilization for energy production. However, it is not known if endogenous lipid reserves (adiposity) can influence protein utilization efficiency in fed fish as well. The aim of this study was to determine the effect of whole-body adiposity of brown trout fry on its voluntary feed intake (VFI), growth, lipid and protein retention efficiency during a 30-day growth test when fed Chironomidae sp. larvae at different rations at 12±0.5 °C. First, two groups of fry (initial body weight 0.85g) were fed for a 34-day conditioning period on a “low fat”(12%)-diet (LF) or the same diet enriched with 20% fish oil to give a “high fat” (32%)-diet (HF). Ration was adapted so that fry grew at similar rate. As expected, fry had similar BW in both groups at the end of the conditioning period (1.9g/fish, P>0.05) and whole-body lipid content was higher in the HF group compared to the LF group (+69%, P<0.01). After the conditioning period, the LF and HF groups were distributed in twenty-four 15-l aquaria (65 fry/aquarium) and fed Chironomidae larvae at four rations of 1.3, 2.6, 3.9, 5.2 % of body weight per day (2 aquaria/group*ration) or at apparent satiation (4 aquaria/group) for a 30-day period. VFI was measured daily and fish body weight and proximal composition were measured after 0, 10, 20 and 30 feeding days. When fed ad libitum, VFI was deeply depressed in the HF group compared to the LF group (-14%, P<0.05). This depression was high at the beginning of the trial (-50%), progressively decreased with time and was positively correlated to the difference of adiposity between the groups (P<0.01). Growth rate, N and lipid gains were linear functions of the ration (r²>0.97, P<0.001). The slopes and intercepts of the relationships between N gain and intake (mgN/fish) were not significantly different (LF: y = -9.2 + 0.397X, n=12, r²=0.99, P<0.001; HF: y = -9.9 + 0.406X, n=12, r²=0.99, P<0.001), indicating that adiposity did not affect protein utilization efficiency and protein requirement for maintenance. However, lipid deposition and lipid utilization efficiency (lipid deposition/lipid intake) were considerably higher in the LF group compared to the HF group. In conclusion, our results indicate that fish adiposity depress VFI, lipid deposition and lipid utilization efficiency, but not growth and protein utilization efficiency in brown trout fry.

Keywords: Adiposity; voluntary feed intake; Brown trout; fry; protein retention efficiency

* E-mail address: xavier.rollin@uclouvain.be (X. Rollin).
Partial substitution of fish meal by sesame oil cake (Sesamum indicum) in the diet of brown trout (Salmo trutta fario) and rainbow trout (Oncorhynchus mykiss) fry: an interspecific comparison

Tran Thi Nang Thu¹², Julien Henrotte¹, Yvan Larondelle¹, Xavier Rollin¹*
¹Laboratoire de Pisciculture Huet, Institut des Sciences de la Vie, UCLouvain, route de Bloery, 2, B-1348 Louvain-la-Neuve, Belgium
²Faculty of Animal Sciences and Aquaculture, Hanoi University of Agriculture, Trau Quy, Gia Lam, Hanoi, Vietnam

Abstract

Our objectives were to test the potential replacement of fish meal by sesame oil cake (SOC) in high energy diets fed to rainbow trout (RT, Oncorhynchus mykiss) and brown trout (BT, Salmo trutta fario). Groups of trout (initial body weight (BW) of 1.42 g for RT and 1.48 g for BT) were fed to visual satiety with isonitrogenous (61 g digestible N kg dry matter (DM)⁻¹) high-energy (22.1 MJ kg DM⁻¹) freeze-dried diets, in which fish meal was progressively replaced by SOC (0, 13, 26, 39 and 52%, on a N basis). Apparent digestibility coefficient (ADC) of the diets was determined using the indirect method. A growth trial was conducted over feeding 45 days at 15 ± 0.6°C. Protein digestibility was high (91%) and was unaffected by either diet or species. ADC of energy was reduced by 5.5% in both species when SOC replaced 52% of fish meal protein and was significantly higher in BT compared to RT. Growth rate increased when SOC progressively replaced fish meal until reaching a maximum at a substitution rate of 39% for RT and 26% for BT. In both species, DM intake (%BW day⁻¹) linearly increased with substitution rate and feed efficiency was adversely affected by SOC. Protein and energy retention efficiencies were little affected by diet (except in the 52% SOC diet for RT which showed significantly lower protein retention efficiency), but were significantly lower in BT compared to RT, especially energy retention efficiency (42.5-44.8% in RT vs. 32.7-35.8% in BT). The positive effect of SOC on growth was mainly explained by a general increase in feed intake (2.54 and 3.23 % BW day⁻¹ in RT and 1.73 and 2.10% in BT, for the fish meal control diet and the 52% SOC diet, respectively). From our results, it is concluded that SOC can substitute at least 52% of fish meal protein both in RT and in BT fry without negative effect in growth rate.

Keywords: Sesame oil cake; Rainbow trout; Brown trout; Digestibility; Voluntary feed intake

* E-mail address: xavier.rollin@uclouvain.be (X. Rollin).
Growth and gut transit time of Atlantic halibut larvae reared under shifting light regime

Shou Wang1*, Torstein Harboe2, Ivar Ronnestad1

1Department of Biology, University of Bergen, Pb 7800, 5020 Bergen, Norway
2Institute of Marine Research, Austevoll Research Station, 5392 Storebo, Norway

Abstract

High mortality rates and large variations in larval quality are commonly observed in Atlantic halibut fry production. High economic loss and animal welfare issues are closely related to rearing conditions in this critical stage. Continuous light condition is used in commercial juvenile Atlantic halibut farming in Norway, in contrast to diurnal light and darkness periods in nature environment. A pilot study demonstrated dramatically reduction of incomplete eye migration in Atlantic halibut under shifting light regimes using single meal based feeding. The aim of this study is to reveal gut ingestion and evacuation at selected stages during development until metamorphosis in Atlantic halibut in order to assess gut transit time. Three experiment tanks were set up under 7 h of darkness and 17 h of light during a 24 h cycle. Fish in a period from 8-30 days post first feeding were sampled for number of Artemia in the gut by direct counting. All larvae were fed short time enriched Artemia supplied two times daily. As seen in earlier studies, large variations in number of Artemia in the gut were observed. The time to evacuate the gut was estimated more than 4 hours, as calculated by another study based on a model for the same species. Age affects the gut transit rates in larvae sampled. It was also observed that evacuation somehow stopped during the second hour after light was switched off. The effects of prey availability and light will be discussed in relation to appetite, gut transit time and absorption of critical nutrients.

Key words: Atlantic halibut; Gut transit; Larvae; light regimes;

*E-mail address: shou.wang@student.uib.no
Effect of different selenium sources on growth performance and oxidation resistance of *Litopenaeus vannamei*

Yu wei12, Junming Cao1, Hongxia Zhao1, Yanhua Huang1, Xiaohua Liu3, Bing Chen1, Hanbing Lan1

1 Institute of Animal Science, Guangdong Academy of Agriculture Sciences
2 South China Agricultural University, College of Animal Science
3 Huazhong Agricultural University, Fisheries College

Abstract

The effect of two selenium sources on growth performance and oxidation resistance of *Litopenaeus vannamei* was studied. 0.10, 0.30 and 0.60 mg/kg of Na2SeO3 (inorganic selenium) as well as 0.10, 0.30 and 0.60 mg/kg of methionine selenium (organic selenium) were added to the half-purified basal feed, to carry out a feeding experiment for *Litopenaeus vannamei* juvenile prawns with average weight of 0.54±0.02 g for 8 weeks. 3 repetitions were set for each feed group and each repetition had 40 prawns. The results showed that the relative growth rate (RGR) in the group of 0.6 mg/kg inorganic selenium and the group of 0.3 mg/kg organic selenium was significantly higher than that in control group (P<0.05). Except the treatment group of 0.3 mg/kg organic selenium, the survival rate in other treatment groups was all higher than that in the control group, but the difference was not significant (P>0.05). The feed efficiency (FE) in all treatment groups was higher than that in the control group, and except the treatment group of 0.1 mg/kg organic selenium, other treatment groups all reached the significant levels (P<0.05). The effect difference of supplemented selenium in feeds on whole body composition of *Litopenaeus vannamei* was not significant (P>0.05). The supplementation of inorganic and organic selenium in feeds could all improve the selenium retention in the muscle of *Litopenaeus vannamei*, of which the retention rate of organic selenium was superior to that of inorganic selenium. After comparing the effects of these two selenium sources on blood serum and hepatopancreas T-AOC, GPx, GR and CAT of *Litopenaeus vannamei*, it was found that the T-AOC activity of the organic selenium groups was higher than that of inorganic selenium groups in case of the same dose levels; the low dose of organic selenium improved GSH-Px activity more highly than inorganic selenium; in case of low and medium dose of organic selenium, MDA content was lower than that of inorganic selenium; the liver GR activity of the organic selenium groups with all doses was higher than that of the inorganic selenium groups; the blood serum CAT activity of all the organic selenium groups was higher than that of the inorganic selenium groups, and the liver CAT activity of 0.6 mg/kg organic selenium groups was the highest, higher than that of all the inorganic selenium groups.

Keywords: *Litopenaeus vannamei*; selenium; growth performance; oxidation resistance; immunity

* E-mail address: junmcao@163.com
Effects of dietary nucleotides level on growth performance and immune activities for juvenile *Litopenaeus vannamei*

Dan-dan Xu ¹²*, Jun-ming Cao ¹, Han-bing Lan ¹, Yan-hua Huang ¹,Guo-li Li ¹, Hong-xia Zhao ¹, Bing Chen ¹

¹ Institute of Animal Science, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, China
² College of Animal Science, South China Agriculture University, Guangzhou 510642, China

Abstract

A feeding trial was conducted to test the effects of different dietary mix-nucleotides (mix-NT) level on the weight gain rate (WGR), survival rate (SR), feed conversion rate (FCR), hepatosomatic index (HSI) and ability of anti-WSSW in *Litopenaeus vannamei*. The shrimps (IBW 1.0±0.01g) were fed a basal diet (as control) and 7 diets supplemented with 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 g/kg mix-NT, being named G0.1, G0.2, G0.4, G0.6, G0.8, G1.0 and G1.2 respectively. After 7 weeks feeding, 13 shrimps of each repetition were injected with white spot syndrome virus (WSSV) suspension with 50ul, while 10 shrimps of each repetition were injected with Shrimp Saline with 50ul as control. After 72 hours of artificial infection, the 2- steps WSSV PCR were used to detect the infection of WSSV. The results showed that with increased dietary mix-NT level, WGR reached the highest level at G0.6 and FCR of G0.6 decreased by 4.24% in comparison with the control group. But the differences between the control and the other groups in WGR, SR and FCR of *Litopenaeus vannamei* were not significant (P>0.05). HSI was the highest in shrimp fed 0.6g/kg diet, being significantly higher than that of shrimp fed the control diet (P<0.05). After chal lenged with WSSV 72 hours, The 2- steps WSSV PCR detection showed that the effected *Litopenaeus vannamei* were WSSV positive. The higher Relative Percent Survival (RPS) was obtained in the group G0.1~G0.8, showing significantly higher than that in the control (P<0.05c). With increasing of dietary mix-NT, the activities of muscle anti-0’₂ and hepatopancreatic SOD significantly increased (P<0.05). Injected WSSV had no significant effect on activities of anti-0’₂ and SOD, while the activities of T-AOC in the group G0.4 were significantly higher than the control (P<0.05). When compared with the groups injected Shrimp Saline, the activities of SOD and anti-0’₂ were both increased in the groups of injected WSSV, while the activities of T-AOC were decreased. In conclusion, appropriate levels of mixed-NT supplemented to the diet could improve the growth performance, to a certain extent, and increase the ability of anti-WSSV in *Litopenaeus vannamei*.

Key words: *Litopenaeus vannamei*; nucleotides; growth; white spot syndrome virus; immune activities

*E-mail address: dandxu2008@163.com*
Effects of dietary β-glucan and glycyrrhizin on non-specific immunity and disease resistance of white shrimp, *Litopenaeus vannamei* challenged with *Vibrio alginolyticus*

Jie Chang, Wen-Bing Zhang*, Kang-Sen Mai, Hong-ming Ma, Zhi-guo Liufu, Xiao-jie Wang
The Key Laboratory of Mariculture (Ministry of Education), Ocean University of China, 5 Yushan Road, Qingdao 266003, P.R. China

Abstract

The white shrimp *Litopenaeus vannamei* fed immunostimulant-free, 0.2% β-glucan and 0.06% glycyrrhizin diets for 18 days, respectively, were challenged with *Vibrio alginolyticus* at 6.4×10^4 cfu shrimp^−1^ The total haemocyte count (THC), phenoloxidase (PO) activity, respiratory burst (RB), superoxide dismutase (SOD) activity changes for a 120 h period were investigated, and shrimp mortality was also recorded. Results showed that PO activity, RB, SOD activity were significantly higher in shrimp fed two immunostimulants diets after 18 days than those in shrimp fed immunostimulant-free diet. The THC and SOD activity decreased significantly after 24-96 h and 12-72 h, respectively. The values for PO activity and RB increased after 12-72 h and 24-48 h, respectively. Furthermore, all immune parameters returned to the normal level at 96 h except RB, which recovered at 72 h. After 120 h, treatments fed immunostimulant had high value in PO activity and RB. Compared with the shrimp fed control diet, mortality after being challenged with Vibrio alginolyticus significantly decreased in shrimp fed with β-glucan or glycyrrhizin. It was concluded that dietary β-glucan and glycyrrhizin increased the shrimp immune ability. Furthermore, β-glucan increased some immune parameters 12 h earlier than glycyrrhizin after *V. alginolyticus* challenged.

Keywords: β-glucan; glycyrrhizin; *Litopenaeus vannamei*; immunity; *Vibrio alginolyticus*

*E-mail address: changjieouc@163.com*
**P-114**

**Effect of Dietary Vitamin E on the non-specific immunity of Chinese Mitten-Handed Crab, *Eriocheir sinensis***

Ai Chun-xiang12, Chen Li-qiao*, Liu Xiao-ling1, Gao Lu-jiao1

1College of Life Science, East China Normal University, Shanghai 20062 China

2College of Oceanography and Environmental Science, Xiamen University, Xiamen 361005 China

**Abstract**

A experiment was conducted to determine the effects of non-specific immunity on the Chinese mitten-handed crabs (*Eriocheir sinensis*) (body weight of 37.52±2.29g) fed the diets containing different vitamin E levels (0, 10, 20, 40, 60mg/100g diet respectively). The crabs were distributed into the glass tanks of 200L capacity using a completely randomized design with five treatments respectively and each treatment was stocked with 10 crabs and was run in triplicate. The activities of phenoloxidase (PO), antibacterial performance (*Ua*), lysozyme (*UL*), superoxide dismutase (SOD), alkaline phosphatase (AKP) and acid phosphatase (ACP) were determined at the end of the 60-day feeding trials. The results indicated that the effects of dietary VE on the activities of PO, *Ua*, *UL*, SOD, AKP and ACP in the serum, muscle, hepatopancrea and ovary of *Eriocheir sinensis* were significant enhanced with VE supplement increasing in the range from 20mg/100g diet to 40mg/100g diet (p<0.05 or p<0.01). However, the activities of AKP were declined at 60mg/100g diet VE. The activities of SOD in the serum, muscle, hepatopancrea and ovary of *Eriocheir sinensis* were significantly lowered with VE Supplementation. VE was shown to increase non-specific immunity when crabs were fed diets containing 20mg-40mg/100g diet VE.

**Keywords:** Chinese mitten-handed crab (*Eriocheir sinensis*), vitamin E, non-special immunity

*E-mail address: Chunxai@xmu.edu.cn*
Effects of Cr\textsuperscript{6+} Stress on the non-specific immunity of *Scylla paramamosain*

Ai Chunxiang*, Xu Hua, Chen Yufeng, Li Shaojing
College of Oceanography & Environmental Sciences, Xiamen University, Xiamen Fujian 361005, China

Abstract
A experiment was conducted to determine the effects of different concentrations of water-borne Cr\textsuperscript{6+} (0.5mg/L , 1.0mg/L, 2.0mg/L, 4.0mg/L, 8.0mg/L) stress and nature seawater (don’t add Cr\textsuperscript{6+}, as the control) on the immune parameters (total haemocyte count (THC), Phenoloxidase, Lysozyme, Ca\textsuperscript{2+}-ATPase and Na\textsuperscript{+},K\textsuperscript{-}-ATPase, superoxide dimutase (SOD), acid phosphatase (ACP), alkaline phosphatase (AKP)) of mud crab *Scylla paramamosain*. The results showed that THC of *S. paramamosain* decreased significantly when exposed to different concentrations of Cr\textsuperscript{6+} for 1d (\(P<0.05\)), the variation of THC was positive related to the Cr\textsuperscript{6+} concentration, and showed time-dose-effect relationship. The THC of *S. paramamosain* in experimental groups were restored gradually to the THC of the control group after 9d Cr\textsuperscript{6+} exposure (\(P>0.05\)). Phenoloxidase activity was significantly suppressed in hemolymph when exposed to different concentrations of Cr\textsuperscript{6+} for 1d (\(P<0.05\)), but the variation of PO activity was not relates to Cr\textsuperscript{6+} concentration. Lysozyme activity was significantly elevated in hemolymph of *S. paramamosain* when exposed to the concentrations of 4.0 mg/L and 8.0 mg/L Cr\textsuperscript{6+}. The activation depended on exposure time of Cr\textsuperscript{6+}(time-effect relationship), which suggested a decrease in activation with prolongation of exposure time. The antibacterial activity was significantly inhibited in hemolymph of *S. paramamosain* (\(P<0.05\)), and showed the time-dose-effect relationship. The activities of Ca\textsuperscript{2+}-ATPase and Na\textsuperscript{+},K\textsuperscript{-}-ATPase were significant decreased by Cr\textsuperscript{6+} exposure in gills of *Scylla paramamosain* after 1d (\(P<0.05\)). The activities of Ca\textsuperscript{2+}-ATPase and Na\textsuperscript{+},K\textsuperscript{-}-ATPase were stimulated by Cr\textsuperscript{6+} with exposure time prolonging. The superoxide dimutase (SOD) activity were significant increased by Cr\textsuperscript{6+} exposure in gills, hepatopancreas, muscle of *S. paramamosain* (\(P<0.05\)). The superoxide dimutase (SOD) activities in serum, gills, hepatopancreas, muscle of *Scylla paramamosain* were significant increased by Cr\textsuperscript{6+} exposure (\(P<0.05\)). Acid phosphatase activities in gills, hepatopancreas, muscle of *Scylla paramamosain* were not significantly effect by Cr\textsuperscript{6+} exposure (\(P>0.05\)), while the alkaline phosphatase activity in gills, hepatopancreas, muscle of *Scylla paramamosain* activated by Cr\textsuperscript{6+} exposure. The effect of water-borne Cr\textsuperscript{6+} exposure on gill and hepatopancreas microstructure of *S. paramamosain* put up the dose-time effect. Some changes were observed in histolopathological microstructure of gill and hepatopancreas after 9d. The irregularly thickened gill lamellae epithelium disassembled were the other histolopathological effects of 8.0mg/L Cr\textsuperscript{6+} after 9d. In hepatopancreas, the basement membranes injury, the boundary between hepatic tubules was unclear, increasing hemocytes was observed. The ACP activity in gill of *S. paramamosain* increased significantly after 1d Cr\textsuperscript{6+} exposure (\(p<0.05\)), however, hepatopancreas, muscle of *S. paramamosain* was insignificantly change to Cr\textsuperscript{6+} exposure, the ACP activities in hepatopancreas of *S. paramamosain* in the groups of 2.0mg/L, 4.0mg/L, 8.0mg/L Cr\textsuperscript{6+} concentrations were significantly decreased (\(p<0.05\)) after 9d Cr\textsuperscript{6+} exposure. The AKP activity in gill of crabs in different Cr\textsuperscript{6+} concentrations groups had been significantly increased (\(p<0.05\)) after 5d Cr\textsuperscript{6+} exposure, there were no significant difference of the AKP activities in gills of *S. paramamosain* among the groups of 0.5mg/L, 1.0mg/L, 2.0mg/L Cr\textsuperscript{6+} concentration and the control group (\(p>0.05\)) after 9d exposure, while the AKP activities in group of 4.0mg/L, 8.0mg/L Cr\textsuperscript{6+} concentrations were still significantly higher than that of control group (\(p<0.05\)). The AKP activities in hepatopancreas, muscle in the groups of 02.0mg/L, 4.0mg/L, 8.0mg/L Cr\textsuperscript{6+} concentration significantly increased after 1d Cr\textsuperscript{6+} exposure (\(p<0.05\)). In conclusion, the effects of Cr\textsuperscript{6+} stress on the non-specific immunity of *S. paramamosain* was significant.

Keywords: chromium (Cr\textsuperscript{6+}); *Scylla paramamosain*; immune parameter

* E-mail address: Chunxai@xmu.edu.cn
Effect of long-term administration of dietary \( \beta-1,3 \)-glucan on growth, physiological and immune responses in *Litopenaeus vannamei* (Boone, 1931)

Hong-Xia Zhao\(^1\)\(^2\), Jun-Ming Cao\(^1\)*, An-Li Wang\(^2\), Chao Xia Ye\(^2\), Han-Bing Lan\(^1\), Xiao-Hua Liu\(^1\), Guo-Li Li\(^1\), Shui-Hua Liu\(^1\)

\(^1\) Institute of Animal Science, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, P R China
\(^2\) Key Laboratory of Ecology and Environmental Science in Guangdong Higher Education, College of Life Science, South China Normal University, Guangzhou 510631, P. R. China

Abstract

\( \beta-1,3 \)-glucan at different dietary doses was administrated to Pacific white shrimp, *Litopenaeus vannamei*, to enhance its growth, immunity, and survival rate against nitrite stress. Four different diets supplemented with 0, 250, 500, or 1000 mg of \( \beta-1,3 \)-glucan kg\(^{-1} \) were fed to *L. vannamei*. Growth performance (weight gain and survival rate), physiological conditions (blood total protein, glucose, lactate, triacylglycerols, and cholesterol levels) and immunological responses (superoxide dismutase, catalase, lysozyme, acid phosphatase, and alkaline phosphatase activities) were recorded after 84 d feeding period and a 120 h exposure to nitrite-N. After the prescribed feeding period, the 250 mg kg\(^{-1} \) \( \beta-1,3 \)-glucan diet resulted in better weight gain (\( P < 0.05 \)). Before imposing the nitrite stress, the blood lactate, triacylglycerols, and cholesterol levels in shrimp fed with 250 mg kg\(^{-1} \) \( \beta-1,3 \)-glucan diet were significantly higher than those observed in shrimp fed with other diets (\( P < 0.05 \)). Higher activities of catalase, lysozyme, and alkaline phosphatase were observed in shrimp fed with 500 or 1000 mg kg\(^{-1} \) \( \beta-1,3 \)-glucan diet as compared to those obtained in shrimp fed with other diets (\( P < 0.05 \)). After 120 h of nitrite stress, the blood protein, lactate, superoxide dismutase, catalase, and alkaline phosphatase activities in shrimp fed with 500 or 1000 mg kg\(^{-1} \) \( \beta-1,3 \)-glucan were significantly higher than those observed in shrimp fed with other diets (\( P < 0.05 \)). Glucose and triacylglycerols levels of shrimp fed with 500 or 1000 mg kg\(^{-1} \) \( \beta-1,3 \)-glucan were significantly lower than those observed in other diets (\( P < 0.05 \)). In shrimp fed with 500 and 1000 mg kg\(^{-1} \) \( \beta-1,3 \)-glucan, 120 h after the nitrite stress, the mortality rate was significantly lower than that observed in shrimp under controlled conditions. Thus, results show that administration of 250 mg kg\(^{-1} \) \( \beta-1,3 \)-glucan diet was recommended for the growth demands of *L. vannamei*, and long-term administration of 500 mg kg\(^{-1} \) \( \beta-1,3 \)-glucan contributes to the improvement of shrimp’s immune ability and resistance against nitrite stress. Evidences demonstrate that shrimp fed with a high dose of \( \beta-1,3 \)-glucan can contribute more energy derived from glycolytic, anaerobic metabolism, and lipid oxidation to meet immunity demand under stress.

Keywords: \( \beta-1,3 \)-glucans; *Litopenaeus vannamei*; Growth; Physiological response; Immune response; nitrite stress

E-mail address: zhaohongxia8866@163.com (Hong-Xia ZHAO)
P-117

Effects of two types of vitamin B\textsubscript{1} on growth Performance, Body Composition and Biochemical Indices of \textit{Litopenaeus vannamei}

Xiao-ying CHEN\textsuperscript{1,2} , Jun-ming CAO\textsuperscript{*}, Yan-hua HUANG\textsuperscript{1}, Guo-li LI\textsuperscript{1}, Bing Chen\textsuperscript{1}, Hong-xia ZHAO\textsuperscript{1}, Han-bing LAN\textsuperscript{1}

1 Institute of Animal Science, Guangdong Academy of Agricultural Sciences, Guangzhou, 510640, China; 2 College of Animal Science, South China Agricultural University, Guangzhou, 510642, China

Abstract

A feeding trial was conducted to investigate the effects of two types (encapsulated and ordinary) of vitamin B\textsubscript{1} on growth performance, body composition and biochemical indices of \textit{Litopenaeus vannamei}. The shrimps, with initial body weight of 1.0 g, were fed a basal diet containing 1.54 mg/kg vitamin B\textsubscript{1} (as control) , 4 diets supplemented with 17.2, 37.4, 53, 71.2 mg/kg diet of encapsulated vitamin B\textsubscript{1} and 3 diets with 33.2, 50.6, 73.6 mg/kg diet of ordinary vitamin B\textsubscript{1}, respectively. After 6 weeks feeding, there was no significant difference (P>0.05) in weight gain rate, specific growth rate and feed conversion ratio between the group of adding encapsulated vitamin B\textsubscript{1} and the group of adding ordinary vitamin B\textsubscript{1} when added the similar level. The shrimps fed encapsulated vitamin B\textsubscript{1} showed somewhat higher weight gain rate in comparison with those fed ordinary vitamin B\textsubscript{1}. The pyruvic acid content in hepatopancreas and muscle reached the highest level in the group of shrimps fed 17.2 mg/kg encapsulated vitamin B\textsubscript{1} being significantly higher than that of the control and the group of 50.6 mg/kg ordinary vitamin B\textsubscript{1}. With increasing of dietary vitamin B\textsubscript{1}, serum pancreatic protein content increased. The lowest level was found in the control group, showing significantly different with the other groups of vitamin B\textsubscript{1}. The results described above showed that, in the experimental conditions, the effects of encapsulated vitamin B\textsubscript{1} on growth performance and biochemical indices in \textit{Litopenaeus vannamei} were better than those of ordinary vitamin B\textsubscript{1}.

Key words: \textit{Litopenaeus vannamei}; vitamin B\textsubscript{1}; growth performance; body composition; biochemical indices

* E-mail address: junnmcao@163.com (J.M Cao)
Influence of different dietary 18:3n-3/18:2n-6 ratio on growth performance, body composition and fatty acid composition in Pacific white shrimp, *Litopenaeus Vannamei*

Sui-hua Liu 1, 2, Jun-ming Cao 1*, Yan-hua Huang 1, Hong-xia Zhao 1, Guo-li Li 1, Han-bing Lan 1, Li Liu 2

1 Institute of Animal Science, Guangdong Academy of Agriculture Sciences, Guangzhou 510640, China;
2 Academy of Animal Science, South China Agriculture University, Guangzhou 510642, China;

Abstract

This study aimed to evaluate the effect of different 18:3n-3/18:2n-6 ratio on growth performance, body composition and fatty acid composition on juvenile shrimp, *Litopenaeus Vannamei*. Four diets in which content of crude fat and n-3HUFA was similar, were formulated using 3 different lipid sources (fish oil, linseed oil and soybean oil) in variable proportions in order to prepare diets with different 18:3n-3/18:2n-6 ratios: 0.17, 0.30, 0.56 and 0.68 for SLO1, SLO2, SLO3 and SLO4, respectively. The trial lasted 8 weeks. Results demonstrate that an increase proportion of 18:3n-3 and 18:2n-6 in the diet does not affect growth performance and carcass lipid content of juvenile shrimp. In hepatopancreas, the percentages of LOA were higher in SLO1 and SLO2 fed shrimp than in SLO3 and SLO4 (P<0.05). Compared to the respective hepatopancreas composition, the percentages of LOA in muscle were lower. The content of LOA in muscle was higher in SLO1 fed shrimp than others (P<0.05). the percentages of LNA in hepatopancreas and muscle were higher in SLO3 and SLO4 fed shrimp and lower in SLO1 and SLO2 (P<0.05). In hepatopancreas, the percentage of EPA in SLO4 was markedly higher than in SLO2 fed prawn and the content of DHA in SLO4 was significantly higher than others (P<0.05). In muscle tissue, the percentages of EPA and DHA had no difference among treatments (P>0.05), however, these FA contents was highest in SLO2. Compared to the respective diet, ratios of ∑n-3/∑n-6 in hepatopancreas and muscle was significantly lower in SLO1 than in others treatments (P<0.05). Analyses of tissue fatty acid profiles of juvenile shrimp indicate shrimp has the capacity for bioconversion of 18:2n-6 and 18:3n-3 to HUFA by desaturation and elongation. It shows that the 18:3n-3/18:2n-6 ratio of the SLO2 diet may be more adapted to this bioconversion. According the examination of the two-dimensional relationship between the growth and the ratio between the two fatty acids, as was y=−1333.2x^2+1175.5x+785.66, R^2=0.7238. While the ratio is 0.44 in the diet, juvenile shrimp gain the biggest growth (1045.7%).

Keywords: 18:3n-3/18:2n-6 ratio; Pacific white shrimp; Growth performance; Body composition; Fatty acid composition

* E-mail address: junmcao@163.com (J.M Cao)
Effects of two types of vitamin B₆ on growth Performance, Body Composition and Serum Biochemical Indices of Litopenaeus vannamei

Wei-liang Jiang¹²*, Jun-ming Cao¹, Yan-hua Huang¹, Jun-ru Hu¹, Hong-xia Zhao¹, Bing Chen¹, Han-bing Lan¹
¹ Institute of Animal Science, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, China
² South China Agricultural University, College of Animal Science, Guangzhou 510640, China

Abstract
An 8 weeks feeding trial was conducted to determine the effects of two types (encapsulated and ordinary) of vitamin B₆ on growth performance, body composition and serum biochemical indices of Litopenaeus vannamei. A semi-purified basal diet was formulated using vitamin-free casein, gelatin and fishmeal as protein resources. The shrimps (IBW 0.36g) were fed the basal diet and 7 test diets supplemented with 30, 60, 90, 120mg/kg diet encapsulated vitamin B₆ (Ⅰ,Ⅱ,Ⅲ,Ⅳ,Ⅴ) and 30, 60, 90mg/kg diet ordinary type vitamin B₆ (Ⅵ,Ⅶ,Ⅷ), respectively. Upon analyzed, the content of vitamin B₆ in 8 diets was 0.22, 24.3, 52.7, 83.7, 108.6, 30.4, 52.7 and 77.6 mg/kg diet. The results showed that survival rate, weight gain rate, protein efficiency ratio, feed conversion rate, HSI and ALT activities, TP, GLU and HDL content in serum and body composition were not significantly different (P>0.05) between the group of adding encapsulated vitamin B₆ and the group of adding ordinary type vitamin B₆ when added the same levels. Weight gain rate reached the peak in group Ⅳ, but increased in group Ⅷ. Survival rate and protein efficiency ratio reached the peak in group Ⅲ, but increased in group Ⅷ. Feed conversion rate and HSI reached the lowest in group Ⅲ, the former decreased in group Ⅷ and the later increased in group Ⅷ. Activities of ALT and content of TP reached the highest level in group Ⅲ, decreased in group Ⅷ. The results indicated that optimal supplementation of encapsulated vitamin B₆ in Litopenaeus vannamei was estimated to be 60mg/kg diet.

Keyword: Litopenaeus vannamei; vitamin B₆; FCR; body composition; Serum Biochemical Indices

* E-mail address: jiangweiliang126@126.com
Apparent digestibility coefficients of selected feed ingredients for white shrimp *Litopenaeus vannamei*, Boone

Yi-Rong Yue*, Yong-Jian Liu, Li-Xia Tian, Hui-Jun Yang, Gui-Ying Liang, Lian Gan, Yu-Jie Gao, Wen-Jia Luo
Nutrition Laboratory, Institute of Aquatic Economic Animals, School of Life Science, Sun Yat-sen University, Guangzhou 510275, P.R. China

Abstract

Apparent digestibility coefficients of dry matter, crude protein, gross energy, phosphorus, and amino acids in steam dried fish meal (SDFM), local fish meal (LFM), Peruvian FAQ fishmeal (PFFM), shrimp meal (SM), soybean meal (SBM), defulled soybean meal (DSBM), peanut meal (PM), cottonseed meal (CSM), canola meal (CM), corn gluten meal (CGM) and wheat flour (WF) were determined for white shrimp (0.28 ± 0.0 g, mean ± SEM). A reference diet and test diets (consisting of 70% reference diet and 30% of the feedstuff) were used with 0.01% Y2O3 as an external digestibility marker. The juvenile white shrimp were stocked in 300-l fiberglass tanks at a density of 30 shrimp per tank. The shrimp were fed to apparent satiation three times a day and the feeding experiment lasted for 8 weeks. ADMD coefficients of test ingredients ranged from 49.0% to 84.6% and appeared to be related to the fibre, ash and starch content of the ingredient. For crude protein, ADCs exceeding 90% were observed for SDFM, and protein digestibility coefficient of CGM was the lowest among all the treatments. Soybean meal, peanut meal and dehulled soybean meal were utilized significantly better than the other plant ingredients tested in this study with average protein ADC contents of 81.3%, 82.2% and 78.6% respectively. These values were close to, or equal to, those of Peruvian FAQ fishmeal, local fish meal and shrimp meal 78.2%, 83.6% and 81.0%. The apparent phosphorus digestibility ranges of animal and plant feedstuffs were 29.2-73.8% and 13.0-24.8%, respectively. The lowest phosphorus digestibility was observed in CSM for plant sources and in PFFM for animal sources. The trend of ADCs for energy was similar with those of dry matter and protein, the highest and lowest energy digestibility coefficients were found in SDFM and CGM, respectively. In general, amino acid availability reflected protein digestibility; however, for some ingredients, there were some differences in availability of different amino acids. These results provide more precise information concerning nutrition and energy utilization of white shrimp and will allow ingredient substitutions in practical feed based on levels of available nutrients.

Keywords: White shrimp; Digestibility; Feed ingredients; *Litopenaeus vannamei*

*E-mail address: yrongyue@163.com (Yi-Rong Yue).
Expression of fusion protein of fortin, TAT and GFP in *Pichia pastoris* and effects on immune activities of haemocyte of white shrimp *Litopenaeus vannamei*

Yi Zhou, Wenbing Zhang, Kangsen Mai, Xiaojie Wang, Hongming MA, Wei Xu
The Key Laboratory of Mariculture (Ministry Education of China), Ocean University of China, Qingdao 266003, P.R. China

Abstract

Crustacean fortin or the product of the translationally controlled tumor protein (TCTP) gene is well conserved and has a Ca$^{2+}$ binding domain. Fortin has anti-apoptotic properties, and can protect shrimp from white spot syndrome virus infection. The protein transduction domain (PTD) of TAT (Trans-activator transcription) protein, which was expressed by AIDS virus, is able to deliver protein across bio-membrane. The fortin, TAT and GFP (green fluorescence protein) were combined as a fusion gene with overlap PCR approach and the fusing gene was sub-cloned into pGAPZαA vector. Then, five recombinant yeast expression vectors pGAPZαA-GFP (G), pGAPZαA-Fortin-GFP (FG), pGAPZαA-GFP-Fortin (GF), pGAPZαA-TAT-Fortin-GFP (TFG) and pGAPZαA-GFP-Fortin-TAT (GFT) were constructed. Sequencing analysis verified that the target genes were correctly inserted in right reading frame. The construct was linearized and was integrated into the yeast chromosome by electroporation under the selection of Zeocin. Subsequently, PCR was performed to identify the genomic DNA of these transformants. Several recombinant strains that could express the fusion gene were found. The expression of fusion protein was detected by SDS-PAGE. These results showed that fusion genes was successfully expressed in *Pichia pastoris*. The construction and expression of Fortin yeast expression vector laid a theoretical foundation for further research of the protein function and development of new shrimp feed.

Hematopoietic (Hpt) cell cultures of the white shrimp (*Litopenaeus vannamei*) were incubated with different concentrations of the lyophilized culture supernatants of above recombinant yeasts. Then, the respiratory burst (release of superoxide anion), phenoloxidase (PO) activity, and nitric oxide synthase (NOS) activity were measured. Significant increase in PO activity was observed in all treatments than that in control ($P<0.05$). Hpt cell incubated with 500µg ml$^{-1}$ FG and all concentrations of GFT showed significant increase in NOS activity ($P<0.05$). 100, 500µg ml$^{-1}$ FG and 500µg ml$^{-1}$ GF showed significant increase in respiratory burst activity ($P<0.05$), however, significant lower respiratory burst activity was noticed in 100, 500µg ml$^{-1}$ GFT treatment group. It is therefore concluded that those recombinant proteins could affect the immune activity of shrimp haemocyte.

Keywords: Fortin, TAT, Yeast, Recombinant Expression, *Litopenaeus vannamei*, Haemocyte, Immunity

*E-mail address: zyjc1314@yahoo.com.cn*
Influence of salinity on growth and survival of *Litopenaeus vannamei* and *L. setiferus* reared at 24°C

Xuezhi Zhu, D. Allen Davis, Martin Perez-Velazquez, YongJian Liu, LiXia Tian, KangSen Mai, ShiXuan Zheng, Yan Li

Abstract

Temperature and salinity of the culture water are two environmental factors that can have a major impact on survival and growth of shrimp. An understanding of the response of shrimp to various salinities at sub-optimal temperatures is critical for the prediction of growth and survival. Two growth trials were conducted to evaluate the effect various salinities (2 ppt, 8 ppt, 16 ppt and 32 ppt) on growth and survival of juvenile *Litopenaeus vannamei* and *L. setiferus* at 24°C. Four replicate of 15 shrimp per tank (150 L) were cultured in recirculating systems using a commercial feed. After eight weeks of culture, the survival and final weights of juvenile *L. vannamei* ranged from 98.3% to 100% and 1.78g to 2.1g and were not significantly influenced by salinity. After four weeks, the survival and final weights of juvenile *L. setiferus* ranged from 61.7% to 75% and 0.32g to 0.44g and were not significantly different under the conditions of this study. The results demonstrate that the two species of shrimp can tolerate a range of salinities, however, the survival of *L. setiferus* was lower than that of *Litopenaeus vannamei* at 24°C, suggesting that *L. setiferus* may not be as tolerated to low temperatures.

Keywords: temperature, salinity growth, *Litopenaeus vannamei, L. setiferus*
Effects of combined polychaete extracts on reproductive performance of kuruma shrimp, *Marsupenaeus japonicus*

Binh Thanh Nguyen\(^1\)*, Saichiro Yokoyama\(^2\), Manabu Ishikawa\(^2\), Shunsuke Koshio\(^1,2\)

\(^1\) The United Graduate School of Agricultural Sciences, Kagoshima University. 1-21-24 Korimoto, Kagoshima 890-0065, Japan
\(^2\) Faculty of Fisheries, Kagoshima University. 4-50-20 Shimoarata, Kagoshima 890-0056, Japan

**Abstract**

A two-week feeding trial was conducted to examine effects of combined polychaete extracts on reproductive performance of wild caught broodstock kuruma shrimp, *Marsupenaeus japonicus*. Three dietary treatments such as non-feeding (NF), basal diet supplemented with 0.5% polychaete trichloroacetic acid-soluble and neutral lipid extracts (NA) and fresh polychaetes (FP) were applied to unilaterally ablated shrimp with 70 g initial body weight.

Survival rates were similar between NA and FP groups, and the lowest survival rate was observed in NF group. NF treatment showed the decreased trend of ovarian shadow ratios (% of the ovary shadow width over the body width) with time and there was no spawning. On the other hand, NA and FP groups showed no significant difference on ovarian maturation and spawning rates. Multiple spawning was observed in both NA and FP groups although the hatching rates and the number of nauplii per spawn observed in NA group were significantly higher than those of FP group.

In shrimp hepatopancreas and ovaries, NA group showed significantly higher 20:5n-3 and 22:6n-3, and significantly lower 20:4n-6 compared to those of FP group. However, ovarian 20:5n-3 of FP group increased when ovarian maturation progressed.

The present study demonstrated that both polychaete trichloroacetic acid-soluble and neutral lipid extracts supplemented formulated diets promoted ovarian maturation and spawning. Furthermore, combination of two extracts may have synergetic effects on reproductive performance such as hatching rate and number of nauplii for matured female kuruma shrimp.

**Keyword**: *Marsupenaeus japonicus*, kuruma shrimp, maturation, polychaetes

\(^*\) Email address: binh2912@yahoo.com (B.T. Nguyen)
Glutamate dehydrogenase and Na+-K+ ATPase mRNA expression and growth of pacific white shrimp, *Litopenaeus vannamei*, fed on different dietary protein

Erchao Li¹², Leticia Arena³, Gabriel Lizama³, Gabriela Gaxiola³, Gerard Cuzon⁴, Carlos Rosas³, Liqiao Chen¹, Alain Van Wormhoudt²

¹ School of Life Science, East China Normal University, 200062 Shanghai, China
² UMR5178, Station de Biologie Marine du Muséum National d’Histoire Naturelle, BP225, 29900 Concarneau, France
³ Unidad Multidisciplinaria de Docencia e Investigación, Facultad de Ciencias, Universidad Nacional Autónoma de México, Puerto de abrigo s/n, Sisal C.P.97350, Hunucmá, Yucatán, Mexico
⁴ IFREMER, Tahiti, French Polynesia

Abstract

Experiments were conducted to investigate the effects of dietary protein levels on the growth performance, glutamate dehydrogenase (GDH) mRNA expression and activity, and Na⁺-K⁺ ATPase mRNA expression of the white shrimp, *Litopenaeus vannamei*. The response of growth to ambient salinity change was also evaluated. Three experimental diets were formulated containing 25%, 40% and 50% dietary protein respectively, and were fed to the shrimp at 25 ppt salinity for 20 days. Results showed that no significant difference was observed in weight gain after acclimation for 20 days to the three experimental diets. But increasing tendencies in GDH mRNA expression and activity and Na⁺-K⁺ ATPase mRNA expression were found when the dietary protein level increased. Then shrimp fed on diets with 25% and 50% dietary protein were transferred into both 38 ppt and 5ppt salinities, respectively, and were fed to the shrimp at 25 ppt salinity for 20 days. Results showed that no significant difference was observed in weight gain after acclimation for 20 days to the three experimental diets. But increasing tendencies in GDH mRNA expression and activity and Na⁺-K⁺ ATPase mRNA expression were found when the dietary protein level increased. Then shrimp fed on diets with 25% and 50% dietary protein were transferred into both 38 ppt and 5ppt salinities, respectively, while shrimp fed 40% protein at 25 ppt were kept constant serving as a control because is considered the optimal condition for the white shrimp. The shrimp were sampled at weeks 1 and 2 to estimate the growth responses to the change of ambient salinity. No matter transferred to 38 or 5 ppt, the shrimp fed 50% dietary protein showed significantly higher growth performance, and no significant differences were found with the control. While for shrimp fed 25% dietary protein at both 38 and 5 ppt had the significantly lower weight gain values after two weeks compared with shrimp fed other two diets. These results could indicate that dietary protein level increase could improve the osmoregulation ability of the white shrimp by providing more energy source by increasing GDH activities and expression. Also, ambient salinity change alerted the hepatosomatic index, which increased after ambient salinity change in the first week, and then recovered to a relatively normal level after two weeks, indicating that the shrimp’s special nutrient and energy demands stressed by ambient salinity change were met by accelerating the nutrient metabolism.

Keywords: *Litopenaeus vannamei*; Gene expression; Glutamate dehydrogenase; Na⁺-K⁺ ATPase; Protein; Salinity

* E-mail address: lqchen@bio.ecnu.edu.cn (Liqiao Chen)
P-125

Effect of dietary protein reduction with synthetic amino acids supplementation on growth performance, digestibility and ammonia nitrogen excretion of Pacific white shrimp, *Litopenaeus vannamei*

Ming-yan Huai1,2*, Yong-jian Liu1, Li-xia Tian1, An-long Xu1, Hui-jun Yang1, Gui-ying Liang1

1School of Life Sciences, Sun Yat-sen University, Guangzhou 510275, P.R. China
2Alltech biological products (China) Co.Ltd, Beijing 101407, P.R.China

Abstract

This experiment was conducted to evaluate the effect of lowering fish meal inclusion rate and crude protein level by using synthetic amino acids supplements in practical diets on growth performance, body composition, apparent digestibility coefficient (ADC) and ammonia nitrogen excretion of *Litopenaeus vannamei*. Six diets were formulated with diet 1 as the control with 363.3 g kg\(^{-1}\) crude protein, diets 2-6 were formulated to contain 353.2, 341.2, 328.3, 315.5 and 304.1 g kg\(^{-1}\) of crude protein with synthetic amino acids were added to ensure the level of lysine, methionine plus cystine, threonine and arginine similar to that in the diet 1. Shrimp (initial body weight: 0.99±0.02g) were randomly assigned to triplicate tanks per diet with 30 shrimp per tank in salinity water 7.0-8.0 g L\(^{-1}\). After 50-day feeding trial, it was found that reducing dietary crude protein from 363.3 g kg\(^{-1}\) to 328.3 g kg\(^{-1}\) did not influence growth performance, feed utilization and survival (\(P<0.05\)), but shrimp fed with 315.5 g kg\(^{-1}\) and 304.1 g kg\(^{-1}\) had poorer performance and survival than other treatments (\(P<0.05\)). The proximate analysis of whole body and muscle, hepatosomatic index and ADCs were not affected by dietary crude protein level (\(P>0.05\)). The value of total ammonia nitrogen (TAN) discharged by the shrimp 12 h after feeding were significantly reduced with the dietary crude protein decreasing (\(P<0.05\)). Maximum TAN was obtained for shrimp fed control diet, and was significantly higher than other treatments (\(P<0.05\)). This study indicated that dietary crude protein can be decreased from 363.3 g kg\(^{-1}\) to 328.3 g kg\(^{-1}\) by using synthetic amino acids at low adding dosage to keep the main limited essential amino acids consistent, and reducing dietary crude protein can reduce TAN excretions and improve the water quality.

Keywords: *Litopenaeus vannamei*; Crude protein, synthetic amino acids, fish meal

* E-mail address: huaaimy@126.com (Ming-Yan Huai).
P-126

The effects of dietary Chinese herbs on growth, digestive enzyme and immunity of *Litopennum vannamei*

Li Zhuo-Jia\(^1\), Lu Xin\(^{1,2}\), Lin Hei-Zhao\(^1\), Yuan Feng-Hua\(^1\)

\(^1\) South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Guangzhou 510300, China; 
\(^2\) School of Life Science and Technology, Dalian Fisheries University, Dalian 116023, China;

Abstract

Chinese herbs were supplemented to a basal diet at five levels of 0.0%, 0.1%, 0.2%, 0.4% and 0.8%, respectively. The five diets were fed to *Litopennum vannamei* (initial body weight 4.14±0.06g) for 8 weeks. The results showed that the survival rate of tested groups (diets contained Chinese herbs at levels of 0.1%, 0.2%, 0.4% and 0.8%) was higher than that of control group (diet contained Chinese herbs at level of 0.0%). The survival rate of shrimp in 0.2%-Chinese herbs diet group was significantly higher than that of shrimp in the control group \((P<0.05)\). Weight gain (WG) of shrimp in 0.2%- and 0.8%-Chinese herbs diets groups was significantly higher than that of shrimp in control group \((P<0.05)\). The AKP of shrimp in 0.4%-Chinese herbs diet group was significantly higher than that of shrimp in the other diets groups \((P<0.05)\). The SOD of the tested groups was higher than that of shrimp in the control group. The POD was decreased with increasing the supplemental Chinese herbs. The digestive enzyme activities in the liver and intestine of shrimp in the tested groups were higher than that of shrimp in the control group. The liver protease and liver amylase of shrimp in the 0.2%-Chinese herbs diet group were significantly higher than that of shrimp in control group. The intestinal amylase and the intestinal protease of shrimp in 0.1%- and 0.4%-Chinese herbs were significantly higher than that of shrimp in the control group. In conclusion, the appropriate dietary herbs could enhance the growth, digestive enzyme activities and immune function of *Litopennum vannamei*.

Key words: Chinese herbs; *Litopennum vannamei*; Growth; Digestive; Immunity

\*E-mail address: zhuojiali609@163.com
P-127

Effects of feeding timing of dietary Chinese herbs on digestive and immune enzymes of *Litopenaeus vannamei*

Lin Hei-Zhao\(^1\)*, Lu Xin\(^1,2\), Li Zhuo-Jia\(^1\), Yuan Feng-Hua\(^1\)

\(^1\)South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Guangzhou 510300, China;
\(^2\)School of Life Science and Technology, Dalian Fisheries University, Dalian 116023, China;
\(^3\)Guangdong Ocean University, Zhanjiang 524000, China)

Abstract

A 3-week experiment was conducted to assess the effect of dietary Chinese herbs on digestive and immune enzyme of *Litopenaeus vannamei* at 0, 7, 14 and 21-day, respectively. Chinese herbs were supplemented to a basal diet at four levels of 0.0%, 0.1%, 0.2% and 0.4%, respectively. The diets were fed to *Litopenaeus vannamei* for 21 days. The results showed that the digestive enzymes of shrimp in the tested groups (diets contained Chinese herbs at levels of 0.1%, 0.2%, 0.4%) were higher than that of shrimp in the control group (diet contained Chinese herbs at level of 0.0%). The liver protease increased at the first week, then declined with time lasting; however, the intestinal protease declined during the 3-week feeding trial. The liver amylase was decreased and intestinal amylase was increased gradually with time lasting. The digestive enzymes of *Litopenaeus vannamei* were fluctuated with time lasting. AKP and SOD increased first, and then declined as time lasting. In conclusion, periodic feeding the dietary Chinese herbs could enhanced the digestive enzymes and the immunity of *Litopenaeus vannamei*.

Key words: Chinese herbs; *Litopenaeus vannamei*; Digestive; Immunity

*E-mail address: linheizhao@163.com*
Effect of seven different carbohydrates on the growth, survival, nutrients composition and enzymes activities of liver 6-phosphogluconate Dehydrogenase and glucokinase

Niu Jin1*, Lin Heizhao1, Liu Yongjian2, Tian Lixia2, Chen Xu1, Huang Zhong1
1 South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Guangzhou, 510300, P.R. China
2 Nutrition Laboratory, Institute of Aquatic Economic Animals, School of Life Science, Sun Yat-sen University, Guangzhou

Abstract

Penaeus monodon (average weight 1.9 g) were fed with seven diets for 74 days to observe the effect of seven carbohydrates (wheat starch, sucrose, potato starch, cornstarch, dextrin, glucose and maltose) on the growth, survival, nutrients composition and RNA expression of liver 6-phosphogluconate Dehydrogenase (6PGDH) and glucokinase (GK). Seven formulated diets all contained 20% carbohydrate content with seven carbohydrate sources. All shrimp in glucose and maltose diets groups were dead completely after two weeks. After the 74 days feeding trial, weight gain (WG, %) and specific growth rate (SGR, % day⁻¹) in wheat starch diet groups were significant higher than that in cornstarch and dextrin diet groups (P<0.05), sucrose and potato starch diets groups exhibited the intermediate results of WG and SGR. The survival of shrimp in wheat starch, sucrose, potato starch and cornstarch diets groups were significantly higher than that in dextrin diet groups (P<0.05), but no significantly difference was found among wheat starch, sucrose, potato starch and cornstarch diets groups (P>0.05). Different carbohydrate sources also had significant influence on nutrients composition of whole body and muscle. The wheat starch diet groups had the highest whole body protein and muscle protein, dextrin diet groups had the lowest whole body protein and muscle protein, sucrose, potato starch and cornstarch exhibited the intermediate results of whole body protein and muscle protein, but no significant differences were found in whole body lipid and muscle lipid among all five diets groups (P>0.05). As for the apparent digestibility coefficient (ADC) of dietary protein and carbohydrate, the wheat starch, sucrose and potato starch diets groups had the highest ADC of dietary protein, dextrin diet groups had the lowest ADC of dietary protein, cornstarch diet groups exhibited the intermediate result of ADC of dietary protein, the ADC of dietary carbohydrate in wheat starch and sucrose diets groups were significantly higher than that in cornstarch and dextrin diets groups (P<0.05), potato starch diet groups exhibited the intermediate result of ADC of dietary carbohydrate. As for the enzymes activities of liver 6PGDH and GK, the wheat starch diet groups had the highest liver 6PGDH and GK activities, potato starch, cornstarch and dextrin diets groups had the lowest liver 6PGDH and GK activities, and sucrose diet groups exhibited the intermediate activities of liver 6PGDH and GK activities. Corresponding to the liver 6PGDH and GK activities, the RNA expression of liver 6PGDH and GK in the five diets groups were wheat starch>sucrose>potato starch and cornstarch>dextrin.

Keywords: Penaeus monodon; Carbohydrate sources; Growth; Survival; Nutrient composition; 6-phosphogluconate Dehydrogenase; Glucokinase

* E-mail address: gzniiuin0203@163.com
P-129

Effects of stocking density on growth and optimal dietary protein requirement of Pacific white shrimp, *Litopenaeus vannamei*

Li-Ping Shang*, Ni-Na Gou, Li-Rong Bai, Yu-Tao Miao, An-Li Wang

Key Laboratory of Ecology and Environment Science in Guangdong Higher Education, Guangdong Provincial Key Laboratory for Healthy and Safe Aquaculture, College of Life Science, South China Normal University, Guangzhou 510631, People’s Republic of China

Abstract

It is presumed reared at different stocking-density, shrimp’s optimal requirements for some specific nutrients, such as protein, may be different. However, few investigations have been conducted in this area of study. The present study aims to investigate the interactive effects of stocking density and dietary protein level on growth, survival and body composition of Pacific white shrimp, *Litopenaeus vannamei*. Stocking densities of 15, 30, and 45 shrimp/tank and levels of dietary crude protein of 26, 30, 34, 38 and 42% were tested in a 3 × 5 factorial experiment. A significant effect of stocking density on growth of shrimp was detected, with the growth responses (weight gain, SGR) ranked in the order 15 shrimp per tank (4.52 g, 165.87%) > 30 shrimp per tank (3.86 g, 120.93%) > 45 shrimp per tank (3.63 g, 118.30%). No effects of dietary protein level or stocking density on survival of shrimp were observed under the experimental conditions of this study. There was an inverse relationship between stocking density and PER, PER was decreased as dietary protein level increased. No effect of stocking density, protein, or their interaction was observed on the protein content and final water content of whole shrimp. Additionally, the activity of SOD, POD, AKP, ACP were significantly higher than that of other treatments in animals fed the protein content 34%. Meanwhile, the activity of POD did not vary significantly among treatments.

Keyword: Stocking density; Pacific white shrimp; Dietary protein level; Growth

*E-mail address: 183940968@qq.com (L. P. Shang)
1Corresponding author: wanganl@scnu.edu.cn (A. L. Wang)
P-130

The dietary effect of xylooligosaccharides and immune response of *Litopenaeus vannamei* on low temperature stress

Yang Yang*, Yu-Tao Miao, Jun-Wa Huang, Xiao-Chun Wu, Dan Jiang, An-Li Wang

Key Laboratory of Ecology and Environment Science in Guangdong Higher Education, Guangdong Provincial Key Laboratory for Healthy and Safe Aquaculture, College of Life Science, South China Normal University, Guangzhou 510631, People’s Republic of China

Abstract

In recent years, the acutely change of temperature has caused greatly loss in farming of shrimp, so a lot of work has been carried to solve this problem. This study was designed to decrease this loss in nutritional immunology method. The weight of the Xyl(Xylooligosaccharides) in diet is 0,0.1,0.15,0.2,0.25‰. After 10 weeks feeding, all the shrimp were divided into different groups facing the low Temp(14±1) challenge, the samples including haemocyte, hepatopancreas, intestine were collected at 0,1,6,12,24 hours after challenge. The shrimp fed the Xyl were higher than the one fed basic diet in weight gain. THC, AKP decreased from 0h to 1h, and then increased slowly. Otherwise, superoxide anion and MDA are just reverse. CAT and SOD increased significantly after low temp stress. It is the first time to discuss the possibility of using oligosaccharides to improve resist of low temp in farming shrimp, which may raise health management of shrimp.

Keywords: *Litopenaeus vannamei*, Low Temperature, Xylooligosaccharides, Immune Response

*E-mail address: yangyang2002226@126.com (Y. Yang)

1Corresponding author: wanganl@scnu.edu.cn (A. L. Wang)
Flow cytometry as a tool to study the effects of dietary zinc on Litopenaeus vannamei under nitrite stress

Chaoxia Ye, Anli Wang, Jian’an Xian, Weina Wang, Yutao Miao, Shaoan Liao
Key Laboratory of Ecology and Environment Science in Guangdong Higher Education, College of Life Science, South China Normal University, Guangzhou 510631, People’s Republic of China

Abstract

A study was conducted to investigate the effects of dietary zinc on labile zinc concentration, respiratory burst of haemocytes and percentage of apoptotic haemocytes in white shrimp under nitrite stress. Practical diets supplemented with 0, 20, 40, 60, 80 and 120 mg kg⁻¹ zinc were fed to white shrimp (mean initial weight: 1.33 ± 0.04 g) for 20 weeks. After the feeding trial, the white shrimp were placed in water containing concentration of nitrite-N at 25 mg l⁻¹, and the nitrite stress lasted for 24 hours. The zinc-sensitive fluorescent probe FluoZin-3 was used to quantify the amount of labile zinc in haemocytes of white shrimp under nitrite stress. Estimations were based on the general equation \([\text{Zn}} = K_d \times \left(\frac{F-F_{\text{min}}}{F_{\text{max}}-F}\right)\) with \(K_d(\text{Zn}) = 15 \text{ nM}\). \(F_{\text{max}}\) and \(F_{\text{min}}\) was determined by the addition of zinc in the presence of the ionophore pyrithione, or the membrane-permeant chelator tetrakis-(2-pyridyl-methyl) ethylenediamine, respectively. As a result, the intracellular concentrations of labile zinc were estimated to be 2.5-7.3 nM in haemocytes of white shrimp. And labile zinc was not significantly affected by dietary zinc under nitrite stress. Shrimp fed diets without zinc supplement showed significantly lower level of respiratory burst and higher percentage of apoptotic haemocytes, suggesting zinc plays an important role in cell apoptosis. The flow cytometric method developped during this work allows accurate and rapid measurement of haemocyte functions, and should improve progress in understanding crustacean cellular immunity.

Keywords: Zinc; Shrimp; Nitrite; Respiratory burst; Apoptosis; Flow cytometry

*E-mail address: chaoxia_ye@yahoo.com.cn.
Quality control of raw materials for juvenile *Litopenaus vannamei* diets: in *vitro* prediction of protein digestibility

Fanny Yasumaru¹, Daniel Lemos¹*

¹Marine Aquaculture Laboratory, Department of Biological Oceanography, University of São Paulo, São Paulo, Brazil.

Abstract

Current and future global trends of availability and consumption of feed raw materials indicate the need to increase flexibility in developing nutritional and cost-effective aquafeed formulations taking into account nutrient content and availability of ingredients. The choice for appropriate raw materials for feed manufacture requires detailed screening of their potential nutritional value and variability at the industrial level. *In vitro* digestion of feedstuffs by standardized enzymes extracted from the target species has been recently correlated with *in vivo* apparent protein digestibility (APD) in juvenile *Litopenaeus vannamei* for ingredient from different types and sources (animal, plant, marine-origin; n = 26; R² = 0.86; P = 0.001) as well as for experimental diets (n = 25; R² = 0.96; P = 0.007). It suggests a close relationship between *in vitro* peptide bond breakage by hepatopancreas digestive proteases (degree of protein hydrolysis, DH) and the apparent nitrogen assimilation in shrimp, and this may be a potentially useful tool for rapid nutritional information. The present survey reports *in vitro* predicted protein digestibility (PPD) for 132 samples of raw materials from different origin and types, including rendered animal by-products, marine meals, soybean meal and other plant materials. *In vitro* DH of samples was determined according to standard procedures using enzyme extracted from pond-reared *L. vannamei* shrimp (7-10 g wt) sampled from a commercial farm. Predicted protein digestibility of raw materials was calculated by the model y = (a + bx)/(1 + cx + dx²) that relates APD (y) and DH (x). Protein digestibility varied significantly among samples of a same ingredient. Ranges for blood meal (n = 13), feather meal (n = 16), fish meal (n = 15), poultry by-product meal (n = 12) and soybean meal (n = 40) corresponded to 70.4-82.4%, 74.2-87.1%, 83.2-93.0%, 86.8-91.4% and 85.0-90.3% PPD, respectively. For broken rice, corn gluten meal, gelatin, krill meal, meat and bone meal, meat solubles, porcine meat meal, rice bran, squid meal, and wheat flour (n = 2-4), PPD variation was 87.9-89.0%, 77.7-84.2%, 95.2-96.6%, 76.6-78.3%, 89.3-92.0%, 94.1-94.7%, 88.1-90.1%, 92.7-94.3%, 81.4-83.0% and 93.2-95.5%, respectively. Single represented samples as casein, cottonseed meal, crab meal, DDGS, hydrolyzed poultry protein, rapeseed meal, full-fat soy soybean meal, soybean protein isolate, silkworm pupae meal, fish solubles and wheat gluten registered PPD of 98.3, 93.6, 87.9, 83.8, 93.5, 85.1, 81.8, 93.5, 77.9, 93.4 and 95.4%, respectively. Present values may assist in the comprehension of quality and variability of market available feedstuff for shrimp diets. Variation verified in raw materials commonly included at high dietary levels would entail continued quality assessment for feed manufacture. The combination of amino acid profile and *in vitro* PPD may be useful in assisting feed industry to achieve effective formulations at the current scenario of decreasing levels of fish meal in shrimp diets.

Keywords: Ingredient, digestibility, protein, *in vitro*, shrimp

*E-mail address: dellemos@usp.br (D. Lemos).*
Dietary administration of *Spirulina platensis* increased the immune response of white shrimp *Litopenaeus vannamei*

Chyng-Hwa Liou, Carina Miranda Tayag, Yong-Chin Lin, Jiann-Chu Chen*
Department of Aquaculture, National Taiwan Ocean University, Keelung, Taiwan

Abstract

White shrimp *Litopenaeus vannamei* which had been fed diets containing *Spirulina platensis* at 0, 3%, 6%, and 9% for 4 weeks were challenged with *Vibrio alginolyticus*, and then placed in seawater. Survival rate of shrimp that fed diets containing *S. platensis* were significantly higher than that of control shrimp after 48–96 h. In a separate experiment, the hyaline cell count, granular cell (including semi-granular cell) count, total haemocyte count, phenoloxidase activity, respiratory burst, superoxide dismutase activity, glutathione peroxidase activity, lysozyme activity, phagocytic activity and clearance efficiency were measured when shrimp were fed diets containing *S. platensis*. These parameters directly increased with the concentration, and significantly increased when shrimp were fed diets containing *S. platensis*. It was concluded that *L. vannamei* that fed diets containing *S. platensis* showed enhanced immunity and increased resistance against *V. alginolyticus* infection.

Keywords: *Litopenaeus vannamei; Vibrio alginolyticus; Spirulina platensis*; Health; Resistance

* E-mail address: jcchen@mail.ntou.edu.tw (J.C. Chen).
Effect of the polychaetes extracts on growth, stress tolerance and immune responses of kuruma shrimp (*Marsupenaeus japonicus*) juveniles

**Shogo Harakawa**1*, Shunsuke Koshio2, Manabu Ishikawa2, Saichiro Yokoyama2

1 Graduate School of Fisheries, Kagoshima University, 4-50-20 Shimoarata, Kagoshima 890-0056, Japan
2 Laboratory of Aquatic Animal Nutrition, Faculty of Fisheries, Kagoshima University, 4-50-20 Shimoarata, Kagoshima 890-0056, Japan

**Abstract**

A 60-day feeding trial was conducted to investigate effects of polychaete extracts on the growth performance, stress tolerance and immune response of kuruma shrimp juveniles (0.85±0.01g). Trichloroacetic acid (TCA) soluble (P-TCA), neutral lipids (P-NL) and polar lipids fractions were extracted from powdered polychaete and supplemented to basal diet (BD). BD supplemented with soybean lecithin phospholipids (SBL-PL) was used as a positive control. At the end of the feeding trial, body weight gain (BWG) and feed conversion ratio (FCR) were calculated and shrimp were subjected to a low salinity (0ppm) stress test. Hemolymph was taken to measure total hemocyte counts and phenoloxidase activity. Lipid class compositions of shrimp were also analyzed. BWG was significantly (P<0.05) higher in P-TCA and SBL-PL than that in other dietary groups, and FCR was the highest in shrimp fed P-NL. In low salinity stress test, P-TCA and SBL-PL showed high cumulative mortality index compare with that of other dietary groups. Total hemocyte count and phenoloxidase activity were significantly higher in the groups of supplemented with polychaete extracts and SBL-PL than those in the BD group. In lipid class analysis, cholesterol level was significantly higher in P-NL than that in other treatments, while phosphatidylcholine was significantly higher in P-TCA and SBL-PL than in other treatments. Results showed that polychaete extracts enhanced growth ratio performances, tolerance to stress and improved immune responses of kuruma shrimp.

**Keywords:** Kuruma shrimp; *Marsupenaeus japonicas*; Polychaete; Growth; Immune response

*E-mail address: field_river8419@yahoo.co.jp (S. Harakawa)*
Effect of Tetrodotoxin from Puffer Fish Meal on Growth Performance and Health Condition in Pacific White Shrimp (*Litopenaeus vannamei*)

Wutiporn Phromkunthong¹*, Manthana Jaiyen¹, Amonrat Sermwatanakul², Mali Boonyaratpalin², Paiboon Bunlipatanon² and Patcharee Soonson²

¹Department of Aquatic Science, Faculty of Natural Resources, Prince of Songkla University, Hat Yai, Songkhla, 90112, Thailand
²Department of Fisheries, Ministry of Agriculture and Cooperative Affair, Jatujak, Bangkok, 10900

Abstract

The effects of tetrodotoxin (TTX) from puffer fish meal on growth performance and health condition was conducted in Pacific white shrimps (*Litopenaeus vannamei*). This trial comprised 6 treatments with 4 replications each. Twenty shrimps were released in each aquarium and the water volume was maintained at 180 liters. Completely randomized design was employed in the study in which the TTX levels were adjusted by the inclusion of puffer fish meal in the normal fish meal. The TTX concentrations were 0, 1.31, 1.81, 1.97, 2.18 and 2.64 ppm for treatments 1 to 6, respectively. A control diet was prepared using normal fishmeal without the inclusion of puffer meal. The results obtained from this study suggested that the inclusion of TTX at the level of 2.64 ppm provided the lowest weight gain as well as the worst feed efficiency. Furthermore, shrimp fed diets with TTX supplementation 2.64 ppm caused the reduction in total blood cell counts, protein in haemolymph and phenoloxidase activity. At the termination period, TTX was not detected in the whole body of shrimp. Histological changes were observed as for pathological condition of liver in specimens given 1.81, 1.97, 2.18 and 2.64 ppm tetrodotoxin. Atrophy of hepatic tubules and R-cell resulted as a consequence of degeneration of kidney tubules and necrosis of blood forming cells. However, more severe changes occurred in hepatic tissue with severity correlated with levels of toxins and time of exposure. No histological changes were observed for hemopoietic tissue, gill tissues and body musculature during the study.

Keywords: tetrodotoxin; puffer fish; Pacific white shrimp; *Litopenaeus vannamei*; blood cells; histology

*E-mail address: wutipomp@yahoo.com
Effects of dietary protein requirement in red swamp crayfish (Procambarus clarkii) of different body size

Xucheng Xiao*1, 2, Xiaoming Zhu1, Yunxia Yang1, Dong Han1, Shouqi Xie1
1 State Key Laboratory of Freshwater Ecology and Biotechnology; Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China
2 Graduate School of the Chinese Academy of Sciences, Beijing 100039, China

Abstract

A 56d growth trial with red crayfish fry Procambarus clarkia (initial body weight: 1g) and 62d growth trial with juveniles (initial body weight: 10g) were conducted to investigate the effects of dietary protein levels on the growth performance of red crayfish of different body size. Using fish meal and soybean meal as protein sources, fish oil as lipid source, seven diets were formulated to contain 20, 25, 30, 35, 40, 45 and 50% dietary crude protein (CP). Each diet was randomly assigned to triplicate groups of red crayfish.

The results showed that feeding rate of the fry was lowest at 40% CP group while that of the juveniles was lowest at 25% CP group. Specific growth rate and moulting rate of the fry was highest at 40% CP while that of the juvenile was highest at 35% CP. Feed efficiency of the fry was highest at 40% CP while that of the juvenile was highest at 45% CP. Protein efficiency ratio of the fry was highest at 25% CP while that of the juvenile was highest at 30% CP. Survival was not affect by dietary protein in fry or juvenile. Growth was observed to be linearly correlated with moulting rate. In conclusion, optimal dietary protein requirement for the maximal growth of fry and juvenile Procambarus clarkii was estimated to be around 35% based on the second-order polynomial regression between specific growth rate and dietary protein.

Keywords: Dietary protein; Red swamp crayfish; Body size

* E-mail address: 20030901009xiao@163.com
The effects of dietary supplementation of algae and synthetic astaxanthin on body astaxanthin, survival, growth and low dissolved oxygen stress resistance of tiger prawn, *Penaeus monodon*

Wen-Chung Shiau, Yew-Hu Chien*
Department of Aquaculture, National Taiwan Ocean University, Keelung, Taiwan

Abstract

Natural carotenoids from astaxanthin containing algae *Haematococcus pluvialis* (H) and a non-astaxanthin carotenoid-containing algae *Spirulina pacifica* (S), and a synthetic astaxanthin Carophyll Pink (A) were supplemented in a formulated diets at two concentrations, 50 (I) and 100 (II) mg kg⁻¹, resulting in seven pigmented diets HI, SI, AI, HII, SII, AII, and HS (H-50 mg kg⁻¹ +S-50 mg kg⁻¹). Formulated diet without carotenoid supplementation served as a control (C). These diets were fed to juvenile tiger prawn *Penaeus monodon* for 8 weeks. Dietary carotenoid effects on survival, growth, pigmentation, and resistance to low dissolved oxygen stress were compared by the treatment individually or collectively. The survival and growth of the pigmented groups were not significantly different from those of the control. Pigmentation of the pigmented groups was higher than that of control. Comparisons of pigmentation among the pigmented groups showed that 100 mg kg⁻¹ (II) groups were higher than 50 mg kg⁻¹ groups, synthetic pigment groups (A) were higher than natural pigment groups (H and S), and astaxanthin groups (A and H) were better than β-carotene groups (S). In the low oxygen stress experiment, prawn fed various diets were not different in survival time and oxygen consumption rate, showing that pigment supplement had no effects on survival and oxygen consumption of prawn when subjected to low oxygen stress.

Keywords: Astaxanthin; β-carotene; *Haematococcus pluvialis; Spirulina pacifica; Penaeus monodon*; growth; survival; pigmentation; stress resistance

* E-mail address: yhchien@mail.ntou.edu.tw
Dietary Vitamin B₁ Requirement of Prawn, *Litopenaeus vannamei*

He Zhi-jiao¹²³, Cao Jun-ming¹, Chen Bing¹, Lan Han-bing¹, Huang Yan-hua¹, Chen Xiao-ying¹², Pan Qing¹

¹Institute of Animal Science, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, China
²College of Animal Science, South China Agricultural University, Guangzhou 510642, China
³Ocean and Fishery Bureau of Maoming City, Maoming 525000, China

Abstract

A feeding trial was conducted to determine the requirement of dietary vitamin B₁ (Thiamine, TN) for juvenile *Litopenaeus vannamei*. Semi-Purified basal diets were formulated using vitamin-free casein, gelatin and fish meal as protein resources. Graded levels (0, 20, 40, 60, 80, 120, 200 and 400 mg TN/kg diet) of TN were added to the basal diet which upon analyzed contained 0.83, 19.7, 37.4, 50.8, 65.3, 94.2, 163, 323 mg/kg diet, respectively. Each diet was fed to three replicate tanks (300 L) of shrimp (mean weight of 0.5 g) for 42 days. Results indicated that the weight gain, specific growth rate (SGR) and feed efficiency of shrimp fed diets supplemented with thiamine were significantly higher than those of shrimp fed non-supplemented diet (0.83 mg TN/kg diet). Shrimp fed 19.7-50.8 mg TN/kg diets had a significantly higher survival rate. There were no significant differences in body composition and hepatopancreatic amylase among the groups. The activities of serum transketolase (TKA) were the highest in shrimp fed 50.8 mg/kg diet, significantly higher than those of shrimp fed the non-supplemented diet (0.83 mg TN/kg), but not significantly different with other groups. SGR and TKA in serum were found to be suitable indicators, analyzed by broken-line regression indicated that the dietary vitamin B₁ requirement of *Litopenaeus vannamei* was estimated to be 23.9 and 23.7 mg/kg diet, respectively.

Keywords: *Litopenaeus vannamei*; VitaminB₁; Growth performance; Requirement

*E-mail address: chenbing114@163.com (CHEN Bing)*
Dietary Vitamin B$_6$ Requirement of Prawn, *Litopenaeus vannamei*

He Zhi-jiao$^{1,2,3}$, Cao Jun-ming$^1$, Chen Bing$^1$, Lan Han-bing$^1$, Huang Yan-hua$^1$, Jiang Wei-liang$^{1,2}$, Pan Qing$^1$

$^1$Institute of Animal Science, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, China
$^2$College of Animal Science, South China Agricultural University, Guangzhou 510642, China
$^3$Ocean and Fishery Bureau of Maoming City, Maoming 525000, China

Abstract

A feeding trial was conducted to determine the requirement of dietary vitamin B$_6$ (pyridoxine, PN) for juvenile *Litopenaeus vannamei*. Semi-purified basal diets were formulated using vitamin-free casein, gelatin and fish meal as protein resources. Graded levels (0, 30, 60, 90, 120, 150, 210 and 420 mg PN/kg diet) of PN were added to the basal diet which upon analyzed contained 0.42, 32, 52.9, 76.8, 105, 128, 181 and 333 mg/kg diet, respectively. Each diet was fed to three replicate tanks (300 L) of shrimp (mean weight of 0.5g) for 56 days. The results showed that with increasing supplement of PN in diets, specific growth rate (SGR) of *Litopenaeus vannamei* increased at first and decreased later. Shrimp fed 128 mg/kg and unsupplemental diet (0.42 mg PN/kg) got the highest and the lowest SGR respectively, but there was no significant differences over the dietary PN levels. The survival rate was the lowest in the shrimp fed 333 mg PN/kg diet, and significantly lower than the other groups. Shrimp fed 128 mg PN/kg diet had the highest feed efficiency, and significantly higher than those of the unsupplemented diet (0.42 mg PN/kg) and 333 mg PN/kg diet. The body crude protein content in the shrimp fed 76.8-181 mg PN/kg diets was significantly higher than the unsupplemental diet. The AST and ALT activity in serum were not significantly different among the groups. The hepatopancreatic ALT activity was the highest in the shrimp fed 76.8 mg PN/kg diet, significantly higher than the unsupplemental diet (0.42 mg PN/kg). The hepatopancreas vitamin B$_6$ concentration in the shrimp fed 52.9-105 mg PN/kg diet was significantly higher than the unsupplemental diet (0.42 mg PN/kg). The hepatopancreatic ALT activity and vitamin B$_6$ concentration could be taken as the sensitive indicators. The hepatopancreatic ALT activity analyzed by broken-line regression indicated that the dietary vitamin B$_6$ requirement of *Litopenaeus vannamei* was 71.16 mg PN/kg, and the hepatopancreatic vitamin B$_6$ concentration analyzed by multiple regression indicated that the dietary vitamin B$_6$ requirement of *Litopenaeus vannamei* was 90.3 mg PN/kg.

Keywords: Shrimp; *Litopenaeus vannamei*; Vitamin B$_6$; Growth performance; Requirement

*E-mail address: chenbing114@163.com (CHEN Bing)*
P-140

Effects of antibacterial peptides of musca domestica on growth performance and immune-related indicators in *Litopenaeus vannamei*

Chen Bing, Cao Jun-ming, CHEN Ping-jie, Zhao Hong-xia, Lan Han-bing, Zhu Xuan
Institute of Animal Science, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, China

Abstract

An 8-weeks growth trial was conducted to investigate the dietary effects of different levels of antibacterial peptides extractives of musca domestica (0, 1000, 2000, 3000, 4000, 5000 mg/kg respectively) on growth performance and immune-related indicators of *Litopenaeus vannamei* with initial body weight of 0.86±0.01g. Each diet was fed to four replicates of 40 shrimps. The results showed that antibacterial peptides extractives of musca domestica could enhance survival rate, weight gain rate, specific growth rate and feed efficiency of *Litopenaeus vannamei* significantly (*P*<0.05). When antibacterial peptides extractives were 2000~3000 mg/kg, the shrimps had the maximal survival rate, weight gain rate, specific growth rate and feed efficiency, significantly higher than the control. By using the model based on WGR, an optimum dietary antibacterial peptides extractives level of 2900 mg/kg was calculated. The shrimps fed on the diets with 2000~3000 mg/kg antibacterial peptides extractives had significantly higher body crude protein content and body ash contents. The body crude lipid content was not significantly affected by the supplemental levels of antibacterial peptides extractives. The number of haemocytes in shrimp fed on the diets with 2000~5000 mg/kg antibacterial peptides extractives was significantly higher than the control. The highest phagocytic rate was obtained in the shrimps fed on the diet with 3000 mg/kg antibacterial peptides extractives. When antibacterial peptides extractives were 2000~3000 mg/kg, the shrimps had a higher level in activities of PO, POD, AKP, LZM and T-AOC in serum. The activities of SOD in serum were not significantly different among all the groups (*P*>0.05). There were no significant differences among all the groups in the activities of AKP, SOD, T-AOC in hepatopancreas. The highest activities of LZM in hepatopancreas were obtained in shrimp fed on the diet with 3000 mg/kg antibacterial peptides, significantly higher than the others. The results suggested that the dietary supplementation of certain level of antibacterial peptides extractives could improve the growth performance and immune-related indicators in *Litopenaeus vannamei*.

Keywords: Antibacterial peptides; *Litopenaeus vannamei*; Growth performance; Immunity

*E-mail address: chenbing114@163.com (CHEN Bing)*
Effect of dietary supplementation of compound Chinese herbal medicine on phagocytosis and lysozyme activity of *Litopenaeus vannamei*

Chen Cheng-xun*, Bai Dong-qing, Xing Ke-zhi, Guo Yong-jun, Zheng Yan
Tianjin Key Laboratory of Aquaculture and Aquaculture, Fisheries Science Department, Tianjin Agricultural University, Tianjin, 300384 China

Abstract
To investigate dietary compound Chinese herbal medicine (CCHM) on nonspecific immunity of *Litopenaeus vannamei*, four types of CCHM were prepared. Type I CCHM (CCHM-I) was composed of radix Astragalus, radix Atractylodis Macrocephalae, radix Glycyrrhizae and rhizoma Alisma. Type II CCHM (CCHM-II) was composed of rhizoma Picrorhizae, Scrophularia ningpoensls and loosestrife. Type III CCHM (CCHM-III) was composed of Lonicera japonica and Gardenia jasminoides. Type IV CCHM (CCHM-IV) was composed of Rheum Raphonticum, Scutellaria baicalensis and Phellodendron chinense Schneid. Each type of CCHM was supplemented in basal diet at three levels (8‰, 11‰ and 15‰). Control groups were fed basal diet without CCHM. Each diet was fed to nine groups of shrimp (triplicate in each level, initial body length 10.4±1.2 cm) for 40 d. Phagocytic rate (PR) and phagocytic index (PI) in haemolymph and lysozyme activity in visceral mass and muscle was assayed after feeding trial. Results showed that PR and PI were enhanced significantly in CCHM supplemented groups in the following order: 8‰>11‰>15‰>control groups. No significant difference was occurred in 11‰ or 15‰ level among four types of CCHM. PR in shrimp fed CCHM-I was statistically significant than that in the other types. PI was lowest in shrimp fed CCHM-I, intermediate in shrimp fed CCHM-II and CCHM-IV, and highest in shrimp fed CCHM-III. Lysozyme activity in visceral mass and muscle in shrimp fed CCHM-I (11‰), CCHM-II (8‰ and 15‰) and CCHM-III (15‰) was significantly higher than that in control groups. No significant difference in lysozyme activity was observed in shrimp fed CCHM-IV. These results indicated that the four types of CCHM enhanced nonspecific immunity of *Litopenaeus vannamei* in varying degrees.

Keywords: *Litopenaeus vannamei*; Compound Chinese herbal medicine; Phagocytic ability; Lysozyme

*E-mail address: ccxnx@163.com (Chen Cheng-xun).*
Optimal dietary copper, zinc requirement for juvenile Chinese mitten crab, *Eriocheir sinensis*

Shengming Sun, Ming Chen, Liqiao Chen*, Haibo Jiang, Erchao Li
School of Life Science, East China Normal University, Shanghai 200062, China

Abstract

Two consecutive feeding trials were conducted to determine the optimum dietary copper and zinc requirement of juvenile Chinese mitten crab (from megalopae to the juvenile of crab at stage III) reared in indoor flow-through and aerated aquaria. In experiment I, juvenile Chinese mitten crab (initial weight=6.86±0.34mg) were fed one of six experimental diets for 25 days which were formulated to add Cu sulfate (CuSO$_4$·5H$_2$O) at 0mg/kg, 15mg/kg, 30mg/kg, 45mg/kg, 60mg/kg and 75mg/kg respectively. In experiment II, juvenile Chinese mitten crab (initial weight=7.16±0.48mg) were fed one of six experimental diets for 25 days, and the experimental diets were formulated to add Zn sulfate (ZnSO$_4$·7H$_2$O) at 0mg/kg, 25mg/kg, 50mg/kg, 100mg/kg, 200mg/kg and 400mg/kg respectively. Results from the two feeding trials indicated that the weight gain rate (WGR), survival rate (SR) and activity of cytochrome oxidase were significantly affected by dietary copper level (P<0.05). Although no significant differences were observed on the SR of juvenile crab fed diets with increasing zinc level, there were significant difference on the WGR and activity of carboxypeptidase A among those dietary treatments. According to above results, broken-line regression analysis of WG against dietary copper and zinc levels indicated that the optimal dietary copper and zinc requirement for maximum growth of juvenile crab is about 41mg Cu/kg and 84 mg Cu/kg dry diet.

Keywords: *Eriocheir sinensis*; Copper; Zinc; Requirement

*E-mail address:* lqchen@bio.ecnu.edu.cn (Liqiao Chen)
Effects of dietary vitamin B\textsubscript{6} and protein levels on growth, biochemical composition, and amino transferases activities of Juvenile Chinese mitten crab, \textit{Eriocheir sinensis}

Hongbo Jiang, Liqiao Chen*, Erchao Li
School of Life Science, East China Normal University, Shanghai 200062 China

Abstract

A feeding trial was conducted to evaluate the effects of dietary protein and vitamin B\textsubscript{6} levels on growth performance, body biochemical composition, and the Glutamic Pyruvic Transaminase (GPT) and Glutamic Oxalacetic Transaminase (GOT) activities of juvenile Chinese mitten crab, \textit{Eriocheir sinensis}. Six isocaloric experimental diets were formulated with three dietary protein levels (25, 35 and 45 \%) crossing two vitamin B\textsubscript{6} levels (0 and 200mg/kg diet), and were fed to the juvenile crabs (6.0±0.5g) for twelve weeks. The results showed that increase of dietary protein levels significantly increased the weight gain, GOT and GPT activities, while had no significant effects on survival, hepatopancreas protein and vitamin B\textsubscript{6} of the crabs. Supplementation of vitamin B\textsubscript{6} (200mg/kg diet ) in the diets significantly improved the weight gain, survival rate, hepatopancreas vitamin B\textsubscript{6} contents, GOT and GPT activities of the crabs, while no significant effects were found in the hepatopancreas protein of the crabs, indicating that the vitamin B\textsubscript{6} could improve the efficiency of the nutritional quality of dietary protein by increasing the aminotransferases activities directly. However, there were no significant interactions on all the parameters tested in this study, indicating that it is necessary to meet the dietary protein and vitamin B\textsubscript{6} requirement at the same time, and it is not feasible to try to meet and increase one of those two nutrients to get the maximum growth and survival rate of \textit{E.sinensis} with the aim to spare the other nutrient.

Keywords: \textit{Eriocheir sinensis}; Vitamin B\textsubscript{6}; Protein; Interaction; Growth

* E-mail address: lqchen@bio.ecnu.edu.cn (Liqiao Chen).
P-144

Effects of dietary protein levels on biochemical composition of the Chinese mitten crab, Eriocheir sinensis, during maturation

Hongbo Jiang, Liqiao Chen*, Erchao Li
School of Life Science, East China Normal University, Shanghai, 200062 China

Abstract

Experiments were conducted to investigate the effect of dietary protein levels on the ovarian biochemical compositions during the ovarian maturation of the female Chinese mitten crab, Eriocheir sinensis, broodstock. Three diets containing 25, 35, and 45 % dietary protein respectively were formulated, and were fed to the female E. sinensis with initial weight of 40±5g for 120 days. The GSI and HSI of the crabs feeding 45% protein were the highest, but significant difference were not observed when compared with other two groups (P>0.05). Dietary protein levels only could affect crab ovarian protein and vitellin contents significantly at phase IV; ovarian protein content of crabs feeding 45% protein was significantly higher than those feeding 25% protein (P<0.05), while no significant difference was found when compared with those feeding 35% protein (P>0.05). Crab of ovarian vitellin content of 45% protein group was significantly higher that other two groups (P<0.05), while significant difference between 35% and 25% protein group was not observed. Total content amino acids in ovary and the hepatopancreas of the crabs feeding 25% protein was significantly lower that those of other two groups (P<0.05), and the total essential amino acids content was also significantly lower than those feeding 45% protein (P<0.05). GPT and GOT activities of the crabs feeding 25% protein were significantly lower (P<0.005) than other two dietary protein treatments. All these findings could suggest that dietary protein is of vital importance for the ovary maturation and the biochemical composition accumulation which play important role in the process of production, and the sufficient amount for normal ovary maturation must be met.

Keywords: Protein; Eriocheir sinensis; Ovary; Biochemical composition; Maturation

* E-mail address: lqchen@bio.ecnu.edu.cn (Liqiao Chen).
P-145

Dietary Vitamin B₁ requirement of juvenile Chinese mitten crab, *Eriocheir sinensis*

Yue Wang, Liqiao Chen*, Erchao Li
School of Life Science, East China Normal University, Shanghai 200062, China

Abstract

A feeding trial was conducted to determine the optimal dietary vitamin B₁ requirement of juvenile Chinese mitten crab, *Eriocheir sinensis*. The basal diet was formulated using vitamin B₁-free casein as the protein source, and eight graded levels (0, 10, 20, 40, 80, 160 and 320mg vitamin B₁/kg diet) of vitamin B₁ were added to the basal diet. Each diet was fed to three replicate groups of *E.sinensis* (1.32 ± 0.15 g) for 10 weeks. Dietary vitamin B₁ significantly increased the weight gain, protein efficiency ratio, crude protein content and hepatopancreas vitamin B₁ content, and meanwhile decreased feed efficiency and crab whole body water content. When the dietary vitamin B₁ reached to 80mg/kg treatment, weight gain and protein efficiency ratio of the crab reached to a significant higher level than the other group (except 20mg/kg), and feed efficiency and water content of the crab reached to the lowest levels. Crab fed diets with 80 and 160mg/kg vitamin B₁ had significant higher crude protein contents than the crabs fed diets with 0, 10 and 20mg/kg vitamin B₁. With the increase of dietary vitamin B₁, hepatopancreas amylase activity increased significantly, and the maximum amylase activity was observed in crab fed the 80 mg vitamin B₁/kg diet, and it was significantly higher than the crabs fed diets with 0, 10,160 and 320 mg B₁/kg diet. The hepatopancreas lactate content had the opposite variation tendency with amylase activity. The maximum amylase activity was observed in crab fed the 80 mg vitamin B₁/kg diet, and it was significantly higher than the other groups. No significant differences were found in survival rate, crude fat and ash content. Broken-line regression analysis showed that the optimum dietary thiamine requirement for obtaining maximum weight gain, hepatopancreas vitamin B₁ content and amylase activity of *E. sinensis* under these experimental conditions was 55.08-66.71mg vitamin B₁/kg diet.

Keywords: *Eriocheir sinensis*; Vitamin B₁; Growth; Amylase; Requirement

* E-mail address: lqchen@bio.ecnu.edu.cn (Liqiao Chen)
Effects of dimethylhydantoin and dietary carbohydrate levels on growth and immune factors of *Litopenaeus vannamei*

WANG Xing-qiang*, CAO Mei
Jiangsu Key Laboratory of Marine Biotechnology, Huaihai Institute of Technology, Lianyungang 222005, China

Abstract

Three experiments were conducted to determine the effects of dimethylhydantoin and dietary carbohydrate levels on growth and immune factors of *Litopenaeus vannamei* under the low-salinity conditions. The effect of dimethylhydantoin on survival were investigated in experiment 1 and the result indicated that the survival rate of *Litopenaeus vannamei* exhibited decreased trends with increasing dimethylhydantoin concentrations at salinities of 0.2‰, 5‰ and 20‰. The safety concentrations of dimethylhydantoin increased with increasing salinities. The effects of salinity, dimethylhydantoin and dietary carbohydrate levels on growth and immune factors were investigated in experiment 2 and 3, and the result showed that under the low-salinity conditions, living in long-term dimethylhydantoin environment could lead to stress in *L. vannamei*, more immune factors in vivo were consumed, phenoloxidase and superoxide dismutase activity decreased, and the growth rate slowed down. An adequate level of carbohydrate provided in diets could not only improve growth of *L. vannamei* significantly, but also have a protein sparing action.

Keywords: *Litopenaeus vannamei*; Dimethylhydantoin; Carbohydrate; Growth; Immune factor

*E-mail address: xqwangcaomei@yahoo.com.cn*
Effect of partial replacement of dietary fish meal by corn gluten meal on growth, feed utilization and apparent nutrient digestibility of white shrimp *Litopenaeus vannamei*, Boone

Qihui Yang1,2*, Qicun Zhou1, Beiping Tan1, Xiaoqiu Zhou2, Xiaohui Dong1, Shiyuan Chi1

1Laboratory of Aquatic Economic Animal Nutrition and Feed, College of Fisheries, Guangdong Ocean University, Zhanjiang 524025, China
2Institute of Animal Nutrition, Sichuan Agricultural University, Ya’an 625014, China

Abstract

An experiment was conducted to evaluate the use of corn gluten meal (CGM) as a protein source and partial replacement for fish meal (FM) in diets for juvenile *Litopenaeus vannamei* Boone (initial mean body weight: 0.71±0.01g) were cultured in 18 glass fiber tanks (0.5 m³) with 40 shrimp for each. Six kinds of iso-caloric, iso-nitrogenous diets were formulated to contain corn gluten meal 12.00% (the control), 15.14%, 18.29%, 21.44%, 24.59% and 30.0%, replacing 10%, 20%, 30%, 40% and 60% of the protein derived from fish meal were fed to triplicate groups, respectively. All experiment diets were supplemented with crystal amino acid according to the amino acid requirement of *Litopenaeus vannamei* Boone. After 8 weeks feeding trial with juvenile shrimp was conducted and digestibility of the diets was also determined.

The results showed that there were significant differences (P< 0.05) in weight gain (WG), specific growth rate (SGR), feed coefficient ratio (FCR). There was no significant in growth within the 30% replacement level of CGM (P>0.05). WG and SGR decreased rapidly in excess of 30% CGM replacement of FM protein, and FCR was the opposite changes with the different replacement level (P<0.05). The composition of crude protein, phosphorus and lipid of whole body for white shrimp were significantly affected (P<0.05) by dietary treatments, but no significant treatment effects in crude ash for juvenile white shrimp (P>0.05). Digestibility of nutrients and energy of the diets decreased with the increase in levels of dietary corn gluten meal. Protein digestibility values for 24.59% and 30.0% CGM dietary treatments (85.29% and 84.45%, respectively) were significantly lower than that of the control diet (91.73%) and others diets. Digestibility of lipids ranged from 97.01% for the control diet to 88.34% for the 30.0% CGM diet, decreased as the level of CGM increased (P<0.05), while phosphorus apparent digestible was not significantly effect among treatments (P>0.05). Amino acid availability values for all diets, there was a general trend toward reduced growth among the shrimp fed the diets containing levels of CGM replacement of fish meal, including the total amino acid availability. In summary, based on broken-line regression analysis of WG, 32.2% of FM can be replaced by CGM with no significant effect on growth performance, feed utilization of *Litopenaeus vannamei*.

Keywords: *Litopenaeus vannamei* Boone; Fish meal; Corn gluten meal; Growth; Apparent nutrient digestibility

*E-mail address: qihuiyang@yahoo.com.cn*
Effect of conjugated linoleic acid on immune-related genes changes in the Pacific white shrimp *Litopenaeus vannamei*

Weijing Zhong, Shengpeng Zhang, Weipei Huang, Jinfeng Li, Anli Wang

Key Laboratory of Ecology and Environmental Science in Guangdong Higher Education, Guangdong Provincial Key Laboratory for Healthy and Safe Aquaculture, College of Life Science, South China Normal University, Guangzhou 510631, PR China

Abstract

This experiment was conducted to investigate the effect of conjugated linoleic acid (CLA) on immune-related genes expressions in the Pacific white shrimp *Litopenaeus vannamei* (*L. vannamei*). Dietary 0, 0.5%, 1%, and 2% CLA was added to the basic diet of shrimps to replace fish oil. The feeding period was 2 months. After the experiment, it is indicated that dietary 2% CLA significantly enhanced cytosolic manganese superoxide dismutase (cMnSOD) gene expressions of *L. vannamei* in the haemocyte and hepatopancreas, as compared with control group (P<0.05). In addition, glutathione peroxidase (GPx) gene transcript in the hepatopancreas was increased markedly in *L. vannamei* consuming 2% CLA (P<0.05). Shrimps fed 2% CLA significantly elevated high density lipoprotein/β-glucan binding protein (βGBP-HDL) and lipopolysaccharide- and β-1,3-glucan-binding proteins (LGBP) genes expressions in the muscle of *L. vannamei* in comparison with control group (P<0.05). Dietary 0.5% and 1% CLA had no significant effect on immune-related genes expressions, when compared with control group. Together, dietary administration of 2% CLA could improve immune status of *L. vannamei* by enhancing antioxidant enzymes genes expressions (cMnSOD and GPx) and promote lipid transport by increasing βGBP-HDL gene transcripts.

Keywords: Conjugated linoleic acid; *Litopenaeus vannamei*; Immune response; Lipid transport; Antioxidant enzyme

*E-mail address: wanganl@scnu.edu.cn (A. L. Wang).*
Effect of dietary protein level on growth, water quality, immunity and eco-nutrition requirement for *Litopenaeus vannamei* in high density culture system

Yong Li 12, Su D. Xia 12, Wen Q. Wang3, Shi Q. Tang1, Hua Wang12, Guo X. Sun12*

1 Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China
2 Graduate University of the Chinese Academy of Sciences, Beijing 100049, China
3 Animal Science Department, Qingdao Agriculture University, Qingdao 266109, China

Abstract

An experiment was conducted for 60 days to determine the relationship between the different dietary protein content and the growth, water quality, and immunity of *Litopenaeus vannamei* in high density culture system. White shrimps 450 (average initial weight 6.2 ± 0.2g) were assigned randomly to five treatments with three replicates within each treatment and were fed diets with 5 different protein levels(% as diet) 31, 35, 39, 43 and 47(group A-E) respectively in average density 3.1 kg/m³. The results were as follows: (1) Shrimps in group D had the maximal special growth ratio (1.24) and the minimal feed conversion ratio (2.54), there was no significant difference when the protein level was above 39%. Comparing with group A, the SGR in groups C, D, E was enhanced by 24.4%, 27.1%, 24.9% separately, the FCR was decreased by 17.3%, 19%, 14.9% separately. (2) Seawater factors such as NH₄⁺-N, NO₂⁻-N, PO₄³⁻-P and SS were affected significantly by dietary protein levels, and all of them were enhanced with increasing protein levels. From 3 to 15 hours after feeding, setting group A as the benchmark, the NH₄⁺-N excretion were enhanced by 24.7-78.4% for group B, C, D, E; the PO₄³⁻-P were enhanced by 32.7-73.4% for group C, D, E. (3) The total hemocyte counts, T-AOC activity, POD activity, total protein content, white protein content in haemolymph were increased at first and then decreased with the increase of protein content in the diet. Comparing with group A, the above 5 indicators of 43% protein content which was the best group were enhanced from 16.8% to 33.9% (P<0.05). The SOD activity and oxyhemoly content reached a peak in group E and C separately. (4) The innovative dynamic relationship, between nitrogen excretion in the environments and the nitrogen gain in the body as the increasing dietary protein levels, had been built. So that the exact requirement of protein can be determined, which push a further development of animal eco-nutrition from theory to practice. Based on the relationship, 43.73% may be recommended as the conventional requirement of protein for the biggest growth for adult white shrimp in high density culture system; however, 40.42% as the eco-nutrition requirement of protein for the optimum growth and nitrogen excretion.

Keywords: *Litopenaeus vannamei*; protein levels; environment factors; immunity; eco-nutrition requirement; high density;

* E-mail address: lyzhy678@hotmail.com (Y. Li).
P-150

Effects of continuous and alternate administration of β-glucan and mannan-oligosaccharide on the growth, immunity and resistance against Vibrio splendidus of sea cucumber Apostihopus japonicus

Nan Bai*, Wenbing Zhang, Kangsen Mai, Hongming Ma, Qinghui Ai, Xiaojie Wang, Min Gu

Key Laboratory of Mariculture (Ministry of Education), Ocean University of China, Qingdao 266003, PR China

Abstract

A four-week growth trial was conducted to compare continuous administration of β-glucan (0.15g Kg⁻¹ diet), mannan-oligosaccharide (MOS, 0.2g Kg⁻¹ diet) and alternate feeding of β-glucan and MOS on the growth, immunity and resistance against Vibrio splendidus of sea cucumbers Apostihopus japonicus (6.80±0.30g). Three strategies of alternateness were set: sea cucumbers were fed with diet containing β-glucan five days following diet containing MOS two days; with diet containing β-glucan two days following diet containing MOS five days; with diet containing β-glucan seven days following diet containing MOS seven days. The sea cucumbers fed with immunostimulants showed higher special growth rate (SGR) and lower cumulative incidence than those of sea cucumbers fed with diet without immunostimulants (control). In all groups, the highest SGR was observed at the group of feeding sea cucumbers with diet containing β-glucan seven days following diet containing MOS seven days while sea cucumbers fed with diet containing β-glucan two days following diet containing MOS five days alternately showed the lowest cumulative incidence. In all sea cucumbers fed with diets containing immunostimulants, the four analyzed immune parameters (total coelomocytes counts, superoxide anion, superoxide dismutase activity and phagocytic rate) were significantly higher than those of the control group on the 4th day except that total coelomocytes counts (TCC) and superoxide anion of the sea cucumbers fed with diet containing MOS were significantly higher than those of control group on the 15th day and 11th day, respectively. However, with the experiment prolonging, TCC and superoxide anion of the sea cucumbers fed with β-glucan continuously decreased and were no significant differences compared with those of control group on the 18th day. TCC and superoxide anion of the sea cucumbers fed with MOS continuously were not significantly different compared with those of the control group on the 29th day, though they were still higher than those of the control group. The sea cucumbers fed with β-glucan and MOS alternately showed all four immune parameters significantly higher than those of the control group during the experiment and the phagocytic rate was significantly higher than that of the sea cucumbers fed β-glucan or MOS continuously. Overall, alternate feeding β-glucan and MOS could take advantage of both to enhance the growth, immunity and resistance to V. splendidus. In three alternate strategies, feeding diet containing β-glucan two days following diet containing MOS five days alternately was most suitable for sea cucumbers.

Keywords: Apostihopus japonicus, β-glucan, mannan-oligosaccharide, alternateness, Vibrio splendidus

*E-mail address: bainan668@163.com (N. Bai).
Effects of short-term feed deprivation and refeeding on growth performance, body composition and serum parameters of juvenile black sea bream, *Sparus macrocephalus*

Jin Xing Xiao\(^1\)*, Qing Jun Shao\(^1\), Fan Zhou\(^1\), Bergo Owari Ngandzali\(^1\), Yuan Jian Xu\(^2\) and Jun Zhuo Xu\(^2\)

\(^1\)College of Animal Sciences, Zhejiang University, Hangzhou 310029, P.R. China
\(^2\)Marine Fisheries Research Institute of Zhejiang Province, Zhoushan 316100, P.R. China

**Abstract**

An 8-week feeding trial was conducted to investigate the effects of short-term feed deprivation and refeeding on growth performance, body composition and plasma parameters of juvenile black sea bream, *Sparus macrocephalus* (initial weight 9.56 ± 0.12 g, mean ± SD) in fifteen indoors flow-through circular fibreglass tanks (300 L per tank) provided with sand-filtered aerated seawater. Feeding treatments consisted of the following five regimes of 1-week duration: satiate feeding (C), S1, S2, S3, S4 experienced 0, 1, 2, 3 and 4 days of starvation then feeding to apparent satiation for the remainder days, respectively. Each treatment was assigned to triplicate groups of 20 fish in a completely randomized design. The results showed that survival was insignificant difference \((P > 0.05)\) among treatments. Fish subjected to the 1-day starvation had a significantly higher specific growth rate (SGR) and weight gain (GR) than the fish in control group, while all the other treatments did not catch up the control treatment. The results showed that the feed intake decreased with increasing starvation days \((P < 0.05)\). The highest Feed efficiency ratio (FER) and protein efficiency ratio (PER) were observed in S1 group, FER and PER showed a declining tendency with the starvation days increasing. The muscle crude protein and crude lipid content of fish in S1 and C were significantly higher \((P < 0.05)\) than other treatment, while on significant difference was found on moisture and ash content \((P > 0.05)\). The concentrations of total protein, nonesterified fatty acid, triglyceride and cholesterol in serum decreased significantly with increasing starvation days \((P < 0.05)\). The serum growth hormone (GH) concentrations also showed a similarly tendency \((P < 0.05)\). Serum thyroxine (T\(_4\)) and triiodothyronine (T\(_3\)) were equally depressed by feed deprivation \((P > 0.05)\). The concentrations of T\(_4\) and T\(_3\) of S1 and C were significantly higher than other treatments, and had significant differences \((P < 0.05)\). Inconsidering these results, it could be concluded that juvenile black seabream had the ability to over-compensatory growth in the group starvated 1-day in 1-week (S1), while other treatments did not have complete compensatory growth.

**Keywords:** Black sea bream; *Sparus macrocephalus*; Growth performance; Feed utilization; Body composition; Serum parameters; Starvation and refeeding.
Feeding strategies for turbot (*Scophthalmus maximus*) juvenile

Keke Zheng*, Mengqing Liang, Qing Chang, Wei Fang
Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Qingdao, Shandong Province 266071, China

Abstract

The effect of feeding strategies on growth, feed utilization and immune function of turbot juvenile (initial weight=29g) was evaluated in three growth trials. A commercial diet (46% protein, 10% lipid) was fed in each trial. In trial 1, seven treatments of feeding ratio were used: 100% (satiation), 90%, 80%, 70%, 60% and 50% of satiation. Fish were hand fed twice daily for 45d. The results showed that weight gain of fish fed to 100%, 90% and 80% of satiation were not significantly different but significantly \( P<0.05 \) higher than those of fish fed to 70%, 60% and 50% of satiation. Growth and feed efficiency of fish fed to 80% of satiation were not significantly different from those fed to 100% of satiation. The activities of lysozyme, superoxide dismutase and alkaline phosphatase of fish were not significantly affected by feeding ratio. The optimum feeding ratio for turbot juvenile could be lowered to 80% of satiation without growth suppression. In trial 2, three different feeding frequencies (once a day, twice a day, three times a day) were evaluated. Growth, feed utilization and immune response of turbot juvenile were not significantly affected by feeding frequencies in this study \( P>0.05 \). Turbot juvenile can be fed only once a day. In trial 3, daily feeding rhythms of turbot juvenile were investigated. Growth and feed utilization were significantly affected by feeding time. Growth and feed efficiency of fish fed at feeding time 15:00, 18:00 h were significantly higher than those fed at feeding time 0:00, 6:00, 9:00, 12:00 and 21:00 h \( P<0.05 \).

Keywords: feeding strategy; turbot; feeding ratio; feeding frequency; feeding rhythms

*E-mail address: zhengkk@ysfri.ac.cn*
Variation of amino acid levels in fishmeal – a review on amino acid analysis

Andreas Lemme1* and Mark S. Redshaw1
1 Evonik Degussa GmbH, Rodenbacher Chaussee 4, 63457 Hanau, Germany

Abstract
In many diets for aquaculture species fishmeal is used as a valuable source of nutrients although nutritional value can vary. One of the major constituents of fishmeal is protein and amino acids, respectively. We have analysed amino acid content in over 15000 fish meal samples over the last five years, providing a unique insight in to the variation of fish meal quality. Based on 1129 fishmeal samples analysed from 2005 to 2009 by wet chemistry, the variation of amino acid levels and their potential impact on diet formulation and economics is demonstrated. For example total methionine (Met) content ranged from 0.35% to 2.74% and averaged at 1.46%, crude protein (CP) ranged between 30% and 86% with an average of 61%. Respective coefficients of variation were 30.3% for Met and 16.9% for CP. Although it was possible to develop a regression equation allowing for estimation of amino acid levels in fishmeal from protein content, using those calculated numbers includes a relatively high inaccuracy with $r^2$ ranging from less than 0.50 to 0.88 across a range of essential amino acids. When expressed in % of protein methionine content of the 1129 samples varied between 0.86% and 3.24% suggesting that not only absolute contents but also amino acid profile of fishmeal varies widely and this is not considered in the regression approach. Much of the variation might be explained by origin of fishmeal. For example, analyses of blue whiting samples revealed average Met and CP contents of 2.01% and 69.7%, respectively, with variation coefficients of only 2.7% and 2.2%. There are, however, also examples that even within defined materials such as sardine meal the variation coefficient is high (37.5% Met, 19.3% CP). Clearly, processing of fish meal does not lead to standardization resulting in high variation. In order to better meet amino acid specifications in feed formulation and thus reducing safety margins composition of the used fishmeal should be known. While wet chemistry is the reference for amino acid determination, it is a time consuming and expensive method. A solution for this dilemma is the use of rapid NIR technique. Evonik-Degussa developed calibrations with high robustness and precision for amino acid determination of fish meal. AMINONIR® has been recognised by the feed industry to be very valuable as demonstrated by the tripling of the annual number of samples analysed from about 2200 in 2005 to more than 7300 in 2009.

* E-mail address: andreas.lemme@evonik.com
P-154

Effect of mixed feeding schedules versus regular feeding diet on Nile tilapia Oreochromis niloticus fry performance: Reevaluation using constant ingredient composition.

Ashraf Suloma1*, Rania S Mabroke1, Osama M. El–Husseiny1
1Animal Production Department, Faculty of Agriculture, Cairo University, Egypt.

Abstract
The concept of mixed protein where a high-protein diet was alternated with a low-protein schedule, primarily based on the observation that protein utilization varies on a day-to-day basis in a certain rhythmic manner in Nile tilapia (Oreochromius niloticus). By scanning previous studies, it was observed that none of the authors examine the effect of mixed feeding schedules using equal nutrients in all treatment. Four dietary treatments were tested in triplicate, regular feeding with continuously diet (30%CP) served as controls (C). The three mixed feeding schedules were 1A/1B, 2A/2B, and 3A/3B, where A refers to low protein diet (20%) and B refers to high-protein diet (40%), and the numerical value refers to the number of days that a particular diet is offered. The average of the ingredients composition of the two mixed feeding schedules diets were similar to C in order to minimize the confounding effect of variables other than mixed feeding schedules which not considered in previews studies. Control showed the highest growth performance and feed utilization followed by fish fed mixed protein schedule 2A/2B and 3A/3B, respectively. Fish fed on mixed protein schedule (1B: 1 A) presented the lowest feed growth and feed performance. The results showed that the positive effect mixed feeding schedules in previous studies may be due to other factors which need more work to be explained.

Keywords: Mixed feeding schedules; Tilapia; Protein; Reevaluation

E-mail address: suloma2001@yahoo.com.
The effects of feeding rate in juvenile olive flounder, *Paralichthys olivaceus*, fed commercial diet at the low temperature season

Namyong Hwang, Jun-Ho Lee, Seunghyung Lee, Young Chul Kim, Jun-Young Bae, Okorie E. Okorie, Mahmoud Mohseni, Gun Hyun Park and Sungchul C. Bai*

Dept. of Aquaculture / Feeds and Foods Nutritional Research Center (FFNRC)
Pukyong Nat’l University, 599-1, Deayeon-3-dong, Nam-gu, Busan 608-737, Korea

Abstract

Production of olive flounder (*Paralichthys olivaceus*) ranked the top among the Korean mariculture finfish species. Most of Korean aqua-farmers rely on farm-made raw fish-based diets (moist pellets, MP). They believe that moist pellets give better growth than do extruded pellets (EP). However, the use of moist pellets is being discouraged due to the water pollution resulting from such a practice and shortage of resources for making MP. Korean government also recommends the use of extruded diets in aquaculture and encourages the development of environmentally friendly diet with optimum feeding regimes. Of particular interest in the feeding practice is the optimum feeding rate, which has been found to be influenced by several factors among which is the rearing water temperature. Low temperatures in the winter season cause stress; reduce resistance to disease; and decrease feed intake and digestibility. This ongoing study is conducted to investigated the effects of feeding rates in juvenile olive flounder fed commercial diet (extrude pellet) at the low temperature season (below 15°C). Three replicated groups of fish (initial weight 5.3±0.22g) are fed commercial diet at the feeding rates of 0, 0.5, 1, 1.5 and 2% body weight (BW)/day and satiation. The feeding trial is conducted by using a semi-recirculating system with eighteen 30-L aquaria receiving filtered seawater from a center tank. The duration of the trial is four weeks. The effects of feeding rates on growth performance, body composition and histology will be discussed later

Keywords: Feeding rate, olive flounder, low temperature, winter season

*Corresponding author: sebai@pknu.ac.kr
Effects of feeding modes of chitosan on growth and natural killer cells activity of *Ctenopharyngodon idella*

**Hua Xueming**, Han Jiafeng, Wang Jun, Yu Ning, Zhou Hongqi  
Key Laboratory of Exploration and Utilization of Aquatic Genetic Resources, Ministry of Education, Shanghai Ocean University, Shanghai, 201306, China

**Abstract**

Grass carp *Ctenopharyngodon idella* (body weight 19.46±0.04g) were fed for 60 days by three modes (mode A, the grass carp were fed the basal diet; mode B, the grass carp were fed the experimental diet, supplemented with 0.5% chitosan in the basal diet; mode C, the grass carp were first fed the experimental diet and then the basal diet at a interval of 15 days). The growth indexes and activity of natural killer cells (NK cells) in peripheral blood, head-kidney, spleen were measured at 0, 15, 30, 45 and 60 days in order to investigate the relationship between growth, NK activity and feeding modes of chitosan in grass carp. The length gain and weight gain of grass carp had increased according to sampling days at 0, 15, 30, 45 and 60 days. The results showed that both mode B and C could promote the growth of the grass carp but the latter was more effective. The NK activity of mode B and C were significantly higher than those of mode A at 15 and 45 days. Similarly, the NK activity of mode B was significantly higher than those of mode A and mode C at 30 days. The mode B had a higher NK activity than those of mode A and mode C in peripheral blood or the NK activity of mode B and C were significantly higher than those of mode A in head-kidney and spleen at 60 days \(P<0.05\). To consider the same feeding mode, mode B could raise the NK activity in different tissues but to be a time- dependence and the NK activity was no longer raise or even to bring down after 30 days. On the contrary, NK activity had a regular change, first up then down, reached the max. at 45 days and caught up with the level of mode B at the end of 60 days in mode C. Given economical efficiency and practicability, chitosan by incontinuous feeding mode will be suggested to promote growth and increase the activity of NK cells of grass carp.

**Keywords:** Chitosan; Grass carp (*Ctenopharyngodon idella*); Feeding modes; Growth; activity of NK cells

\*E-mail address: xmhua@shou.edu.cn(Hua Xueming)
Effects of protein and satiation degrees on growth and immunity of *Cynoglossus Semilaevis Günther* in industrial culture

Yong Li 1,2*, Mei Q. Wang 1, Ting T. Gao 1, Hua Wang 1,2, Su D. Xia 1,2, Guo X. Sun 1,2

1 Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China
2 Graduate University of the Chinese Academy of Sciences, Beijing 100049, China

Abstract

The 5×3 two-factor random animal experiment was conducted for 108 days to determine the effects of protein levels and satiation degrees on growth performance and immunity activity of juvenile *Cynoglossus Semilaevis Günther* (with average initial weight 110±25g) in the industrial culture condition with closed recirculation seawater. The trial fish were randomly allotted into 15 treatments with triplicates, 5 protein levels diets (43%, 46%, 49%, 52%, 56%, with group A-E), 3 feeding satiation degrees (100%, 90%, 80%, with degree I, II and III). The results indicated below: (1) there was the best weight gain both in the highest protein level and the highest satiation degree. Weight gain ratio in group E was significant higher than the other groups 13.75-50.16%, that in degree I was significant higher than degree II and III 7.57% and 14.08%, respectively; higher protein efficiency ratio and the minimum mortality appeared in the intermediate protein level, mortality ratio of group C decreased than others 50-75%; the highest feed conversion ratio and protein efficiency ratio were present in the lowest satiation degree, degree III was higher than others 4.78% and 5.32%, respectively. (2) the intermediate protein level and the highest satiation degree were stimulative to enhancing SOD activity, group C was significantly higher than other groups 4.2-34.79%, degree I was significantly higher than degree II and III 15.27% and 25.70%, respectively; the intermediate protein level and 90% satiation were good for promoting LZM activity, group C was significantly higher than the other groups 6.1-18.07%, degree II was significantly higher than degree I and III 12.03% and 4.58%, respectively; the intermediate and higher protein level and the highest satiation degree were stimulative to enhancing complement C3, C4 activity; (3) the protein level of feed for maximum growth of juvenile *Cynoglossus Semilaevis Günther* in the industrial culture condition was 56%, and for optimal immunity activity and protein efficiency ratio was 49-52%.

Key words: *Cynoglossus Semilaevis Günther*; Protein levels; Satiation degrees; Growth performance; Immunity activity

*E-mail address: lyzhy678@hotmail.com (Y. Li).*
Effects of feeding diets of different quality within one day on survival, growth and benefit of Grass Carp, *Ctenopharyngodon idella*

SHUAI Ke * GAO Qi-ping  
Fisheries Research Institute of Tongwei Co., Ltd., Chengdu, Sichuan, P.R.C. 610041

Abstract  
This experiment was carried out in 3 ponds covering about three mu each operated by Tongwei Fisheries Research Institute from mid-April to Sept. 30, 2008. Grass carp fingerlings (initial weight of 156.07g) of the same size were weighed and randomly stocked in 3 ponds with density of 660 per mu. During the experimental period, the average water temperature was 27.0℃, ranging from 19.0 to 32.9℃ of three ponds. Minimum at 8:00 AM, and then gradually increased to the maximum at 5:00 PM. Similarly, the average dissolved oxygen (DO) level was 8.8 mg·L⁻¹, the minimum level at 8:00 AM, 4.6 mg·L⁻¹ on average, the maximum at 5:00 PM, 12.9 mg·L⁻¹ on average. There were three test diets for the experiment. Diet A1 was formulated to contain 30% quality crude protein and 4.5% crude lipid with high quality forages. Diet A2 was formulated to contain 30% quality crude protein and 2.5% crude lipid with low quality forages. Diet A3 was formulated with the mean value of Diet A1 and Diet A2. The Diet A1 and A2 were combined to feed fish. The fish in pond 1 was fed with Diet A1 in the morning and A2 in the afternoon, while fish in pond 2 was fed with Diet A2 in the morning and Diet A1 in the afternoon. And fish in pond 3 was fed with Diet A3 within one day as control group. The same feeding rate was kept for fish in every pond (2.5174% on average in the whole process), 4 times daily. The result indicated there is no significant difference among groups in survival rate, daily weight gain and harvested size. The grass carps in pond 2 showed significant minimum FCR of 1.9326, which was 8.36% lower than that of control group at 2.0942; and the feed cost for one-kilogram gain (CPG) was 4.75 yuan, which was 8.42% lower than that of control group at 5.15 yuan. The FCR of Group 1 was intermediate at 2.0007 and CPG was 4.92 yuan. In conclusion, the breeding result by feeding with the combination of Diet A1 and Diet A2 daily is superior to that by feeding Diet A3 within whole day. It can improve feed utilization of grass carp by feeding lower grade diet at both lower temperature and dissolved oxygen level.

Keywords: Grass Carp (*Ctenopharyngodon idella*), Feed Combination, Growth Performance, Benefit

* E-mail address: shuaik@tongwei.com
P-159

The effect of different feeding rate on the grow performances of Jian carp (Cyprinus carpio Var.Jian)

SHUAI Ke  LI Yun-lan  *  GAO Qi-ping
Fisheries Research Institute of Tongwei Co., Ltd., Chengdu, Sichuan, P.R.C. 610041

Abstract

The trial was conducted to determine the optimum feeding rate for adult Jian carp (Cyprinus carpio Var.Jian). 600 Jian carps with initial weight 508g were averagely allocated to 5 treatments with 3 replications per treatment. The fishes were maintained in cement pits(3.4m3) at 25℃, dissolved oxygen levels at least 5.0mg/l. Jian carps were fed 1.1, 1.6, 1.8, 2.2 and 2.4% of theirs body weight per day with a complete feed (CP 35%, EE 5.0%) for 11 weeks. The results showed that the weight gain, specific growth rate (SGR) and feed conversion ratio (FCR) were significantly affected by different feeding rate (P<0.01). The weight gain and SGR increased gradually when the feeding rate increased from 1.1 to 2.2%, but decreased when feeding rate hit 2.4%. Compared with 1.1, 2.2% of feeding rate could increase the weight gain and SGR by 84.36% and 56.02% respectively. The FCR was lowest at 1.6%, but not significantly different with 1.6, 1.8 and 2.2%. Neither 1.1% nor 2.4% could get excellent grow performances. when fish was fed 1.1% their body weight nutrient intake was deficient . The result indicated that exorbitant feeding rate result more waste, too. In order to get the maximum body weight and minimum FCR, we suggest the optimum feeding rate for adult Jian carp (508-918g) is 1.8-2.2% of body weight per day.

Keywords: feeding rate; adult jian carp (Cyprinus carpio Var.Jian); grow performances.

* E-mail address:lily@tongwei.com
Comparison of the activity of gut enzymes of wild and aquacultured groper (*Polyprion oxygeneios*) using fluorogenic methylumbelliferyl substrates

Anna Yu. Kilimnik*, Sally A. Anderson, Vicky L. Webb
National Institute of Water and Atmospheric Research Ltd., 301 Evans Bay Parade, Kilbirnie, Wellington, 6022, New Zealand

Abstract

The groper *Polyprion oxygeneios* has been selected as a high value aquaculture species in New Zealand. There are limited data on the diet of wild, adult groper, and no data on the diet of larval or juvenile groper. To optimize the diet and healthiness of juvenile groper in aquaculture, a comparison was made of the digestive enzyme capacities between wild and aquacultured groper. The activities of 6 digestive enzymes (trypsin, chymotrypsin, ala-aminopeptidase, chitinase, lipase, and b-glucosidase) from groper stomach, pyloric caeca and intestine were measured in 15 (3 groups of 5) wild and 13 (2 groups of 5 and 1 group of 3) aquacultured groper. For all enzymes, except b-glucosidase where no activity was found, activities varied enormously between individuals, and for proteases spanned over 5 orders of magnitude. High enzyme activities were found in extracts from the pyloric caeca and intestine, while most stomach extracts had zero or only traces of enzyme activities. Averaged data for trypsin and chymotrypsin activities were 5 and 50 times higher (respectively), in wild groper than the activities of the same enzymes in aquacultured groper. In contrast, averaged data for ala-aminopeptidase and chitinase activities were 20 and 10 times higher (respectively) in aquacultured than in wild groper. Averaged lipase activities varied less, but were 2 times higher in wild than in aquacultured groper. Further experiments are required to establish factors (seasonal variations, quantity of gut content and gut bacterial community) in wild and aquacultured groper that impact on enzyme variabilities.

Keywords: New Zealand groper; Trypsin; Chymotrypsin; Ala-aminopeptidase; Chitinase; Lipase; B-glucosidase

* E-mail address: a.kilimnik@niwa.co.nz
Combined effect of vitamin C and vitamin E microdiets for gilthead sea bream, *Sparus aurata*

E. Atalah*, C. M. Hernández Cruz¹, E. Gauza¹, T. Benítez-Santana¹, R. Ganga¹, J. Roo¹, H. Fernández-Palacios¹, and M.S. Izquierdo¹

¹Grupo de Investigación en Acuicultura (ICCM&IUSA). P. O. Box 56, 35200, Telde, Las Palmas de Gran Canaria, Spain. phone 34928132900, Fax 34928132908

Abstract

Vitamins C (AA) and vitamin E (α-T) are two of the most important micro-nutrients for fish aquaculture especially larvae aquaculture. An experiment was carried out to study the effect of AA with several levels of α-T. Gilthead sea bream larvae were fed five formulated experimental diets MM, MH, HL, HM, HH (AA/α-T) combining two different dietary levels of α-T (M: medium 1500 mg kg⁻¹, H: high 3000 mg kg⁻¹) with up to three different levels of AA (L: low 0 mg kg⁻¹, M: medium 630 mg kg⁻¹, H: high 1260 mg kg⁻¹) using triplicates.

The survival of fish fed HL diet was significantly lower than those fed HM diet. After two weeks feeding, elevation of AA levels from M to H in presence of M α-T, and the elevation of AA from L to M or to H in presence of H α-T, respectively, significantly improve the final total length, whole body dry weight, SGR and final biomass.

In exposure to stressful water temperature (15 °C), larvae fed with MH diet showed a significantly higher survival than those fed with high level α-T with different levels of AA diets. In conclusion, the elevation of AA from M to H mg/kg in presence of medium α-T (1500 mg/kg) and elevation of AA from L to M or H in presence of high α-T (3000 mg/kg) significantly improved growth performance indicating the importance of the ratio vitamin E/ vitamin C.

Keywords: Gilthead sea bream larvae *Sparus aurata*; n-3 HUFA; Vitamin C; Vitamin E

* E-mail address: eyadatalah@gmail.com (Eyad Atalah).
Feeding behaviour of the burbot (Lota lota L.), a new candidate for freshwater aquaculture

Hendrik Wocher¹, Frieder J. Schwarz²*

¹Aquacultural Station Lindbergmühle, District of Niederbayern, Lindbergmühle 40, D-94227 Lindberg, Germany
²Department of Animal Sciences, Technische Universität München, Hochfeldweg 6, D-85350 Freising-Weihenstephan, Germany

Abstract

The burbot (Lota lota) is the only freshwater fish of the family Gadidae. The preference of this benthic and nocturnal fish for cold water temperatures makes the burbot an interesting candidate for coldwater aquaculture. The meat of this fish is low in fat and its liver is considered a delicacy. As a step towards effective aquacultural production, knowledge about the feeding behaviour is required. In a first experiment (exp1) with juvenile burbot (131.7 g ± 24.7), in which the impact of shelter availability on growth and behaviour was investigated, fish were fed for 12 weeks overnight (10 h) with an interval of 30 minutes. The observation of feeding behaviour with a video-based infrared illuminated system demonstrated that fish attacked feed pellets mainly during the first three night hours, independent of shelter availability treatment. The highest percentage (30.2-44.0 %) of burbot attacking feed was observed at the second feeding in all treatments. During the remaining night time the number of fish eating was quite low. A second experiment (exp2) was conducted to verify the observed feeding behaviour of exp1 by growth data. Juvenile burbot (143.5 g ± 24.7) were reared over a 16 week period with different feeding regimes (feeding interval (FI) and feeding frequency (FF)), whereby daily feed ration was divided into four single meals overnight: treatment 1 (FI: 3 h; FF: nightly, all night), treatment 2 (FI: 0.5 h; FF: nightly, during the first two night hours) and treatment 3 (FI: 0.5 h; FF: every second night during the first two night hours). Growth was affected by FI and FF, whereby significant differences occurred only between treatment 1 and 3 (final weight: 210.2 g ± 84.7 vs. 244.4 g ± 82.8; weight gain: 65.8 g ± 7.7 vs. 103.6 g ± 1.8 respectively) and between treatments 1 and 2 and treatment 3 (weight-specific growth rate: 0.33 % ± 0.03 and 0.41 % ± 0.02 vs. 0.49 % ± 0.02 respectively). FI and FF had no impact on coefficient of variation (CV) and feed conversion ratio (FCR). Final condition factor (CF) was highest in treatment 3 (0.69 ± 0.12) and differed significantly to treatment 1 (0.63 ± 0.11). The amount of uneaten feed pellets was high (61-66 %) but without significant differences between the treatments. Due to the results it can be supposed that the feeding regime of treatment 3 was closest to the feed demand of the burbot and it can be considered for commercial culture and further investigations.

Keywords: Burbot; Feeding behaviour; Feeding interval; Feeding frequency; Growth

*E-mail address: schwarzf@wzw.tum.de (Frieder J. Schwarz)
Centre for Feed Technology, Fôrtek: Research opportunities

Zimonja, O.
Department of Animal Sciences, Norwegian University of Life Sciences

Abstract

Centre for Feed Technology, Fôrtek, was established in 1997 with an overall goal to serve the feed industry by carrying out research, educational and developmental assignments in the fields of animal and fish feed nutrition. The Feed Technology Center's pilot plant is equipped with a variety of feed processing equipment designed to test new ingredients in feed formulas and their physical handling characteristics, evaluate variations of formulas to improve nutritional value and product quality. FôrTek's production lines are available for the production of research feed for fish and other monogastric animals as well as for ruminants. Since its establishment, Fôrtek has gained valuable expertise on feed processing, physical quality of feed and how this affects nutritional value of feed throughout the research conducted at the facility. One of our strengths is the ability to combine feed technology with animal nutrition.

FôrTek is co-located with Department of Animal and Aquacultural Sciences (IHA) as a research/production section within Animal Production Experimental Centre (SHF). The interaction between IHA and SHF enables researchers to monitor the complete feed chain; from feed ingredients processing and its technical characteristics to the affects on nutrition, health, welfare of various farm animals. Thus, the main strategy of Fôrtek is to serve as base research institution on know-how principle in order to combine national and international knowledge in area of feed technology and animal nutrition.

Fôrtek as a part of SHF produces feed in agreement with Norwegian regulation policy for feed production, “Regulation of feedstuffs” (“Forskrift om fôrvarer”) dated 7th November 2002, and “Regulation of feed additives” (“Forskrift om tilsettingsstoffer”) dated 12th April 2005. These regulations are in correspondence with EU regulation policy for feed production.

Keywords: Feed processing, Extrusion, Pelleting

*E-mail address: ozren.zimonja@umb.no
Development of a pH-Stat method to measure in vitro degree of protein hydrolysis using enzymes from pyloric caeca of Atlantic cod (Gadus morhua L.)

Sean M. Tibbetts1, Joyce E. Milley1, Neil W. Ross2, Santosh P. Lall1*

1 National Research Council of Canada, Institute for Marine Biosciences, 1411 Oxford Street, Halifax, Nova Scotia, Canada, B3H 3Z1
2 National Research Council of Canada, Industrial Research Assistance Program, 1411 Oxford Street, Halifax, Nova Scotia, Canada, B3H 3Z1

Abstract
Ingredients used in aquafeeds can vary considerably in protein quality which is a concern for feed manufacturers. Rapid and reproducible methods to screen ingredients are needed. In vitro methods, which do not use large numbers of fish and yield data quickly, offer an attractive complement to traditional in vivo methods and may be more suitable for research and industrial applications. Although results obtained using certain in vitro methods for finfish and shellfish are promising, reproducibility of results has been difficult. Additional modifications are necessary to improve existing methods and to standardize the procedure. The method described here involves extraction and partial purification of an enzyme solution from pyloric caeca of Atlantic cod. Activity of the two major proteolytic enzymes (trypsin and chymotrypsin) significantly (P<0.001) increased by 4 to 12-fold throughout the extraction and partial purification steps indicating that the procedure appears suitable for use in pH-Stat assays. Prior to application of this method to test the digestibility of feed ingredients, a study was conducted to determine the most suitable substrate/enzyme (S/E) ratio to use in subsequent degree of hydrolysis (DH) assays. This trial used a standard purified protein source (vitamin-free casein, IFN 5-01-162) with four S/E ratios (0.25, 0.50, 0.75 and 1.00 mg N/mL) and a true blank for each. Direct observation of the curve data combined with best-fit curve modelling with minimum R2 of 0.99 resulted in maximum DH values in a tight range (25.3-27.5%) with no significant differences between S/E ratios tested. The assay duration time and volume of NaOH titrant required to achieve maximum DH was, however, highly influenced by S/E ratio ranging from <5 to <9 hours and <6 mL to >21 mL. Over the 10-hour assay, the casein DH curve of the 0.50 mg N/mL ratio displayed a rapid increase and reached a clear plateau at maximum DH of 27%. This maximum period was also achieved within a moderate assay duration (6-8 hours) and minimal use of titrant (<12 mL). Based on these results, an S/E ratio of 0.50 mg N/mL and minimum assay duration of 8 hours is recommended for subsequent studies. Non-linear regression analysis (DH = [-5.6 × S/E ratio]2 + [5.08 × S/E ratio] + 25.9, R2=0.93) also supports this recommendation (0.45 mg N/mL). It appears that application of this pH-Stat method will result in the generation of ‘species-specific’ predictive regression equations for rapid-screening of protein quality of conventional and novel feed ingredients.

Keywords: Gadoids; Atlantic cod; Protein; Digestibility; In vitro; Feed ingredients

* E-mail address: Santosh.Lall@nrc-cnrc.gc.ca (S.P. Lall).
Enzyme pretreatment of high fibre plant ingredients and effects on feed utilisation in rainbow trout (*Onchorhynchus mykiss*)

Vegard Denstadli¹*, Marie Hillestad², Viviane Verlhac³, Mikkel Klausen⁴, Margareth Øverland¹

¹Aquaculture Protein Centre, Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, 1430 Ås, Norway
²BioMar A/S, Nordre gt 11, 7011 Norway
³DSM Nutritional Products, Animal Nutrition & Health Research Centre, BP170, 68305 Saint-Louis cedex., France
⁴Novozymes A/S, Krogshoejvej 36, 2880 Bagsvaerd, Denmark

Abstract

Non starch polysaccharides (NSP) are assumed to have low nutritional value for carnivorous species, including rainbow trout. Plant protein concentrates are therefore often considered as a better alternative than crude or defatted plant ingredients, in spite of higher costs. Exogenous carbohydrase products have a potential to upgrade high fibre ingredients, and are commonly used in diets for terrestrial monogastrics. Two experiments were conducted to evaluate the effect of enzymatic pretreatment of sunflower cake (SFC), extracted rapeseed meal (RSM) extracted soybean meal (SBM) and whole peas. In the first experiment the individual ingredients were incubated at semi-moist conditions (40% moisture, 45°C, 45 min) in the presence of an enzyme cocktail containing activities of pectinase, β-glucanase and hemi-cellulase (RONOZYME® VP, DSM). The changes observed were moderate, but total NSP was significantly reduced in the enzyme pretreated SBM, SFC and RSM, but with less effect in peas. Total NSP was reduced by 5-10%, where the most prominent effects were seen with arabinose and uronic acid (10-15% reduction). A wet incubation with the VP enzyme product was also performed with samples from SBM and RSM at various temperatures and with longer retention time (85% moisture, 30 - 45 - 60°C, 180 min). Significant reductions were observed (5-12%), but very similar to the changes observed in the semi-moist incubation. In the second experiment, seven diets were produced and fed to 205 g rainbow trout (*Onchorhynchus mykiss*) held in saltwater: a fish meal control diet and six diets with 50/50 combinations of two of the three plant sources: SBM, SFC and RSM with or without enzymatic pretreatment. The feed intake was similar for all groups, and a two-way ANOVA showed that enzyme pretreatment had no significant effect on the digestibility of protein, lipid, starch or energy. Despite of this, the feed utilisation tended to be lower in groups of fish fed the pretreated diets. The latter may be related to release of oligosaccharides during enzyme treatment. Fecal dry matter and pellet durability was significantly higher in fish fed pretreated diets, as well as the apparent Cu absorption. However, a hypothesised positive effect on digestible energy and feed utilisation was not observed in trout despite of lowered NSP content after enzyme pretreatment.

Keywords: High fibre; Enzyme pretreatment; Rainbow trout; Feed utilisation

* E-mail address: vegard.denstadli@umb.no (V. Denstadli).
P-166

Effect of multi-cycled feeding with low dietary protein and optimal dietary protein on growth and feed utilization of hybrid sturgeon (Acipenser ruthenus Linnaeus ♂ × A. baeri Brandt ♀)

Ying Huang1,2*, Xiaoming Zhu1, Wu Lei1, Yunxia Yang1, Dong Han1, Shouqi Xie1
1 State Key Laboratory of Freshwater Ecology and Biotechnology; Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China
2Graduate School of the Chinese Academy of Sciences, Beijing 100039, China

Abstract

A multi-cycle experiment of feeding low dietary protein and optimal dietary protein to hybrid sturgeon (Acipenser ruthenus Linnaeus ♂ × Acipenser baeri Brandt ♀) was conducted to determine the variation of dietary protein contents on growth performance and fish body composition. During the 60d experiment, hybrid sturgeon were divided into 4 groups: the control (fish were fed with 42.9% crude protein diet), L2 (fish were fed cycled 2 days 32.3% crude protein diet+2 days 42.9% crude protein diet) L5 (fish were fed cycled 5 days 32.3% crude protein diet+5 days 42.9% crude protein diet), L10 (fish were fed cycled 10 days 32.3% crude protein diet + 10 days 42.9% crude protein diet).

The results showed that hybrid sturgeon in L5 group and L10 group could obtain similar final body weight compared to the control fish while those in L2 group significantly lower. Specific growth rate in L5 group was significantly lower than that in the control during first period (0-20d) while significantly higher than the control during second period (20-60d). No significant differences in feeding rates or feed conversion efficiencies were observed between different groups.

At the end of the experiment, fish body contents of protein, lipid, energy, ash or water in the fish show no significant difference between groups. Visceral lipid in the fish of L2 and L5 group were significantly lower than that of the control fish. No significant difference was observed in serum SOD activities between groups. L5 and L10 group showed slightly lower feed cost and nitrogen loading.

Keywords: Multi-cycle of dietary protein; Growth efficiency; Economy efficiency

* E-mail address: huangying19822@sina.com (Y. Huang).
Effect of protein restriction with subsequent realimentation on compensatory growth of juvenile Chinese soft-shelled turtles (*Pelodiscus sinensis*)

Quansen Xie*, Junwei Li, Yujuan Li, Zhencai Yang  
College of Life Science, Hebei Normal University, Shijiazhuang 050016, Hebei Province, China

Abstract

We investigated effect of protein restriction with subsequent realimentation on compensatory growth (CG) of juvenile Chinese soft-shelled turtles (*Pelodiscus sinensis*). Crude protein was reduced (weeks 1–4) while the energy content remained constant for four treatment groups: T1 (29.24% protein), T2 (31.42%), T3 (34.19%), and T4 (37.16%); control group (C) was fed an adequate diet (43.31% protein). During subsequent realimentation (weeks 5–11), all groups were fed the adequate diet. After protein restriction, feed conversion ratio (FCR) and apparent dry matter digestibility (ADd) significantly increased, whereas apparent protein digestibility (ADp) and specific growth rate (SGR) significantly decreased in all protein-restricted groups compared to C, final body weight in groups T1 and T2 was significantly lower than controls. After realimentation, final body weight in group T1 was higher compared to group C, no significant differences were found. SGR was significantly increased with protein restriction and was longer lasting with more severe protein restriction. Feed intake (FI) increased and then declined towards the control level, ADd and ADp didn’t increase, and FCR was reduced in early realimentation. We conclude that the CG response induced by protein restriction in *Pelodiscus sinensis* depends primarily upon enhanced FI and decreased metabolism in the early phase.

Keywords: *Pelodiscus sinensis*; protein restriction; protein realimentation; compensatory growth

* E-mail address: xiequansen@163.com (Quansen Xie)
Effects of different binders on physical properties of microdiets and on growth status of Larval turbot, *Scophthalmus maximus*

Xiaobing Chen, Kangsen Mai*, Xiaojie Wang, Qinghui Ai, Wenbing Zhang, Hongming Ma, Hui Xu, Zhiguo Liufu

The Key Laboratory of Mariculture (Ministry Education of China), Ocean University of China. 5 Yushan Road, Qingdao, Shandong 266003, P.R. China.

Abstract

The effects of five different types of binders, sodium alginate (SA), xanthan (Xan), gelatin (Gel), carrageenan (Car), konjac gum (KG), on growth status and vitality of digestive enzyme of turbot (*Scophthalmus maximus*) were studied in this report. A 35-day of feeding trials was conducted to estimate the appropriate binders in the microdiets for larval turbot. Five practical microdiets were formulated to contain 2% of SA, Xan, Gel, Car, and KG as the binder, respectively; compared with no binder group (control) and live feed group (LF). There were no significant difference on survival rates between each groups, but the final weight and the specific growth rate (SGR) were different. The final weight of fish fed with Gel group (525.63±36.3mg) was significantly higher than Xan (379.80±25.4mg), Car (401.80±35.7mg), KG (432.58±32.1mg), Sta (417.26±28.6mg) and LF groups (306.19±9.4mg), but has no significant difference compared with SA group (448±15.6mg). To the specific growth rate, the Gel group (6.7±0.2%day\(^{-1}\)) was significantly higher than Xan (5.9±0.2%day\(^{-1}\)), Car (6.0±0.2%day\(^{-1}\)), Sta (6.2±0.2%day\(^{-1}\)) and LF groups (5.3±0.1%day\(^{-1}\)), but has no significant difference compared with SA (6.4±0.1%day\(^{-1}\)) and KG groups (6.3±0.3%day\(^{-1}\)). Based on the feeding trials results, two types of binders, Gel and SA were suitable as the binders of microdiets of turbot. The results of physical properties of microdiets and digestive enzyme of fish will be analyzed later.

Keywords: Microdiet; Larvae; Turbot; *Scophthalmus maximus*; Binder

E-mail address: smilicecx@126.com (Xiaobing Chen)
Sodium diformate and extruder temperature affects physical quality of barley protein concentrate-based fish feed

Thea Morken1*, Olav F. Kraugerud1, Frederic T. Barrows2, Mette Sorensen1,3, Trond Storebakken1, Margareth Øverland1

1 Aquaculture Protein Centre, Centre of Excellence, Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, P.O. Box 5003, N-1432 Ås, Norway.
2 U.S. Department of Agriculture, Agricultural Research Service, Hagerman Fish Culture Experiment Station, 3059-F National Fish Hatchery Road, Hagerman, ID, 83332, USA.
3 Nofima Marine, P.O. Box 5010, N-1432 Ås, Norway.

Abstract

Extrusion is a complex process, in which raw materials are cooked by applying moisture, pressure, temperature, and shear forces. Cooking initiates a series of physiochemical reactions, including starch gelatinization and protein unfolding, contributing to the binding between feed particles in the dough. Physical quality has been defined as the ability of pellets to withstand attrition during storage, transport, and handling, and is important for reducing overall feed costs in aquaculture. As a consequence of the increased focus on novel feed ingredients in recent years, physical quality of feed has become increasingly important.

This study examined the effects of sodium diformate (Na-diformate) and increasing extruder temperature on physical quality of barley protein concentrate-based (BPC) diets for fish. BPC was included in the diets at 270 g kg\(^{-1}\), with or without the addition of 10.6 g kg\(^{-1}\) Na-diformate. The diets were processed at 110, 126, and 141ºC using a laboratory-scale twin-screw extruder, resulting in six diets. Physical quality of pellets was assessed by hardness, diameter, expansion ratio (ER), water stability index (WSI), and durability during physical attrition.

Extruder temperature significantly affected hardness, WSI, and durability of the pellets. Extrusion at 110ºC resulted in significantly harder pellets than extrusion at 141ºC. For WSI and durability, extrusion at 126ºC resulted in a significantly higher pellet water stability and lower durability than extrusion at 110 or 141ºC. Addition of Na-diformate to diets significantly improved hardness, diameter, ER, WSI, and durability compared to the non-supplemented diets. The combination of increased extruder temperature together with Na-diformate resulted in a significant improvement in WSI and durability. Significant positive correlations were found between hardness of pellets and durability (\(P = 0.01\)), and between durability and WSI (\(P = 0.04\)). These results suggest that physical quality of BPC-based fish diets can be improved by optimizing extruder temperature and by adding Na-diformate.

Keywords: barley protein concentrate; extrusion; fish feed; physical quality; sodium diformate.

* E-mail address: thea.morken@umb.no (T. Morken).
Response to various culture salinities in two native \textit{Brachionus} rotifers from Iran

Reza Malekzadeh Viayeh and Habib Mohammadi

$^1$Artemia and Aquatic Animals Research Institute, Urmia University, Urmia, Iran
$^2$Department of Fisheries and Aquaculture, Faculty of Natural Resources, Urmia University, Urmia, Iran

Abstract

Two native rotifer species, \textit{Brachionus plicatilis} (B. pli) and \textit{B. rotundiformis} (B. rot) sampled from northwest and south Iran were subjected to an experimental culture in order to evaluate their cultural capabilities at different densities. Each rotifer species was cultured at three salinities of 5, 20 and 30 ppt in triplicate. All the animals were fed with \textit{Chlorella vulgaris}, and water temperature was 25 °C and density was 500 ind./ml for all the treatments and replica. The maximum mean ratio of unegged-female (UF) to egged females (EF), mean number of mictic females (MF) and mean number of mictic eggs (ME) (per mL) were observed in B. rot at 20ppt. While, maximum average number of males (M) was in B. pli at 20ppt. The highest number of produced resting eggs (RE) was in B. pli at 30ppt and maximum mean number of parthenogenetic eggs (PF) was in B. rot at 5ppt. ANOVA showed significant differences ($P<0.05$) in the values of the measured parameters among different treatments. Duncan's test revealed that there was significant difference in UF/EF ratio between B. rot at 5 and 20 ppt, between B. rot at 20 ppt and each of the other treatments, and between B. pli at 20 and 30 ppt. There also were significant differences in the number of mictic females of each rotifer species at different salinities and between the two species. However, number of mictic eggs, males, resting and parthenogenetic eggs were not different among B. pli cultured at different salinities. In occlusion, salinity had a significant effect on the life-table and cultural traits of the examined rotifers and both rotifer species showed positive growth responses at lower salinities.
P-171

Effect of waterborne copper exposure on growth, hepatic enzymatic activities and histology in *Synechogobius hasta*

X. J. Liu¹, Z. Luo¹,*, B. X. Xiong¹, X. Liu², Y. H. Zhao², G. F. Hu¹, G. J. Lv¹
¹Fishery College, Huazhong Agricultural University, Wuhan 430070, China
²Postgraduate Research Base, Panjin Guanghe Fishery Co. Ltd., Panjin 124200, China

Abstract

The present study was conducted to determine growth, hepatic enzymatic activities and histology in *Synechogobius hasta* after a 15-day of waterborne copper exposure at the concentrations of 0 (control), 0.15 and 0.3 mg / l, respectively, and explore whether waterborne copper exposure could induce the fatty liver syndrome for the fish species. Growth (WG and SGR) declined, but HSI increased in *S. hasta* with increasing waterborne copper levels (*P* < 0.05). Waterborne copper exposure also significantly increased lipid content and reduced protein content in both whole body and liver, and increased copper accumulation in whole body and vertebrae. Copper exposure changed hepatic enzymatic activities (SOD, CAT, SDH, PK, LDH, LPL and HL) and increased hepatic lipid peroxidation level, impaired the histological structure of the gill and liver in *S. hasta*. Thus, our study demonstrated for the first time that waterborne Cu exposure could induce fatty liver syndrome in fish.

Keywords: Copper toxicity; *Synechogobius hasta*; histology of gill and liver; hepatic metabolism; fatty liver syndrome

*Email address: luozhi99@yahoo.com.cn (Z. Luo).*
P-172

Stress response in seabream (*Sparus aurata*) held under crowded conditions and fed diets with different levels of inclusion of linseed and/or soybean oil

Ganga, R.¹, Bell, J.G.², Montero, D.¹, Atalah, E.¹, Acerete, L.³, Tort, L.³, Benitez Santana, T.¹, Fernández-Vaquero, A.⁴ and Izquierdo, M.S.¹

¹Grupo de Investigación en Acuicultura, Universidad de Las Palmas de Gran Canaria-Instituto Canario de Ciencias Marinas, Transmontañá, s/n 35416 Las Palmas, Spain.
²Institute of Aquaculture, University of Stirling FK9 4LA, Scotland, UK.
³Department of Cell Biology, Physiology and Immunology, Universitat Autonòma de Barcelona, Bellaterra 08193, Spain

Abstract

The physiological response to stressors in fish, including hormonal profiles and associated tissue responsiveness, are less documented. The aim of this study was to evaluate the effect of feeding gilthead seabream (*Sparus aurata*) with diets containing linseed oil (LO) and soybean oil (SO) as a substitute for fish oil (FO) and their effect on fatty acid profile of head kidney and the consequent effect on stress response to a crowding test. Fish were fed with different diets with different levels of substitution 0% (FO), 70% (70LO, 70SO, 20LO50SO and 50LO20SO) and 100% (100LO, 100SO and 50LO50SO) over a period of 8 months. At the end of the feeding trial, samples for head kidney biochemical analysis were collected and the fish were challenged by a crowding test. Samples of plasma for cortisol analysis were collected at different times during the test, 0h, 2h, 5h, 24h, 48 and 1 week. Results showed that basal cortisol levels were significantly increased in fish fed 70LO, 100LO and 50LO50SO. The physiological response to crowding was significantly affected by the diet. After 2 h of crowding, all the treatments showed higher cortisol, being 100LO significantly different registering the maximum value of 131.38 pg/ml compared to 50LO20SO which had the lowest plasma cortisol response with only 18.73 pg/ml. After 5h and 24h, plasma cortisol was reduced in all treatments except for 50LO20SO. After 48 h of crowding, the plasma cortisol was increased in all treatments with the maximum value seen in fish fed 100LO (72.12 pg/ml). These values were decreased after 1 week in fish fed FO, 70LO, 100LO and 50LO50SO, comparing to 70SO, 100SO, 20LO50SO and 50LO20SO. In general, fish fed VO affected the response to stress by crowding.

Keywords: seabream, vegetable oils, fatty acids, stress, crowding, cortisol.

*Email adresse: Rachid.ganga@gmail.com (R. Ganga)
Oxygenated nanobubbles as a means to improve nutrition and the environment of finfish and shellfish in order to increase growth and population density

Phil Hamilton\textsuperscript{2} and Peter Appleton\textsuperscript{3}

\textsuperscript{1} Director, Lois G. Britt Agribusiness Center, Mount Olive College, 652 RB Butler Drive, Mount Olive, NC, 23658. USA.
\textsuperscript{2} Lois G. Britt Agribusiness Center, Mount Olive College, 652 RB Butler Drive, Mount Olive, NC, 23658.

Abstract

Oxygen supply is an economically important limiting factor for aquaculture production when considering nutrition and the environment of finfish and shellfish. Commercial population densities can be increased if more oxygen is available. Fixed costs of aquaculture ponds are normally spread on a cost per unit of production basis dependent on the mass of fish produced per cubic meter of water. If more oxygen can be made available to growing fish, then higher production rates occur.

In order to increase population density without increasing production costs, nanotechnology can be utilized by injecting O\textsubscript{2} in tiny bubbles into the water using a pump utilizing nanotechnology. “Nanobubbles” of oxygenated water reaches the bottom of the water body more efficiently since the smaller bubbles rise to the surface less quickly. Nanobubbles maximizes the amount of time a bubble and O\textsubscript{2} is in contact with the water and begins the restoration process at the bottom of the pond where it is needed most.

In recent independently verified trials involving shrimp production in Mazatlan, Mexico, population densities and growth rates increased. The experiment consisted of a 74 day trial with shrimp density at 15 per cubic meter. Both the experimental group, which had oxygenated water, and control groups were fed the same nutrients on the same daily schedule of three times daily. Samples were weighed once a week after the eighth week (n = 10 minimum).

The experimental (oxygenated) group had an average weight of 10.67 grams while the control group had an average weight of 6.7 grams. The difference of 3.97 grams represented a difference of a 59.25% difference between the oxygenated and control groups.

The value of the increased harvest due to oxygenated water is significant. Based on 2007 price and quantity statistics, an increased total harvest of only 40% (conservative estimate based on the increase of 59.25% test results) and a 2007 average price of $4.55 per pound equates to a potential added $233 million to the US shrimp industry. Individual producers should see losses changed to profits by increasing the density of the fish per cubic meter of pond water. The investment payback can be decreased from five years to three years and a negative Net Present Value (NPV) will become positive by injecting O\textsubscript{2} via nanobubbles. Other economic benefits of nanotechnology may accrue for finfish and shellfish production such as: water conservation, less environmental deterioration, and lower feed and energy costs.

Keywords: finfish, shellfish, growth rate, population density, oxygenation, nanotechnology.

* E-mail address: \texttt{phamilton@moc.edu} (Phil Hamilton).
Effects of crowding stress on environment, growth performance, body composition and some immune factors of *Litopenaeus vannamei*

Ran Guo*, Ya-kun Chen, Hui Xia  
Ocean college, Hebei Agriculture University, Qinghuangdao 066003

Abstract  
There were two trials to evaluate the effects of crowding stress on several aqueous (pH, ammonia-nitrogen, dissolve oxygen), growth performance (weight gain, SGR, survival rate), body composition (crude protein, crude lipid, hepatopancrease lipid) and immune factors in juvenile *L. vannamei*. In the first trial, aqueous factors, growth performance and body composition of shrimp were detected to evaluate the influence of crowding stress (200, 400, 600, 800 shrimp m$^{-3}$ were used respectively). Commercial diets contained 42% protein were fed to satiation to shrimp (*Litopenaeus vannamei* initial mean weight: 0.19±0.02 g) in triplicate tanks (40 cm×50 cm×60 cm) connected to natural seawater (salinity: 30 g L$^{-1}$) for 8 weeks. The results show that there were significant differences in growth performance (weight gain, SGR, survival rate) among the four treatments ($P<0.05$), three index decreasing while breed aquatics density increased, and 200 shrimp m$^{-3}$ treatments got the highest WG (422.57) and SGR (704.29). Body composition, pH and dissolve oxygen had no significant different ($P>0.05$). Ammonia-nitrogen increased with breed aquatics density increased ($P<0.05$), but 200 and 400 shrimp m$^{-3}$ treatments had no significant different ($P>0.05$). The second experiment, a 20-day trial, had the same density as the first trial, but the shrimp had initial mean weight: 1.31±0.07 g. Three shrimp were syringed from pericardial cavity to got haemolymph at 1, 2, 4, 7, 9, 12, 15 and 20 day respectively. Total haemocyte counts (THC), total protein content in haemolymph (TPC), Phenoloxidase activity (PO), respiratory burst (O$_2^-$), AKP and lysozyme activity (LYS) were examined. The results were as follows: (1) PO, O$_2^-$, LYS appeared to decrease with increasing density ($P<0.05$). However, no differences in THC and TPC were observed when the density was above 800 shrimp m$^{-3}$. (2) The THC and TPC did not change during the trial. (3) At day 4, PO, AKP and LYS got peak and decrease from day 9, But the PO activity in the plasma of 800 shrimp m$^{-3}$ treatment was significantly lower than the 200 and 400 shrimp m$^{-3}$ treatments ($P<0.05$). The O$_2^-$ and AKP activities in the hepatopancreas of 200, 400 treatments were higher than the 600 and 800 treatment ($P<0.05$). O$_2^-$ peak at day 9, and then decreased. (4) Based on the results of the SGR, immune indicators and water quality the optimum breed aquatic capacity was 400 shrimp m$^{-3}$.

Keywords: *Litopenaeus vannamei*; immune factor; crowding stress; growth performance

*E-mail address: toguoran@163.com.
Diversity, abundance and spatial distribution of sediment ammonia-oxidizing Beta-proteobacteria in mariculture environment

Li Jing1*, Dang Hongyue1, Geng chunxiang2, Sun xiuhui2
1 Center for Biotechnology and Bioengineering, China University of Petroleum (East China), Qingdao 266555, China
2 Department of Environmental Science and Engineering, China University of Petroleum (East China), Qingdao 266555, China

Abstract

The rapid development of intensive and high density aquaculture in China caused seriously nitrogen accumulation and water pollution. Nitrification is the step in the nitrogen cycle that links the oxidation of ammonia to the loss of nitrogen in the form of dinitrogen gas. To examine the diversity and structure of aerobic obligated beta-Proteobacteria ammonia-oxidizing bacteria (AOB) in mariculture environment, clone library of functional marker gene amoA was constructed and sequenced after screened by PCR-restriction fragment length polymorphism (RFLP). Protein sequences deduced from amoA genes grouped within four distinct clusters which Nitrosomonas lineage dominant in most sites. Real-time fluorescence quantitative PCR analysis also indicated that Nitrosomonas species accounted for more than 90% of total AOB bacteria which ratio of Nitrosomonas specific gene copy numbers compare to amoA were more than 95%. And the ratios of AOB amoA gene copy numbers compare to 16s rRNA in mariculture environment were much higher (ranging from 4.6% to 15.6%) than in natural environment (1.33%). The results indicated AOB community played important role in aquaculture water. Multivariate statistical analyses showed that the community structures of AOB assemblages correlated with environmental parameters of nitrite-N and DO. The results enriched our knowledge of AOB diversity in mariculture environmental sediments and the relationship to the environmental parameters.

Keywords: AOB, Ammonia monooxygenase, Nitrification, Environmental parameter, Mariculture

* E-mail address: lijing@upc.edu.cn (Li Jing).
Accumulation and distribution of heavy metals and total phosphorus in intensive aquatic farm sediments

Wu Xiao-Yi, Yang Yu-Feng*
Institute of Hydrobiology, College of Life Science and Technology, Jinan University, Guangzhou 510632, China

Abstract
This study compares and discusses the accumulation and distribution of eight heavy metals (Cd, Co, Cr, Cu, Pb, Fe, Mn and Zn) and total phosphorus (TP) in surface sediments from five intensive farms of tilapia Oreochromis niloticus × O. aureu, Asian seabass Lates calcarifer and white shrimp Litopenaeus vannamei (n=75), and also their biogeochemistry processing. Results indicate that the sediments were significantly contaminated by Cd, Co, Cr, Cu, Pb, Fe, Mn and Zn as well as P. The mean concentrations of Cd, Co, Cu, Pb, Fe, Mn, Zn and TP in tilapia farm surface sediments were significantly lower than those levels observed in Asian seabass and white shrimp farm surface sediments ($P<0.05$). Heavy metals content of the sediments follow the order: Asian sea bass> White shrimp> tilapia. Concentrations of these heavy metals and total P in 10 cm intervals to 100 cm depth sediments from 30 different stations of white shrimp Litopenaeus vannamei did not show significant variations ($P>0.05$), and also a similar trandency was observed in the pore water of sediments, but the levels of these metals and TP in sediments were significantly higher than the values in pore water ($P<0.05$). Anion concentrations in the pore water follow the order: $\text{Cl}^- > \text{SO}_4^{2-} > \text{NO}_2^- > \text{NO}_3^- > \text{Br}^-$. Generally, Cadmium and Cu in Asian seabass and white shrimp farm sediments would pose the major potential risk, and heavy metals and P in sediments were mostly insoluble. Further, the sediment is highly enriched by Fe, P, Mn and Co.

Keywords: Accumulation; Distribution; Heavy metal; Total phosphorus; Sediment

* E-mail address: wjurk@163.com (Wu Xiao-Yi).
The effect of supplementary feeding on water quality during cage culture practice of Oreochromis niloticus in Lake Kuriftu, Ethiopia

Ashagrie Gibtan 1, Abebe Getahun 1 & Seyoum Mengistou 1
1 Department of Biology, Addis Ababa University, Addis Ababa, Ethiopia

Abstract
The research was conducted to investigate the effect of supplementary feeding on water quality during cage culture practice of Oreochromis niloticus in Lake Kuriftu, Ethiopia. Analysis of some physico-chemical parameters, zooplankton and phytoplankton were done for six months. The fish were fed a composite mixture of mill sweeping, cottonseed, and Bora food complex at 2% of their body weight twice per day using feeding trays in powdered form. The fish were grown in four treatments 50 (50F), 100 (100F), 150 (150F), and 200 (200F) fish per m³. Samples were taken in two sites (site A- around the cage and site B- on the other side of the lake). The zooplankton abundance during the study period varied from $1.124 \times 10^4$ to $1.802 \times 10^4$ and from $1.156 \times 10^4$ to $2.118 \times 10^4$ individuals / m³ at sites A and B, respectively. Abundance was almost the same at both sites. The highest value ($2.118 \times 10^4$ individuals / m³) was recorded at site B. At both sites, rotifers contributed most to the total abundance, followed by copepods and cladocerans. The result showed an insignificant difference in the abundance of zooplankton at the two sites ($P < 0.05$), but there was a significant difference in abundance between sampling dates ($P > 0.05$). Six phytoplankton groups were identified during the study period. The three taxonomic groups, blue greens, diatoms and greens, were dominant in the sense that they represented >90% of the total net phytoplankton. Relatively high proportion was observed at the beginning of the experiment and at the end of the experiment in both sites. There was significant variation ($p > 0.05$) in some physico-chemical parameters.

Keywords: Cage culture, water quality, Lake Kuriftu, Oreochromis niloticus

Correspondence: Ashagrie Gibtan, Addis Ababa University, Department of Biology, P. O. Box 1176, Addis Ababa, Ethiopia. E-mail: livelygib@yahoo.com
Elevated sea temperature push farmed Atlantic salmon to anorexia: Local effects of global warming

Ernst M. Hevrøy1, Rune Waagbo1*, Olav Breck2, Harald Takle3, Sven M. Jørgensen3, Thomas Torgersen4, Sissel Susort5, Leiv Tvenning2, Tom Hansen4
1National Institute of Nutrition and Seafood Research (NIFES), PO Box 2029, Nordnes, N-5817 Bergen, Norway
2Marine Harvest Norway AS, Sandviksbodene 78, N-5035 Bergen, Norway
3Nofima Marin, PO Box 5010, N-1432 Ås, Norway
4Institute of Marine Research, Matre Aquaculture Research Station, N-5984 Matredal, Norway
5Skretting Norway AS, Po Box 319 Sentrum, N-4002 Stavanger, Norway

Abstract

For understanding effects of elevated sea temperatures on adult Atlantic salmon in sea water, an experiment was conducted where 340 immature salmon (Salmo salar L., 1+, ~1.6 kg) were adapted to 14°C. At experiment start, fish (2.0 ± 0.4 kg) were kept in six control tanks (3 m diameter x 0.75 m water depth) on 14 °C, while fish in six tanks were exposed to gradual higher temperature up to 19°C (1°C/day) and kept on 19°C for 56 days, to simulating a warm water period in Western parts of Norway. In such periods fish farmers have observed lower feed intake and growth and poor feed utilization. The fish were reared under simulated natural photoperiod, in 35 g/L seawater, and oxygen level was kept constant at 90% saturation. The fish were given iso-nitrogenous diets (protein ~366 g kg⁻¹) with normal lipid (335 g kg⁻¹, NL) or low lipid levels (298 g kg⁻¹, LL), to investigating a possible dietary strategy to meet periods of elevated sea temperatures. During the experiment, the daily feed intake and growth were reduced with more than 50 % for fish held at 19 °C. Final weights were significantly lower in fish held at 19 °C compared to fish at 14 °C and 24 kg vs. 3.1 for fish fed the NL diet and 2.3 vs. 3.3 kg for fish fed the LL diet. Feed efficiency (FE) was significantly lower when salmon was kept at 19 °C compared to 14 °C. A trend was seen with lower feed utilization when LL diets were fed at both temperatures. Energy homeostasis in form of whole body lipid and muscle lipid where significantly reduced in fish at 19 °C. We have detected that key hypothalamic neuropeptides of the regulators of adiposity signaling are significantly affected in salmon at 19 °C. These findings support development of clinical anorexia in the experiment.

Keywords: Atlantic salmon; Global warming; Dietary strategies; Anorexia

* E-mail address: rune.waagbo@nifes.no (R. Waagbo)
Effects of dietary copper levels on antioxidant defense of the juvenile black tiger shrimp, *Penaeus monodon*, reared in low salinity waters

Jian-An Xian*, Xiao-Dan Chen, Ni-Na Gou, Chao-Xia Ye, Yu-Tao Miao, An-Li Wang

Key Laboratory of Ecology and Environment Science in Guangdong Higher Education, Guangdong Provincial Key Laboratory for Healthy and Safe Aquaculture, College of Life Science, South China Normal University, Guangzhou 510631, People’s Republic of China

Abstract

This study was conducted to evaluate the effects of dietary copper (Cu) on antioxidant defense in the juvenile black tiger shrimp, *Penaeus monodon*, reared in low salinity waters (5‰). Graded levels of supplemental Cu (0, 10, 25, 40, 55 and 110 mg Cu/kg diet) were fed to shrimp juveniles for 8 weeks. Each diet was fed to three replicate groups of shrimp. In plasma, the activities of copper-zinc superoxide dismutase (Cu-Zn SOD) and total SOD were higher in shrimp fed diets with 40 and 55 mg Cu/kg diet than those in shrimp fed diets with ≤25 and 110 mg Cu/kg diet. Total antioxidant capacity (T-AOC) in the plasma was highest in shrimp fed diets 25, 40 and 55 mg Cu/kg diet. Hepatic Cu-Zn SOD activity was significantly increased in shrimp fed diets with 25, 40 and 55 mg Cu/kg diet, and hepatic T-SOD activity was significantly increased in shrimp fed diets with 40 and 55 mg Cu/kg diet, and decreased in shrimp fed diets with 110 mg Cu/kg diet. T-AOC in the hepatopancreas were higher in shrimp fed diets with 10, 25, 40 and 55 mg Cu/kg diet than those in shrimp fed diets with 0 and 110 mg Cu/kg diet. Hepatic thiobarbituric acid reactive substances (TBARS) value was lowest in shrimp fed diets with 25 and 40 mg Cu/kg diet, and highest in shrimp fed diets with 110 mg Cu/kg diet. Based on the activities of Cu-Zn SOD, T-SOD, T-AOC and hepatic TBARS value, the optimum requirement of juvenile shrimp for dietary supplemental Cu was estimated to be 25-40 mg/kg diet. Both deficiency and excess of dietary Cu significantly depressed the immune responses in shrimp.

Keywords: Copper; *Penaeus monodon*; Antioxidant defense; Low salinity

*E-mail address: xian-ja@qq.com* (J. A. Xian)

1Corresponding author: wanganl@scnu.edu.cn (A. L. Wang)
P-180

Effect of Astragalus Polysaccharides on Growth, Nutrient Composition of Body and Immunological indices in *Schizothorax prenanti*

Xiang Xiao*, Zhou Xing-hua, Chen Jian, Wang Wen-juan, Li Dai-jin
Department of Fisheries in Rongchang compust, Southwest University, Rongchang 402460,China

Abstract

The experiment was conducted to the determine the effect of astragalus polysaccharides(APS) on growth performance, nutrient composition of body and activities of immunoenzyme in *Schizothorax prenanti*. 450 healthy *Schizothorax prenanti* [ (6.98±0.43) g] of average weight and [ (9.11±0.25) cm] of average length were randomly divided into five groups and each group included three replicates of 30 *Schizothorax prenanti*. From group C1 to group C5 was supplemented with 0, 0.02, 0.04, 0.06, 0.08% APS respectively. the experiment lasted for 50 days. The results showed that the weight gain ratio(WGR), the specific growth ratio(SGR) and protein efficiency ratio(PER) of fish fed with the control diet were significantly lower than those APS supplemented diet, at the same time, the feed conversion ratio(FCR) of fish was significantly higher (P<0.05). All of the WGR, SGR and PER of *Schizothorax prenanti* were highest(110.31%, 1.86%d⁻¹, 182.07% respectively), at the same time, FCR was the lowest(1.44) when APS content was 0.04%, those were significant difference with other groups (P<0.05). Based on Linear and parabolic regression analysis of the WGR, SGR, PER, FCR, the optimal dietary supplementation APS levels for this diet formulation was 0.045~0.074%; moisture crude Ash in whole body of *Schizothorax prenanti* were no effected by supplemental APS levels (p>0.05), but the crude protein were highest when APS content was 0.06%,but those were no significant difference with APS content was 0.04%,s (p>0.05), and the crude lipid were highest when APS content was 0.04%, those were significant difference with other groups (P<0.05). the immunoenzyme activities of fish fed with the control diet were significantly lower than those APS supplemented diet (P<0.05); the immunoenzyme activities of *Schizothorax prenanti* increased significantly with increasing dietary APS within a certain range. *Schizothorax prenanti* had maximal levels of alkaline phosphatase activity (ALP) when fed 0.04% APS, at the same time, the acid phosphatase activity (ACP) activity were highest when fed 0.06% APS, but the Lysozyme(LSZ) and superoxide dismutase(SOD) activity tended to stabilize when fed 0.06~0.08% APS. It showed that APS could significantly promote the growth and Immunity of *Schizothorax prenanti*. Based on the information above, the optimal dietary APS levels for optimum growth performance and immunological indices in *Schizothorax prenanti* is 0.04~0.074%.

Key words: Astragalus Polysaccharides (APS), Growth, nutrient composition of body, immunity, *Schizothorax prenanti*.

* E-mail address: xiangx@126.com.
The Application of Quorum-quenching Enzyme in Anti-infection immunity of Zebrafish (*Danio rerio*)

Yanan Cao**, Zhigang Zhou*

Key Laboratory for Feed Biotechnology of the Ministry of Agriculture, Feed Research Institute, Chinese Academy of Agricultural Sciences, No. 12 Zhongguancun South Street, Beijing 100081, P. R. China

Abstract

Quorum-quenching enzyme, especially AHL-lactonase, was extensively studied in the biocontrol of plant, but the field of application in aquaculture and its mechanism was carried out less. In this study, AHL-lactonase was used as antimicrobial in aquaculture firstly, and the preliminary study on its mechanism in vivo of zebrafish was investigated. *Aeromonas hydrophilia* (Ah), the main pathogen of explosive epidemic disease in aquaculture, N-acylhomoserine Lactonase (AHL-lactonase) AiiA-B546, and Ah and AiiA-B546 were incorporated into the zebrafish feed respectively. The total RNA, extracted from gut of the zebrafish within 6 h (0, 2, 4 and 6 h) post fed the experimental diets a week acclimated to the control diet, was used as RT-PCR template. The corresponding real time q-PCR were carried on to get the ratio of Ct value from target gene to standard gene (β-Actin for zebrafish and RpoB for Ah) which can reveal the expression value of the important immune factor (TNFα, IL-1β, IFNγ, iNOS2a, IL-10, TLR5) of zebrafish and toxin factor (cytotoxic enterotoxin, hemolysin, aerolysin, protease and cytolytic enterotoxin) of Ah. A 4-week feeding duration was lasted subsequently, and the gut sample was extracted at 3, 7, 14 and 28 d for the further study. The protective effect of AiiA-B546 was calculated from the survival rate in each group. The intestinal bacterial diversity was evaluated with PCR and denaturing gradient gel electrophoresis (DGGE). And the Ah in the gut was counted with q-PCR. This study laid the foundation for the AHL-lactonase as a novel antimicrobial agent in aquaculture.

Key word: N-acylhomoserine Lactonase; Anti-infection immunity; Real time q-PCR

*E-mail address: zhouzhigang@caas.net.cn (Z. Zhou).*
P-182

Effect of dietary glucan extraction from *Ganoderma lucidum* on growth performance, immune response and survival in gibel carp (*Carassius auratus gibelio*) challenged with *Edwardsiella tarda*

Yuhang CHEN*1,2, Xiaoming ZHU1, Yunxia YANG1, Dong. HAN1, Shouqi. XIE1

1 State Key Laboratory of Freshwater Ecology and Biotechnology; Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China
2 Graduate School of the Chinese Academy of Sciences, Beijing 100039, China

Abstract

This study investigated the effects of short (21 days) and prolonged (65 days) administration of glucan extraction from *Ganoderma lucidum* on non-specific immune parameters, growth and the disease resistance in gibel carp (*Carassius auratus gibelio*). Five treatments were tested. The fish were fed the control diet without glucan extract alone for 65 days (C65); the control diet with 0.58% glucan extraction for 21 days and then the control diet for 34 days (L21C34); the control diet with 0.58% glucan extraction for 65 days (L65), the control diet with 2.9% glucan extraction for 21 days then the control diet for 34 days (H21C34); the control diet with 2.9% glucan extraction for 65 days (H65). Each diet was randomly allocated to triplicate groups of fish in 300L tank, and each tank was stocked with 45 fish (initial average weight 6.72±0.01g).

Growth, serum lysozyme, alternative pathway of complement hemolytic activity, blood leucocyte phagocytic activity, respiratory burst, and myeloperoxidase activity were monitored on day 21, 46 and 65. The results showed that specific growth rate (SGR) showed no significant difference between different treatments after 65 days feeding trial (P>0.05). Glucan extraction inclusion could increase the respiratory burst (P<0.05) while weaken the alternative pathway of complement hemolytic activity (P<0.05). Leucocyte phagocytic activity were not significantly different between different treatments (P>0.05). On day 65, H65 group showed highest survival after the challenge of *Edwardsiella tarda* (P<0.05). In conclusion, administration of 2.9% glucan extraction for 65 days is recommended for the enhancement of immunity and survival of *Carassius auratus gibelio*.

Keywords: Glucan; Non-specific immune; *Carassius auratus gibelio*; *Edwardsiella tarda*

*E-mail address: itsmecyh@126.com*
Dietary antibiotics induced changes in the intestinal microbiota alter tilapia physiological responses to infection with *Aeromonas hydrophilas*

Zhigang Zhou1,*, Suxu He1, Yuchun Liu1, Yanan Cao1, Kun Meng1, Bin Yao1, Einar Ringø2, Ilkyu Yoon3

1Key Laboratory for Feed Biotechnology of the Ministry of Agriculture, Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing 100081, China
2Department of Marine Biotechnology, Norwegian College of Fishery Science, University of Tromsø, N9037 Tromsø, Norway
3Diamond V Mills, Cedar Rapids, IA, USA

Abstract

The interaction between host and gut microbiota is presently a hot field, and few information is available in fish. The purpose of the present study was to investigate whether dietary antibiotics induced changes in the intestinal microbiota alter host physiological responses to infection with *Aeromonas hydrophilas* in hybrid tilapia (*Oreochromis niloticus* ♀ × *O. aureus* ♂). After an 8-week induction period with an antibiotics supplemented or non-supplemented diet, 160 hybrid tilapias in 16 tanks were each injected with phosphate buffered saline (PBS) or *A. hydrophilas* (AH) at a dose of one half the LD₅₀ (Four feeding regimes: CC, fed antibiotics non-supplemented diet following injection with PBS; CAH, fed antibiotics non-supplemented diet following injection with AH; AC, fed antibiotics supplemented diet following injection with PBS; AAH, fed antibiotics supplemented diet following injection with AH). Then, all of the diets were changed to a prebiotics supplemented one for the sequential 8-week response period. Parameters including growth, gut microbial diversity and richness, and non-specific immunity were determined at the end of the response period and compared among the four feeding regimes during the response period. *A. hydrophilas* infection had no effects on the growth and diet conversion of tilapia, but the microbial count, visual band number, diversity and evenness of intestinal bacteria in the fish fed the antibiotics non-supplemented diet (CAH) decreased based on PCR-denaturing gradient gel electrophoresis fingerprints. Infection with *A. hydrophilas* reduced the intestinal bacterial evenness but slightly improved the visual band number richness in tilapia fed an antibiotics supplemented diet (AAH). In addition, *A. hydrophilas* infection affected non-specific immunity such as serum lysozyme activity and serum alternative complement pathway (C3 and C4) activities after hybrid tilapia were fed the prebiotics supplemented diets. These changes varied based on the intestinal microbial status of the fish before infection with *A. hydrophilas*.

Keywords: Antibiotics; Intestinal microbiota; *Aeromonas hydrophilas*; DGGE; q-PCR

* Email address: zhou_zg@msn.com (Z. Zhou).
Effects of dietary $\beta$-glucan and glycyrrhizin on non-specific immunity and disease resistance of sea cucumber (*Apostichopus japonicus* Selenka) challenged with *Vibrio splendidus*

Jie Chang, Wen-Bing Zhang*, Kang-Sen Mai, Hong-ming Ma, Wei Xu

The Key Laboratory of Mariculture (Ministry of Education of China), Ocean University of China, 5 Yushan Road, Qingdao, Shandong 266003, P.R. China.

Abstract

The sea cucumber, *Apostichopus japonicus* Selenka were fed with immunostimulant-free basal diet, diet supplemented with 0.2% $\beta$-glucan and diet supplemented with 0.02% glycyrrhizin, respectively, in a recirculated water system for 45 days. After that, the animals were challenged with *Vibrio splendidus* at $1.0 \times 10^8$ cfu sea cucumber$^{-1}$ for 15 days. The phagocytic capacity (PC), intracellular superoxide anion production (ISAP), lysozyme (LSZ) activity, superoxide dismutase (SOD) activity in the coelomic fluid were analyzed on the 0th, 5th, 10th and 15th day, respectively. Results showed that after the 45-day feeding trial, PC, ISAP, LSZ activity and SOD activity in sea cucumber fed with dietary $\beta$-glucan or glycyrrhizin were significantly higher than those in treatment with basal diet. On the 5th day after infection, all the analyzed immune parameters in the sea cucumber injected with *V. splendidus* significantly decreased. On the 15th day, the PC, ISAP and LSZ activity returned to the high levels, which were no longer significant lower than those on the 0th day. For the sea cucumber injected with saline, there were no significant differences in all the analyzed immune parameters and the cumulative mortality during the 15-day challenging trial. After injecting with *V. splendidus*, the cumulative mortality of sea cucumber significantly increased after the 5th day. At the end of the challenging trial, the cumulative mortality of *V. splendidus* challenged sea cucumber fed with immunostimulant-free basal diet was significantly higher than those fed with dietary $\beta$-glucan or glycyrrhizin. There is no significant difference in cumulative mortality between the dietary $\beta$-glucan and glycyrrhizin supplemented treatments.

Keywords: $\beta$-Glucan; glycyrrhizin; *Apostichopus japonicus* Selenka; Growth; Immunity

*E-mail address: changjieouc@163.com*
Effect of varying levels of phosphorus and carbohydrate on growth performance, body composition, nutrient utilization and hepatic enzymes activities of herbivorous grass carp (*Ctenopharyngodon idella*)

J.-J. LIANG*, Y.-J. LIU, L.-X. TIAN, H.-J. YANG, G.-Y. LIANG
Nutrition Laboratory, Institute of Aquatic Economic Animals, School of Life Science, Sun Yat-Sen University, Guangzhou, China

Abstract

Six isonitrogenous (410 g kg⁻¹) diets with three levels of total phosphorus (P 0.4%, P 1.0% and P 1.8%) and two levels of carbohydrate (CHO 20% and CHO 35%) were fed to triplicate groups of 30 fish in indoor recirculation system. Over 8-week-growth trial, best weight gain (WG), specific growth rate (SGR), feed conversion ratio, protein efficiency ratio (PER) and protein production value (PPV) (P < 0.05) were observed in fish fed diet with P 1.0% and CHO 20%. Within both carbohydrate level, fish fed diet with deficient phosphorus (0.4%) tended to produce lower (P < 0.05) WG and SGR, while had higher (P < 0.05) values of viscerosomatic index (VSI), hepatosomatic index (HSI) and intraperitoneal fat ratio (IPF). The whole body lipid, ash, calcium, phosphorus and iron contents were all significantly affected by dietary phosphorus concentration; also, significance were found for phosphorus, iron and copper concentration in liver. The blood chemistry analysis showed that dietary phosphorus had distinct effect on contents of triacylglycerol (TG) and total cholesterol (TC) (P < 0.05). WG, SGR, PER and PPV significantly decreased as carbohydrate concentration increased, while activities of glucokinase, pyruvate kinase, lipid content of liver and whole body, HIS and IPF significantly increased. These results suggest that high dietary carbohydrate (35%) will inhibit the growth performance and cause lipid accumulation. Supplied phosphorus can improve the growth and decrease the whole body lipid, VSI, HIS and IPF, but there is no more effect after the phosphorus is met at 1.0%, so excessive phosphorus (1.8%) can not improve the utilization of carbohydrate.

Keywords: Carbohydrate; Phosphorus; Feed utilization; Grass carp; Growth

*E-mail address: lightlueng@126.com*
Influence of dietary phosphorus levels on growth performance, body composition, and the serum biochemical indicators of juvenile *Pelteobagrus fulvidraco*

C. Wang¹, Q. Liao², J. Zeng¹, L. Xu¹, Q. Sheng¹

¹The Fisheries College, Huazhong Agricultural University, Wuhan, Hubei, China 430070
²Yueyang Zhanxiang Biological Science and Technology Corporation, Yueyang, Hunan, China

Abstract

In order to reduce the phosphorus content in the effluent and adjust the phosphorus content in artificial feed for aquaculture, a growth trial was conducted to estimate the effects of dietary phosphorous levels on growth performance, whole body composition, and serum biochemical indicators of juvenile yellow-headed catfish (*Pelteobagrus fulvidraco*). Three extruded diets (commercial yellow-headed catfish diet-based) were formulated to contain Ca(H₂PO₄)₂ levels at 25, 15, and 5 kg per ton of diet (kg of Ca(H₂PO₄)₂/t), respectively. Each diet was fed to triplicate groups of 15 fish (initial average BW of 3.2 g) over 4 wk. At the end of the trial, specific growth rate (SGR) and weight gain (WG) of the fish fed with the diet containing 15 kg of Ca(H₂PO₄)₂/t was significantly higher than the other groups (P < 0.05). Body crude protein content of the fish fed with the diet containing 25 kg of Ca(H₂PO₄)₂/t had significantly increased compared with those fed the diet containing 5 kg of Ca(H₂PO₄)₂/t (P < 0.05), whereas both of them did not have significant difference between 15 kg of Ca(H₂PO₄)₂/t treatment groups (P > 0.05). Serum superoxide dismutase (T-SOD) of the fish fed with the diet containing 15 kg of Ca(H₂PO₄)₂/t was significantly higher than the other 2 dietary treatments (P < 0.05). There was no significant difference in alkaline phosphatase (AKP), calcium, and phosphorus in serum among 3 dietary treatments (P > 0.05). The results suggested that phosphorus content in the current commercial feed for juvenile yellow headed catfish could be reduced from 25 to 15 kg of Ca(H₂PO₄)₂/t, and the growth performance, body composition, and immunity of this fish would not be diminished.

Keywords: *Pelteobagrus fulvidraco*, dietary phosphorus, growth

E-mail address: cfwang@hzau.edu.cn
P-187

Assessment of dietary supplementation with a multi-strain probiotic on performance, intestinal microflora, non-specific immune response and antioxidant status of rainbow trout (Oncorhynchus mykiss)

Ilias A. Giannenas1*, Efstathios Chronis2, Sophia Stavrakakis1, Maritsa Margaroni3, Pedro Encarnacao4, Elisabeth Mayer4 and Evdokia E. Karagouni3

1Laboratory of Animal Nutrition & Husbandry, Veterinary Faculty, University of Thessaly, 43100 Karditsa, Greece
2C’ Military Veterinary Hospital, 57 001 Thermi, Thessaloniki, Greece
3Laboratory of Cellular Immunology, Hellenic Pasteur Institute, Athens, Greece
4Biomin GmbH, Industriestrasse 21, 3130 Herzogenburg, Austria

Abstract

The manipulation and control of fish intestinal microbiota has been identified as an important area for future developments in aquaculture. An experiment was conducted to evaluate the effects of dietary supplementation with a multi-strain probiotic (Bacillus subtilis, Enterococcus faecium, Pediococcus acidilactici, Lactobacillus reuteri) on growth performance, intestinal microflora, non-specific immune response and antioxidant status of rainbow trout (Oncorhynchus mykiss). Groups of 30 sub-adult trout (113.0 g ±10.4) were randomly allocated into 3 different treatments with three replicates each. The control group was fed a basal diet, while the other two groups were fed diets supplemented with the multi-strain probiotic at levels of 1 and 5 g/kg diet. The fish were fed to apparent satiation for a period of 8 weeks. Body weight, body length and feed intake were recorded weekly. The intestinal bacteria populations (total aerobes, total anaerobes, Lactobacilli spp., Enterobacteriaceae spp., coliforms, E. coli and Aeromonas spp.) were enumerated by conventional microbiological techniques using selective agar media. Antioxidant status of fish was assayed for levels of glutathione reductase, glutathione-s-transferase and malondialdehyde levels in fish fillet at day 0 and 5 after slaughter. Lysozyme and nitric oxide (NO) concentration were evaluated in fish blood serum. Results showed that dietary probiotic supplementation at 1 g/kg diet improved (P < 0.05) growth performance and feed efficiency compared to control diet. Lactobacilli loads were higher in probiotic fed fish at both inclusion levels compared to control; however, other bacteria loads were similar among dietary treatments. Probiotic inclusion decreased significantly (P < 0.05) malondialdehyde formation on day 0 compared to control fish but not on day 5 of refrigerated storage. Probiotic inclusion increased significantly (P<0.05) the activity of glutathione based enzymes at both time points. Lysozyme levels were similar among dietary treatments. Probiotic supplementation at 1 g/kg diet reduced NO serum levels compared to control group. In conclusion, dietary probiotic supplementation, especially at the level of 1 g/kg of diet exerted both a growth promoting and antioxidant protective activity. It also modulated intestinal microbial communities favouring Lactobacilli spp. and affected non-specific immune response by decreasing NO serum levels.

Key words: Rainbow trout; Probiotic; Performance; Antioxidant status; Intestinal microflora; Non-specific immunity

* E-mail address: giannenas@vet.uth.gr (I. Giannenas)
ER stress and ROS play important regulatory roles during development of white adipocytes in *Atlantic salmon*

**TODORČEVIĆ M**\(^{m}\), ŠKUGOR S\(^{AB}\), KRASNOV A\(^{A}\), RUYTER B\(^{A}\)

\(^{A}\)Nofima, Norwegian Institute of Food, Fisheries and Aquaculture Research, P.O. Box 5010, Ås NO-1430, Norway

\(^{B}\)Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, P.O. Box 5003, Ås NO-1432, Norway

**Abstract**

Excessive fat deposition is one of the largest problems faced by the salmon aquaculture industries, leading to production losses due to high volume of adipose tissue offal. In addition, increased lipid accumulation may impose considerable stress on adipocytes leading to adipocyte activation, production and secretion of inflammatory mediators, as observed in mammals. The aim of this study was to investigate adipose tissue development and functions in Atlantic salmon by the use of transcriptome profiling. Primary adipocytes were followed through different developmental stages, from unspecialised stem cells to lipid filled mature adipocytes.

Microarray results showed marked regulation of genes involved in lipid and glucose metabolism during the terminal differentiation stages, also characterised by diverse stress responses. Activation of the glutathione and thioredoxin antioxidant systems and changes in the iron metabolism suggested the need for protection against oxidative stress. Signs of endoplasmic reticulum (ER) stress and unfolded protein response (UPR) occurred in parallel with the increased lipid droplet (LD) formation and production of secretory proteins (adipsin, visfatin). Notably, expression changes of a panel of genes that belong to different immune pathways were seen throughout adipogenesis. The induction of AP1 (Jun, Fos), which is a master regulator of stress responses, culminated by the end of adipogenesis, concurrent with the maximal observed lipid deposition.

Our data point to an intimate relationship between metabolic regulation and inflammatory responses in white adipocytes of a cold-blooded vertebrate. Stress imposed on adipocytes by LD formation and expansion is prominently reflected in the ER compartment and the activated UPR response could have an important role at visceral obesity in fish.

**Keywords:** Atlantic salmon, Adipose tissue; Adipocytes; Development; ER stress; ROS

* E-mail address: marijana.todorcevic@nofima.no
Aflatoxin B$_1$ reduces growth performance, physiological response and disease resistance in Tra catfish (*Pangasius hypophthahus*)

Tu Do Cam$^1$, Pedro Encarnação$^2$, Le Thanh Hung$^1$

$^1$ Nong Lam University, Linh Trung, Thu Duc, 84, Ho Chi Minh City, Vietnam
$^2$ Biomin Singapore Pte. Ltd. 3791 Jalan Bukit Merah #08-08 E-Center@Redhill, 159179 Singapore

Abstract

Contamination of grains with aflatoxin B$_1$ (AFB$_1$) represents a serious problem in tropical areas where many fish species are cultured. There is also mounting evidence regarding the negative effects of mycotoxin contamination on fish performance however, for Tra catfish (*P. hypophthalmus*) this information is scarce. A trial was conducted to determine the sensitivity of Tra catfish to increasing levels of AFB$_1$ contamination in the feed. Triplicate groups of one hundred Tra catfish (8 g±0.2) were fed six test diets (Diet 1 to 6) containing increasing levels of AFB$_1$ (0, 50, 100, 250, 500 and 1000 µg AFB$_1$/kg) achieved by the addition of a natural contaminated source (rice flour contaminated with 23 mg AFB$_1$/kg). Two additional diets (Diet 7 & 8) tested the efficacy of an aflatoxin binder (Mycofix®Secure) at a contamination level of 500 µg AFB$_1$/kg using 1.5 and 2.5 g/kg inclusion level. Initially, fish were fed to near satiety 2 times daily during an 8 week period. Fish fed diets 1 to 4 (0, 50, 100, 250, µg AFB$_1$/kg) were evaluated for an additional 4 weeks period to analyse subclinical effects and disease resistance under challenge with *Edwardsiella ictalurii* (10$^6$ CFU/mL). Results after 8 week feeding period show that Tra catfish is sensitive to AFB$_1$. Reduction in weight gain (P<0.05) was observed for fish fed the 50 µg AFB$_1$/kg and was reduced further with increasing levels of AFB$_1$ in the diet. This reduction in performance however, was not observed for fish fed diets containing the aflatoxin binder (P>0.05). Fish fed diets contaminated with 500 and 1000 µg AFB$_1$/kg showed increased (P>0.05) hepatosomatic index (HSI), while an increase in adipose somatic Index (ASI) was already observed in fish fed 50 µg AFB$_1$/kg and above when compared to the control and binder diets. After 12 weeks, blood serum analysis revealed higher alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in fish fed the 50, 100 and 250 µg AFB$_1$/kg suggesting occurrence of liver damage, in particular for those fish fed the 250 µg AFB$_1$/kg. Disease resistance of fish exposed to *Edwardsiella ictalurii* was also compromised by the presence of AFB$_1$ in the feed and was directly related to the contamination level. Seven days after exposure, survival rates were 50, 41.7, 31.7 and 8.3 % for fish fed control, 50, 100 and 250 µg AFB$_1$/kg respectively. This trial shows that, AFB$_1$ contamination in Tra catfish diets at a level of 50 µg AFB$_1$/kg and above can affect fish performance and disease resistance. Application of an effective binder in the feed (1.5 kg/ton) seems to be useful to prevent the negative effects of AFB$_1$.

**Keywords:** Aflatoxin B1; Tra catfish; growth performance; disease resistance, ALT, AST, aflatoxin binder.

$^*$ E-mail address: pedro.encarnacao@gmail.com
Effects of dietary yeast culture (DVAQUA®) on growth performance, intestinal autochthonous bacterial community and non-specific immunity of hybrid tilapia (*Oreochromis niloticus ♀×O. aureus ♂*) cultured in cages

Shuxu He 1, Zhigang Zhou 1,*, Yuchun Liu 1, Pengjun Shi 1, Bin Yao 1, Einar Ringø 2, Ilkyu Yoon 3

1 Key Laboratory for Feed Biotechnology of the Ministry of Agriculture, Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing 100081, P. R. China
2 Norwegian College of Fishery Science, Faculty of Biosciences, Fisheries and Economics, University of Tromso, Tromso, Norway.
3 Diamond V Mills, Cedar Rapids, IA, USA

Abstract

In the present study, hybrid tilapia (*Oreochromis niloticus ♀×O. aureus ♂*) were fed six iso-nitrogenous and iso-caloric experimental diets in cages. The diets contained six levels of yeast culture: 0 (CK), 0.125 (G1), 0.25 (G2), 0.5 (G3), 1.0 (G4) and 2.0 (G5) g/kg diet. The purpose of this study was to evaluate the effects of dietary yeast culture on growth performance, intestinal autochthonous bacterial community by using 16S rDNA and denaturing gradient gel electrophoresis (DGGE), and non-specific immunity of hybrid tilapia. Supplementation of dietary yeast culture showed no effects on growth performance, diet conversion and survival rate of the hybrid tilapia. However, supplementation of yeast culture affected the autochthonous gut bacteria community. Various potentially beneficial bacteria were stimulated by dietary yeast culture at different feeding period, while potential harmful species such as *Escherichia coli* serotype O20:H42-like, uncultured bacilli bacterium clone MS030A1_F02-like, and *Pseudomonas fluorescens* strain YC0357-like were depressed by dietary yeast culture. Non-specific immunity of hybrid tilapia such as serum lysozyme activity, serum C3 and C4 activities, head kidney macrophage phagocytic activity, and head kidney macrophage respiratory burst activity were improved by dietary yeast culture. The optimum dose of dietary yeast culture for hybrid tilapia determined based on a broken-line approach varies depending on the variables used: 0.226, 0.467, 0.492 or 0.50 g/kg diet for lysozyme, C3, C4, or phagocytic activity, respectively.

Key Words: Yeast culture; Intestinal autochthonous bacterial community; Non-specific immunity; hybrid tilapia; *Oreochromis niloticus ♀×O. aureus ♂*

*E-mail address: zhou_zg@msn.com (Z. Zhou).
P-191

Improved disease resistance in sea bass (Dicentrarchus labrax) fed mannan oligosaccharides (MOS)

Silvia Torrecillas*, Alex Makol1, María José Caballero1, Tibiábin Benítez-Santana1, Daniel Montero1, John Sweetman2, Viswanath Kiron3 and Marisol Izquierdo1.

1Universidad de Las Palmas de Gran Canaria, Grupo de Investigación en Acuicultura (GIA) and Instituto Canario de Ciencias Marinas, P.O. Box 56, 35200 Telde, Las Palmas de Gran Canaria, Canary Islands, Spain.
2Alltech Aqua, Samoli, Livadi, 28200 Lixouri, Cephalonia, Greece.
3Faculty of Biosciences and Aquaculture. Bodø University College. Bodø 8049. Norway

Abstract

The objective of this study was to determine the effect mannan oligosaccharides derived from the outer cell wall of a select strain of Saccharomyces cerevisiae (Bio-Mos, Alltech Inc, USA) on the potential mechanisms related to improved disease resistance and in relation to a stress challenge on European sea bass (Dicentrarchus labrax). Specimens of 40g at initial density of 4.1 kg/m³ were fed for 8 weeks 4‰ dietary MOS inclusion. Weight gain, specific growth rate, mucus production, gut and head kidney prostaglandins levels and microbiota profiles were evaluated. Growth and gut mucus production increased when MOS was supplemented. After the feeding trial, fish were subjected to a direct gut inoculation with the pathogen Listonella anguillarum in combination to a stress panel. Results showed a significant reduction (P ≤ 0.05) on mortality, different patterns of mortality and a significant reduction in the grade of pathogen infection when fish were supplemented with MOS. Prostaglandins production and microbiota profiles were accordingly affected. These results suggest that MOS supplementation increases disease resistance when supplemented before a disease challenge test and this fact could be related with the stimulation of intestinal mucus secretion. The interactions of MOS with the secretory activity of goblet cells could represent new interesting possibility for the manipulation of their important protective function.

Keywords: Sea bass, disease resistance, mucus, prostaglandins levels, microbiota, stress.

*E-mail address: silvia.torrecillas101@masters.ulpgc.es
Dietary plant oils for Atlantic salmon – effects on lens lipid composition and susceptibility to oxidation ex vivo

S. C. Remø¹, P.A. Olsvik¹, B. E. Torstensen¹, H. Amlund¹, O. Breck², R. Waagbo¹
¹ National Institute of Nutrition and Seafood Research (NIFES), P.O. Box 2029 Nordnes, N-5817 Bergen, Norway
² Marine Harvest Norway A/S, Bergen, Norway

Abstract
The present study was performed to investigate the effects of oxidation on Atlantic salmon (Salmo salar L.) lenses ex vivo, with focus on the role of dietary lipid sources and histidine. Oxidation is the most important factor in the epidemiology of cataracts in humans, and may also be a factor in cataract development for farmed salmon. Cataract is visible as opacities of the lens caused by changes in the epithelial tissues surrounding the lens fibres, or the composition and structure of the lens fibres. The histidine compound N-Acetyl-histidine (NAH) has been found to have mitigating effects on the development of cataract in fish. Among its multifunctional nature as osmolyte, metal chelating factor and buffer substance, NAH may also function as an antioxidant. A model for investigating salmon lens oxidation ex vivo was adapted from human research, and cultured lenses were stressed with hydrogen peroxide (H₂O₂). The model was used to investigate the effect of oxidative stress on lenses from Atlantic salmon fed diets based on either fish oil (FO) or vegetable oil (VO). In addition, the effect of dietary mercury (Hg), added as methyl mercury, was investigated as a potential source of additional oxidative stress. Lenses were dissected and cultured for 96 hours in medium added 5mM H₂O₂, in normal or histidine enriched medium. Lenses were analysed for lipid classes and fatty acid composition, histidine, NAH, dry matter, TBARS, glutathione (GSH) and total Hg at the time of sampling. Histidine, NAH, GSH and transcriptional levels of antioxidant enzymes were analysed in cultured lenses. Cataract score was assessed by slit-lamp microscopy, and visible changes during the culture were documented photographically. Lipid class composition of the lenses was not affected by the dietary lipids, however VO fed fish had a decrease in n-3/n-6 fatty acid ratio due to increased n-6. The cultured lenses responded to oxidative stress with loss of transparency, accumulation of autofluorescent compounds and volume increase. GSH was significantly reduced in oxidatively stressed lenses. Histidine level in the lenses was equal, while NAH concentration in stressed lenses was almost entirely depleted; indicating a role for NAH in the antioxidative defence system. The lenses accumulated Hg in response to dietary levels, but the oxidative status was not affected by the mercury concentration. These results indicate that the lipid source does not affect cataract development or susceptibility to oxidative stress in salmon lenses, while the depletion of NAH should be further investigated.

Keywords: A. salmon; cataract; oxidation; N-Acetyl-Histidine; lens culture

* E-mail address: Sofie.Remo@nifes.no (S.C.Remø)
Propolis and Herba Epimedii extracts enhance the non-specific immune response and disease resistance of Chinese sucker, *Myxocyprinus asiaticus*

Guobin Zhang, Shiyuan Gong*, Denghang Yu, Hanwen Yuan
College of Fisheries, Huazhong Agricultural University, Wuhan 430070, China

Abstract

The effect of traditional Chinese medicine (TCM) formulated from propolis and Herba Epimedii extracts at the ratio of 3:1 (w/w) on non-specific immune response of Chinese sucker (*Myxocyprinus asiaticus*) was investigated. Fish were fed diets containing 0 (control), 0.1%, 0.5% or 1.0% TCM extracts for five weeks. The respiratory burst and phagocytic activities of blood leukocytes, lysozyme and natural haemolytic complement activities in plasma were measured weekly. After five weeks of feeding, fish were infected with *Aeromonas hydrophila* and mortalities were recorded. Results of this study showed that feeding Chinese sucker with different dosage of TCM extracts stimulated respiratory burst activity, phagocytosis of phagocytic cells in blood and lysozyme activity in plasma. They had no effect on plasma natural haemolytic complement activity. All dosage of treated groups showed reduced mortality following *A. hydrophila* infection. Feed containing 0.5% TCM extracts was the most effective with the mortality of the fish significantly reduced by 35% compared to the control. The results indicate that propolis and Herba Epimedii extracts in combination enhances the non-specific immune response and disease resistance of Chinese sucker against *A. hydrophila*.

Keywords: Propolis; Herba Epimedii; *Myxocyprinus asiaticus*; Non-specific immune response; Disease resistance; *Aeromonas hydrophila*

* E-mail address: gsy@mail.hzau.edu.cn (S. Gong).
Effects of β-glucan, mannan oligosaccharide and their combinations on growth, immunity and resistance against *Vibrio splendidus* of sea cucumber, *Apostichopus japonicus* in vitro and in vivo

Min Gu**, Hongming Ma, Kangsen Mai, Wenbing Zhang, Qinghui Ai, Xiaojie Wang
The Key Laboratory of Mariculture (Ministry of Education), Ocean University of China, Qingdao 266003, PR China

Abstract

The immune responses of sea cucumber *Apostichopus japonicus* coelomocytes were evaluated after incubated with β-glucan, mannan oligosaccharide (MOS) and their combinations in vitro. Coelomocytes were incubated in L-15 medium containing 0, 10 and 25µg/ml β-glucan alone or in combination with 0, 40 or 80µg/ml MOS respectively. Coelomocytes were sampled at 1 h, 3 h, 6 h, 12 h and 24 h, and immune indices including phagocytosis, superoxide anion production, superoxide dismutase (SOD) activity and total nitric oxide synthase (T-NOS) activity were determined. The results showed that all the four immune indices of coelomocytes incubated with β-glucan alone and combined with MOS were significantly (p<0.05) higher than those of their respective control. MOS could increase phagocytosis, superoxide anion production and SOD activity of coelomocytes significantly (p<0.05), while no difference was observed in T-NOS activity. Synergistic effect of β-glucan and MOS was observed. Combination of 25µg/ml β-glucan and 40µg/ml MOS showed the best effects on increasing the immune indices.

In order to confirm the results in the in vitro experiment, a feeding trial was conducted. Sea cucumbers were fed with basal diets, supplemented 0, 0.075 and 0.15% β-glucan alone or in combination with 0, 0.1 or 0.2% MOS respectively. After 4-week feeding, sea cucumbers were sampled. Growth performance and resistance against *Vibrio splendidus* were determined. During the experimental period, immune indices including total coelomocytes count (TCC), phagocytosis, superoxide anion production, SOD and T-NOS activities were measured on Day 7, 11, 15, 18, 25 and 29. The growth, TCC, phagocytosis, superoxide anion production, SOD activity and infection resistance of sea cucumbers fed with β-glucan, MOS and their combinations were significantly (p<0.05) higher than those of their respective controls. Only 0.15% β-glucan and combinations of β-glucan and MOS could significantly (p<0.05) increase the T-NOS activity. Furthermore, β-glucan and MOS showed synergistic effect on growth performance, immunity and infection resistance of sea cucumbers. Combined use of β-glucan and MOS also prolonged the high levels of immune indices compared with β-glucan or MOS supplementation separately. Combination of 0.15% β-glucan and 0.1% MOS had the best effects on increasing the immune indices.

In conclusion, continuous feeding of β-glucan and MOS, especially their combinations to sea cucumber for 4 weeks showed considerable improvement in the growth, infection resistance and immune response of sea cucumbers. The results of the in vivo experiment are similar to those of the in vitro experiment, which indicate that in vitro experiment could be used as an effective and reliable method to value whether a substance has the potential as an immunostimulant.

**E-mail address:** gumin21@163.com.
Effect of High Carbohydrate Levels in the Dietary on Growth Performance, Immunity and The Expression of HSP70 of Allogynogenetic Crucian Carp (carassius auratus gibelio)

Miao Ling-hong1*, Liu Bo1,2, Xie Jun2, Ge Xian-ping1,2
1. Wuxi Fishery college, Nanjing Agriculture University, Wuxi 214081, China
2. Key Laboratory of Genetic Breeding and Aquaculture Biology of Freshwater Fishes, Ministry of Agriculture, Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences, Jiangsu Wuxi 214081, China

Abstract:
In order to study the capability of utilizing carbohydrate in the diet and the effects of different carbohydrate diets on Allogynogenetic Crucian Carp, artificial purified feed with normal (including 35% carbohydrate) and high (including 50% carbohydrate) level carbohydrate were applied. 168 individuals of Allogynogenetic Crucian Carp (35.60±1.11g) were divided into the two carbohydrate level groups, and each group has three replicates which were cultured in aquarium with automatic temperature control culturing system with recycling water for 10 weeks. After that, indicators of fish growth, immune indicators were measured, and the HSP70 expression level in liver, kidney, heart and spleen was determined by Real-time PCR. The results showed the specific growth rate, weight gain rate were significantly lower than normal carbohydrate group(35%CHO), but no significant difference on feeding rate, and feed conversion rates. The hepatic MDA content of 50%CHO group was significantly (P<0.05) higher, while the AST was significantly (P<0.05) lower. The hepatic SOD, T-AOC, ALT, and AKP was no significant (P>0.05) differences between 35%CHO group and 50%CHO group. It was showed by RealTime PCR the expression levels in liver and spleen of high carbohydrate group(50%CHO) were significantly higher than that of normal group(35% CHO) at 70d, but no significant differences in kidney and heart. Overall, crucian carp has greater capability of utilizing and tolerating carbohydrate than others, but 50% carbohydrate in dietary indeed cause some stresses to Allogynogenetic Crucian Carp (carassius auratus gibelio).

Keywords: Allogynogenetic crucian carp (carassius auratus gibelio); fish growth; immunity; HSP70

* E-mail address: miaolh@ffrc.cn.
P-196

Preliminary study of dietary Angelica polysaccharide on nonspecific immunity of grouper, *Epinephelus malabaricus*

Wang Qing-kui¹*, Chen Cheng-xun¹, Zhao Hai-yun¹, Xing Ke-zhi², Yang Yong-hai², Hu Jin-cheng²

1 Tianjin Key Laboratory of Aquaecology and Aquaculture, Fisheries Science Department, Tianjin Agricultural University, Tianjin, 300384 China
2 Tianjin Seafood Industrial Development Co. Ltd, Tianjin, 300450 China

Abstract

A preliminary study was conducted to determine the effect of dietary Angelica polysaccharide (AP) on nonspecific immunity of grouper, *Epinephelus malabaricus*. Angelica polysaccharide was added to the basal diet at 0, 50, 200, 350 and 500 mg/kg diet. Each diet was fed to duplicate groups of grouper (initial body weight 87.5 ± 12.5 g) in a recirculated rearing system for 4 weeks. Grouper were fed twice daily to near satiation. Nonspecific indices were assayed after feeding trial. Results showed that acid phosphatase (ACP) activity and complement C₄ content in plasma were increased in AP supplemented groups (p>0.05). Plasma complement C₃ content in AP supplemented groups were higher than that in control group. Complement C₃ were higher (p<0.05) in fish fed diets with 50 and 200 mg AP/kg diet than that in control group. Plasma lysozyme activity in AP supplemented groups was higher than that in control. Plasma lysozyme activity in 500 mg AP/kg diet group was significantly higher (p<0.05) than that in control. Blood leucocytes were increased in AP supplemented groups (p>0.05). Blood phagocytic ability was lowest in control group, intermediate in fish fed diets with 50 and 200 mg AP/kg diet, and highest in fish fed diet with 350 and 500 mg AP/kg diet. Differences between each of the three groups were statistically significant (p<0.01). Lysozyme activity in liver and head kidney was increased in AP supplemented groups. Lysozyme activity in liver and head kidney in 350 mg AP/kg diet group was higher (p<0.05) than that in control group.

Keywords: Grouper; Angelica polysaccharide; Nonspecific immunity

*E-mail address: wqkmail@yahoo.com.cn (Wang Qing-kui).*
Effect of injected Angelica polysaccharide on non-specific immune response in grouper, *Epinephelus malabaricus*

Wang Qing-kui¹*, Chen Cheng-xun¹, Zhao Hai-yun¹, Xing Ke-zhi², Zhang Yang¹, Wang Zhen-hui²
¹ Tianjin Key Laboratory of Aquaecology and Aquaculture, Fisheries Science Department, Tianjin Agricultural University, Tianjin, 300384 China
² Tianjin Seafood Industrial Development Co. Ltd, Tianjin, 300450 China

Abstract

A preliminary study was conducted to determine the effect of injected Angelica polysaccharide (AP) on non-specific immunity of grouper, *Epinephelus malabaricus*. The AP was extracted from radix *Angelica sinensis* in the following procedure: boiled the radix powder in water, deposited polysaccharide dissolved in water extract with 80% ethanol after filtration, deproteinized with Sevag reagent several times after decolorization with ethanol, and lyophilized. The AP powder was dissolved in PBS and i.p. injected to fish (initial body weight 80.2±5.1 g) at three dosages (5, 15 and 30 mg AP/kg body weight). Control groups were i.p. injected with PBS. Twenty fish at each dosage were sampled on day 1, 3, 5 and 7 after injection. Serum lysozyme activity in AP injected groups was enhanced on day 3, 5 and 7 and statistically significant in group 15 and 30 mg AP/kg body weight. Serum albumin/globulin ratio in 15 and 30 mg AP/kg body weight was significantly lower than that in control group after injection. Blood leucocyte phagocytic rate in AP injected groups was higher than that in control group. Leucocytes in peripheral blood in AP injected groups were enhanced on day 3, 5 and 7 after injection. Leucocytes in 15 and 30 mg AP/kg body weight group were significantly higher than that in control group on day 5 and 7. These results indicated that AP enhanced non-specific immunity of grouper and recommended i.p. injection dosage was 15 to 30 mg AP/kg body weight.

Keywords: Grouper; Angelica polysaccharide; i.p. Injection; Non-specific immunity

*E-mail address: wqkmail@yahoo.com.cn (Wang Qing-kui).*
Effects of dietary vitamin C and β-glucan on the growth performance, immunity and disease resistance of sea cucumber (*Apostichopus japonicus*)

Qin Zhang, Kangsen Mai*, Hongming Ma, Wenbing Zhang, Zhiguo Liu, Xiaojie Wang, Wei Xu

The Key Laboratory of Mariculture (Ministry of Education of China), Ocean University of China, 5 Yushan Road, Qingdao 266003, P.R. China

Abstract

A feeding experiment was conducted to investigate the effects of dietary vitamin C and β-glucan on the growth performance, immunity, and disease resistance of sea cucumber (*Apostichopus japonicus*) (initial body weight: 5.08 ± 0.08 g, mean ± SD) in a re-circulation system. The results of nine weeks feeding trial showed that dietary vitamin C had a positive effect on specific growth rate (SGR), phenoloxidase (PO) activity, respiratory burst activity, phagocytosis activity and acid phosphatase (ACP) activity when the sea cucumber were fed without β-glucan, but there was no significant difference compared to the control group (P>0.05). The ACP activity significantly increased with the increase of dietary β-glucan when feeding the vitamin C-deficiency diet, and that of the group with 625mg kg⁻¹ β-glucan was significantly higher than the control group (P<0.05). The animals fed with glucan-deficiency diets had significantly lower phagocytosis activity (P<0.05) than those fed with 625mg kg⁻¹ β-glucan when being fed with the diets with 500mg kg⁻¹ vitamin C, and the group fed with 1250 mg kg⁻¹ β-glucan has remarkably decreased the cumulative mortality after 14 days following *Vibrio splendidus* exposure in the presence of vitamin C (P<0.05). The sea cucumber fed with diets supplemented with 1250mg kg⁻¹ β-glucan and 2000 mg kg⁻¹ vitamin C has notably increased SGR, total coelomocytes counts, PO activity, respiratory burst activity, phagocytosis activity and ACP activity (P<0.05), therefore, combination of 1250mg kg⁻¹ β-glucan and 2000 mg kg⁻¹ vitamin C was the optimal combination in the present study.

Keywords: Sea cucumber; Vitamin C; Glucan; Non-specific immunity; *Vibrio splendidus*

*E-mail address: kmai@ouc.edu.cn (K Mai).*
Dietary administration of yeast culture enhances the non-specific immunity of sea cucumber *Apostichopus japonicus* and its resistance against *Vibrio splendidus*

Qin Zhang, Kangsen Mai*, Hongming Ma, Wenbing Zhang, Zhiguo Liu, Wei Xu
The Key Laboratory of Mariculture (Ministry of Education of China), Ocean University of China, 5 Yushan Road, Qingdao 266003, P.R. China

Abstract

An 8-week feeding experiment was conducted to evaluate the effects of yeast culture (Diamond V XP Yeast Culture, XP) on growth, non-specific immunity of sea cucumber (*Apostichopus japonicus* Selenka) as well as its resistance against *Vibrio splendidus*. The basal diet was used as control, the trial diets were designed with 3 added levels of XP which were mixed with the basal diet. The added levels of XP were 0.1%, 0.2% and 0.4%, respectively. Each diet was randomly allocated to triplicate groups of sea cucumber in indoor 50 l tanks with circulating seawater and constant aeration. And each tank was stocked with 30 sea cucumbers (initial average weight 3.78 ± 0.10 g). The results showed that dietary yeast culture have no influence on survival rate of sea cucumber. XP supplemented at 0.2% and 0.4% level significantly enhanced the specific growth rate of sea cucumber (*P*<0.05), whereas the lowest supplementation (0.1%) did not (*P*>0.05). No significant difference in body composition was observed among dietary treatments. Sea cucumbers fed diet with 0.4% XP had significant higher phenoloxidase activity (PO), nitric oxide synthase (NOS) activity and lysozyme (LZM) activity than that fed other diets(*P*<0.05). The respiratory burst activity (NBT), phagocytosis, LZM, NOS, superoxide dismutase (SOD) and acid phosphatase (ACP) activity in sea cucumbers fed diets with 0.2% and 0.4% XP were significantly higher when compared with control and 0.1% XP groups (*P*<0.05). However, no significant difference of the above immunological parameters was observed between control and 0.1% XP groups (*P*>0.05). The challenge experiment showed that sea cucumbers fed the diet with 0.2% and 0.4% yeast culture had significantly lower accumulative mortality compared with the control and 0.1% yeast culture groups (*P*<0.05). And no significant difference was observed between 0.2% and 0.4% yeast culture groups. These results suggested that feeding yeast culture at a dose of 0.4% could enhance growth, non-specific immunity as well as resistance against *V. splendidus* of sea cucumber (*Apostichopus japonicus* Selenka).

Keywords: XP; Growth; Non-specific immunity; Sea cucumber; *Vibrio splendidus*

*E-mail address: kmai@ouc.edu.cn*  (K Mai).
Immune response of sea cucumber *Apostichopus japonicus* coelomocytes to several immunostimulants *in vitro*

Min Gu*, Hongming Ma, Kangsen Mai, Wenbing Zhang, Qinghui Ai, Xiaojie Wang
The Key Laboratory of Mariculture (Ministry of Education), Ocean University of China, Qingdao 266003, PR China

Abstract

The *in vitro* effects of β-glucan, mannan oligosaccharides (MOS), CpG oligodeoxynucleotide (CpG ODN), lactoferrin and vitamin C on the non-specific immune response of sea cucumber *Apostichopus japonicus* coelomocytes were studied. Coelomocytes were cultured in L-15 medium containing different concentrations of β-glucan (0, 5, 25 and 100µg ml⁻¹), CpG ODN (0, 0.5, 2.5 and 5µM), MOS (0, 40, 80 and 120µg ml⁻¹), lactoferrin (0, 5, 25 and 100µg ml⁻¹) and vitamin C (0, 25, 100 and 250µg ml⁻¹). Coelomocytes with each concentration of above immunostimulants were incubated for 1 h, 3 h, 6 h, 12 h or 24 h except Vitamin C for 3 h, 6 h, 12 h, 24 h or 48 h. Coelomocytes incubated without immunostimulants were defined as control at each incubation time. After exposed to these substances for certain time, coelomocytes were sampled and immune parameters viz., phagocytosis, superoxide anion production, superoxide dismutase (SOD) activity and total nitric oxide synthase (T-NOS) activity were determined. All immune parameters were significantly (*P*<0.05) enhanced by β-glucan, MOS and CpG ODN. Lactoferrin induced significant (*P*<0.05) increase in superoxide anion production and SOD activity of coelomocytes but did not affect the phagocytosis and T-NOS activity. Vitamin C significantly (*P*<0.05) enhanced the SOD and T-NOS activities of coelomocytes although no significant effects were observed in phagocytosis and superoxide anion production. The results suggest that β-glucan, MOS, CpG ODN, lactoferrin and vitamin C can enhance the non-specific immune response of sea cucumber *in vitro*.

Keywords: Sea cucumber (*Apostichopus japonicus*); Immunostimulant; Coelomocyte culture; Immune response

*E-mail address: gumin21@163.com.*
The effects of dietary Vitamin C on the oxidative stress of juvenile olive flounder, Paralichthys olivaceus, fed cadmium

Jun-Ho Lee, Seunghyung Lee, Young Chul Kim, Jun-Young Bae, Okorie E. Okorie, Mahmoud Mohseni, Gun Hyun Park, Namyoung Hwang and Sungchul C. Bai*

Dept. of Aquaculture / Feeds and Foods Nutritional Research Center (FFNRC), Pukyong Nat’l University, 599-1, Deayeon-3-dong, Nam-gu, Busan 608-737, Korea

Abstract

The purpose of the present study was to evaluate the effect of a dietary vitamin C supplement on cadmium oxidative stress in juvenile olive flounder, Paralichthys olivaceus. The basal diet with three levels of cadmium (50, 300 and 800 mg Cd/kg) was supplemented with low (0 mg AA/kg), adequate (100 mg AA/kg) and 2× adequate (200 mg AA/kg) of Vitamin C. Nine experimental diets each were fed to triplicate groups of juvenile olive flounder (initial weight: 5.0±0.02 g) in a closed-recirculating rearing system for 8 weeks. Growth performance, Oxidative stress in tissues, cadmium accumulation in each tissues and whole-body proximate composition will be discussed later.

*Corresponding author: scbai@pknu.ac.kr
The potential role of proteinase-activated receptor 2 in soybean meal induced inflammation of the intestine of Atlantic salmon (Salmo salar)

Christian Sahlmann*, Jim Thorsen, Elin C Valen, Trond M Kortner, Åshild Krogdahl
Aquaculture Protein Centre (CoE), Department of Basic Science and Aquatic Medicine, Norwegian School of Veterinary Science, P.O. Box 8146 Dep, NO-0033 Oslo, Norway

Abstract

Atlantic salmon is a highly valued food for human consumption and increased farming calls for a development towards a more sustainable food source. Alternative protein sources from plant material gain a high interest for commercial fish feeds. Soybean meal has been found to be a good food source for various fish species because of its high protein content and favorable amino acid profile. However, when feeding Atlantic salmon with an inclusion of 20% soybean meal (SBM) in the diet the fish react with an inflammation of the distal intestine. It is still unknown what causes this inflammatory response and how the intestinal processes are altered by presence of SBM. In mammals proteinase-activated receptors (PARs) have been identified to play an important role in inflammation of the gastrointestinal system. A subgroup of the PARs, the proteinase-activated receptor 2 (PAR-2), has been shown to mediate proinflammatory effects in the colonic lumen in mammals. For fish this receptor has not received much attention. However, in an earlier study, our group identified PAR-2 in Atlantic salmon and proposed a potential role of PAR-2 in intestinal inflammation in fish. Here we present a study in which we used Real Time PCR (RT-PCR) investigated mRNA expression changes in Atlantic salmon fed a diet with an inclusion of 20% soybean meal for 21 days. The receptor was further localized with immunohistochemistry and immunogold labeling. Results showed an alteration in transcription of PAR-2 after two days of feeding. Our study indicates potential early response of PAR-2 to the plant diet but its importance for the development of enteritis is not clear. Further studies will help to understand the immune response in Atlantic salmon.

Keywords: PAR-2; Aquaculture; Immune response; Distal intestine; mRNA expression; RT-PCR; Fish farming

*E-mail address: christian.sahlmann@nvh.no
Effect of zinc on growth, non-specific immunological responses and resistance to *Streptococcus agalactiae* of sex-reversed red tilapia, *Oreochromis niloticus* x *O. mossambicus*

Kidchakan Supamattaya¹, Boonkob Viriyapongsutee¹, Jirawat Tadkaew¹, Chutima Tantikitti¹*, Wutiporn Phromkunthong¹

¹Aquatic Animal Health Research Center (AAHRC), Department of Aquatic Science, Faculty of Natural Resources, Prince of Songkla University, Hat-Yai, Songkhla, 90112, Thailand.

Abstract

The important role of trace elements such as zinc in health and nutrition has been recently realized in aquatic species. The objective of the present study was to determine effects of zinc amino complex (ZnAA) and ZnSO₄ (ZnS) on growth performance, immune responses and bacterial disease (*S. agalactiae*) resistance of red tilapia. Fish with an initial weight of 2.01±0.00 g were fed with five experimental diets in triplicate for 12 weeks. Diet 1 was the basal diet without zinc supplementation (Zn 0; dietary level 47.32 mg/kg), diet 2 supplemented with zinc amino at 60 mg/kg of diet (ZnAA 60; dietary level 87.83 mg/kg), diet 3 supplemented with zinc amino at 120 mg/kg diet (ZnAA 120; dietary level 129.49 mg/kg), diet 4 supplemented with ZnSO₄ at 60 mg/kg diet (ZnS 60; dietary level 91.63 mg/kg) and diet 5 supplemented with ZnSO₄ at 120 mg/kg diet (ZnS 120; dietary level 134.02 mg/kg). Final body weight and FCR of fish fed diet 5 were the best but liver malonaldehyde level of fish were higher than those fed diets 2 and 4 (P<0.05). No significant differences were observed for glutathione peroxidase activity, lysozyme activity and complement activity among different groups of fish. Fish fed diets 3 and 4 exhibited significantly higher phagocytic index than those fed other diets. After *S. agalactiae* challenge test by injection, fish fed with dietary zinc had higher survival rate (10 days post challenge) than those of the control group.

Keywords: Zinc; Growth; Immune Responses; Survival; Sex-reversed red tilapia

*E-mail address: chutima.1@psu.ac.th*
The combined used of nucleotides and immune-stimulant (Sanictum®) in feed against salmon rickettsia syndrome

José L. Gonzalez Vecino1*, Simon Wadsworth1, Javier Gonzalez2, Kari Ruohonen1
1 EWOS Innovation AS, Dirdal, N-4335, Norway; 2 EWOS Innovation Chile, Puerto Montt, Chile

Abstract

The provision of an exogenous source of dietary nucleotides can increase performance of rapidly-dividing tissues, particularly during critical periods. The addition of exogenous nucleotides to fish feeds is a common practice during disease challenge, rapid growth, physiological stressful periods and reproduction.

Salmon rickettsia syndrome (SRS) is caused by Piscirickettsia salmonis, a non-motile obligate intra-cellular Gram-negative bacteria. This organism occurs in marine waters and affects salmonids during the on-growing process from smolt to harvest. Efficacy of current vaccines against the disease is not satisfactory and better ways to increase survival would be beneficial. Several published articles have shown reduction in mortalities caused by bacteria, virus and parasites when feeding nucleotides. However its combination in the diet with immune-stimulants such as bacterial cell-wall extracts has not been reported against SRS.

Two tank challenge trials were carried out in Puerto Montt, Chile to test the efficacy of feeds supplemented with nucleotides and bacterial cell-wall extract (Sanictum®) against salmon rickettsia syndrome. Different groups of Atlantic salmon were fed for a period of four weeks with diets either control, including nucleotides, Sanictum at different concentrations or a combination of both before challenging against P. salmonis (i.p. injection LD50). Results showed a synergistic effect of the combination of nucleotides and immune-stimulant since survival was significantly better than feeding nucleotides or immune-stimulant separately. A 79% improved survival was achieved once the combination of nucleotides and immune-stimulant in the diet was optimized after the two trials. This level of protection is similar to current available vaccines against salmon rickettsia syndrome.

* Corresponding author: jose.g.vecino@ewos.com (José L. González Vecino)
Interaction of arachidonic acid and vitamin E on the immune response to pathogen challenge in juvenile Atlantic salmon (Salmo salar)

Patricio Dantagnan¹, ²*, Katerina Gonzáles¹, Martín Hevia³, Takahiro Ogura², Adrián Hernández¹, ², Aliro Bórquez¹, ², Javier Alcaino¹
¹Escuela de Acuicultura, Universidad Católica de Temuco, Casilla 15-D, Temuco, Chile
²Centro de Genómica Nutricional Agroacuícola (CGNA), Casilla 58-D, Temuco, Chile
³Centro Experimental Quillaípe, Fundación Chile, Puerto Montt, Chile

Abstract

The role of fatty acids on the immune function of fish has come under serious study within recent decades. However, the effect apparently depends both on the environmental conditions and on the interaction between the different types of fatty acids or other nutrients. The present study examines whether a high incorporation of arachidonic acid in the diet for juvenile rainbow trout, maintaining constant levels of EPA and DHA, might generate better immune response to a selected pathogen and the interaction with the dietary vitamin E content. Eight experimental diets were formulated containing different ratios of arachidonic acid (ARA)/vitamin E. Each diet was tested in triplicate over a period of 96 days in juvenile Atlantic salmon (Salmo salar) with an initial average weight of 6.5 grams. At the end of the feeding trial all groups of fish were subjected to a pathogen challenge test. Growth performance and immune response indicators (lysozyme activity and phagocytic activity of macrophages) were evaluated. The results indicate that an increase of 50% in the ARA content that is normally in the diet significantly improves (P <0.05) the immune response, measured as activity of macrophages, when the vitamin E level is above 180 ppm. Furthermore, cumulative mortality decreased between 22.7% and 63.7%, depending on the dietary level of vitamin E, when fish were exposed to a pathogen (SRS).

Keywords: Atlantic salmon; Fatty acids; Arachidonic acid; Vitamin E; Immune response

*E-mail address: dantagna@uct.cl (P. Dantagnan).
P-206

Fucoidan derived from seaweed give effectiveness on enhancement of non-specific immune response in Japanese flounder *Paralichthys olivaceus*

Takuma Sakurai1*, Saichiro Yokoyama2, Manabu Ishikawa2, Shunsuke Koshio2
1 Graduate School of Fisheries, Kagoshima University, 4-50-20 Shimoarata, Kagoshima 890-0056, Japan
2 Laboratory of Aquatic Animal Nutrition, Faculty of Fisheries, Kagoshima University, 4-50-20 Shimoarata, Kagoshima 890-0056, Japan

Abstract

Recently, onset of disease is a problem in aquaculture. Vaccination and medicines are used as countermeasures against viral and bacterial disease. However, problems on cost and occurrence of resistant bacteria arise. If medicines are excessively used in aquaculture, consumers may have negative impression on cultured fish resulting to lower market price. Therefore, an effective but safe countermeasure such as immunostimulant against disease should be developed. Dietary fucoidan, a sulfated-polysaccharide derived from seaweed, was confirmed to improve growth and immune responses in kuruma shrimp (*Marsupenaeus japonicus*) and *Penaeus monodon*. However, little is known about the effects of fucoidan on non-specific immune response in marine finfish. In addition, studies about dietary immunostimulant must be undertaken with clarified diet ingredient or the individual effect of substances. In this study, crude fucoidan was extracted from seaweed (*Undaria pinnatifida*) by water extraction and semi-purified with dialysis. Crude fucoidan was dissolved into physiological saline (0.85% NaCl) and orally-administrated to juvenile Japanese flounder (*Paralichthys olivaceus*, body weight= 127g) at a level of 0 (control: administrated physiological saline only) and 20mg crude fucoidan/ kg body weight. Twelve test fish in each groups were anesthetized with 200ppm 2-phenoxyethanol, and crude fucoidan suspension was forced administrated into stomach of test fish with dose levels below mentioned above. Fish were then stocked in 500L tank with flow through seawater, and sampled at 0, 3 and 7 day after administration. Non-specific immune responses such as phagocyte activity and lysozyme activity, protein content and bactericidal activity of skin mucus and blood serum were analyzed. Fucoidan administrated group showed significantly (p<0.05) higher phagocytic activity and serum lysozyme activity compared to those of control group at 7 day after the crude fucoidan administration. Fucoidan administrated group also indicated significantly (p<0.05) higher skin mucus lysozyme activity and serum bactericidal activity at 3 day after the crude fucoidan administration. Finally, results suggest that orally-administrated fucoidan enhances non-specific immune responses, and could estimate the period for it to take effect. Our next experiment will focus on the effect of crude and/or purified fucoidan supplemented in formulated diet for health status of marine finfish.

Keyword: Fucoidan; *Paralichthys olivaceus*; Non-specific immune response; Oral administration

*E-mail address: sakutaku610326@yahoo.co.jp (T. Sakurai)
In vivo and in vitro reduced gut bacterial translocation in relation to mucus production and immune activity and disease resistance in sea bass (Dicentrarchus labrax) fed mannan oligosaccharides (MOS)

Silvia Torrecillas*, Alex Makol1, María José Caballero1, Daniel Montero1, John Sweetman2 and Marisol Izquierdo1.

1Universidad de Las Palmas de Gran Canaria, Grupo de Investigación en Acuicultura (GIA) and Instituto Canario de Ciencias Marinas P.O. Box 56, 35200 Telde, Las Palmas de Gran Canaria, Canary Islands, Spain
2Alltech Aqua, Samoli, Livadi, 28200 Lixouri, Cephalonia, Greece

Abstract

The objective of this study was to determine the effect of mannan oligosaccharides derived from the outer cell wall of a select strain of Saccharomyces cerevisiae (Bio-Mos, Alltech Inc, USA) on potential mechanisms related to disease resistance such as mucus production, mucus immune activity, gut morphology and in vivo and in vitro gut bacterial translocation in European sea bass (Dicentrarchus labrax). Specimens were fed 4‰ dietary MOS level of inclusion for eight weeks. Anterior gut mucosal folds height, width and % of total surface area occupied by folds were increased after 8 week of MOS supplementation (P<0.05, n=240). Posterior gut presented shorter folds (P<0.05, n=240) but wider that those fed control diet (P<0.05, n=240) resulting in increased total surface area (P<0.05, n=240). For rectum, fish fed control diet the folds length and width were increased (P<0.05, n=240) compared to fish fed MOS diet. Gut quantitative morphological analyses showed an enhancement in the number of cells secreting acid mucins by unit of area, higher density of acidophilic granulocytes in the mucosa for fish fed MOS together with an improvement in gut mucus lysozyme activity which could be related to the significant improvement found in resistance to bacterial infection in terms of bacterial in vivo and in vitro gut translocation. No differences were found on skin mucus immune parameters. The results of this study denote the implication not only of a higher mucus production, but also of a higher immune activity in enteric mucus of fish fed MOS and their relation in prevention of bacterial traslocation and gut infections.

Keywords: Sea bass, bacterial translocation, mucus, gut morphology, flesh quality.

* E-mail address: silvia.torrecillas101@masters.ulpgc.es
Effects of dietary selenium and vitamin E on glutathione peroxidase activities in soft-shelled turtle, *Pelodiscus sinensis*

Ying-Jhih Lin*, Chen-Huei Huang
Department of Aquatic Biosciences, National Chiayi University, Taiwan

Abstract

Soft-shelled turtle, *Pelodiscus sinensis*, is a high-valued aquaculture species in Asia. This animal is considered an excellent nutraceutical in traditional Chinese medicine. Nonetheless, the nutrient requirements of this reptile have not yet been fully investigated. In experiment 1, basal diet was supplemented with 90 mg Vit E/kg. Soft-shelled turtles with initial mean body weight of 6.2 g were fed diets supplemented with 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.8, and 1.0 mg Se/kg diet for 8 weeks. The result showed that hepatic glutathione peroxidase (GPX) activity of soft-shelled turtles fed diets supplemented with 0.5, 0.8, and 1.0 mg Se/kg diet were significantly \((P<0.05)\) higher than other groups; serum GPX activities of soft-shelled turtles fed diets with 0.5, 0.8, and 1.0 mg Se/kg diet were significantly \((P<0.05)\) higher than those fed diets supplemented with 0 and 0.1 mg Se/kg. The supplemental Se level for optimal GPX activity determined by the polynomial regression model was approximately 8.6 mg Se/kg. In experiment 2, basal diet was supplemented with 20 mg Vit E/kg. Soft-shelled turtles with initial mean body weight of 4.2 g were fed this diet supplemented with 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.8, and 1.0 mg Se/kg diet for 8 weeks. The result showed that hepatic glutathione peroxidase (GPX) activity of soft-shelled turtle fed diets with 0.5, 0.8, and 1.0 mg Se/kg diet were significantly \((P<0.05)\) higher than other groups, but there is no significant difference in serum GPX activity among test groups. The supplemental Se level for optimal GPX activity for turtles fed low Vit E was 8.4 mg Se/kg determined by the polynomial regression model.

Keywords: Se; soft-shelled turtle; GPX

*E-mail address: s0970538@mail.nctu.edu.tw*
Effects of iron and zinc levels on total antioxidant capacity and nitric oxide synthase of Tilapia (Oreochromis niloticus×O. aureus)

Li-Rong Bai*, Yu-Tao Miao, Hong-Yan Kou, Li-Ping Shang, Hai-Lan Lian, Zhao-Hui Zhu, Yong-Xiang He, Jian-An Xian, Wei-Jing Zhong, An-Li Wang

Key Laboratory of Ecology and Environment Science in Guangdong Higher Education, Guangdong Provincial Key Laboratory for Healthy and Safe Aquaculture, College of Life Science, South China Normal University, Guangzhou 510631, People’s Republic of China

Abstract

A 12-week feeding trial with three dietary iron levels (28.37, 159.43, 362.71 mg/kg) and three dietary zinc levels (7.44, 23.87, 326.19 mg/kg) was conducted to determine the effects of total antioxidant capacity and nitric oxide synthase activity on tilapia. All treatments except the medium were set the pair-fed groups. The results showed that: (1) The total antioxidant capacity: according to the 4-week trial, the high iron groups were the lowest in the iron groups, the high iron pair-fed groups were the highest; The same trend can be seen in the zinc groups. In the 8-week trial, the high iron groups had the lowest value among the iron groups, the pair-fed groups with the deficient iron had the highest value; the pair-fed groups which contained high zinc were the lowest in zinc groups, and the pair-fed groups with the deficient zinc were the highest. According to the 12-week experiment, the optimal iron groups were the lowest in iron groups, the pair-fed groups with high iron were 0.3630 U/mg prot which was the highest; whereas the optimal zinc group was the lowest in zinc group, the pair-fed groups with deficient zinc was the highest which was 0.5817 U/mg prot. (2) The constitutive nitric oxide synthase activity increased up to 8 weeks, then decreased with the increase of experimental period. The activity of the fish fed the high iron pair-fed diet was the max when they were kept 4 weeks, and the lowest value appeared in the fish fed high zinc which were fed for 8 weeks. (3) The induced nitric oxide synthase activity decreased up to 8 weeks, then increased with the increase of experimental period. The activity of the fish fed the deficient iron pair-fed diet was the max when they were fed 12 weeks, and the lowest value appeared in the fish fed optimal zinc diet which were fed for 8 weeks. The max activity was 0.4787 U/mg prot, and the lowest was 0.0626 U/mg prot.

Keywords: Iron; Zinc; Total antioxidant capacity; Nitric oxide synthase; Tilapia

*E-mail address: bailirong1152@163.com (L. R. Bai)
Effect of dietary vitamin E and selenium on growth, nonspecific immune responses and disease resistance in japanese flounder (*Paralichthys olivaceus*)

Qing Chang*, Meng-Qing Liang, Jin Zhou
Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Qingdao Key Laboratory for Marine Fish Breeding and Biotechnology, Qingdao 266071, China

Abstract

An 10-week feeding trial was conducted to investigate the effects of four diets containing different combinations of selenium and vitamin E on nonspecific immune responses of japanese flounder (*Paralichthys olivaceus*) and disease resistance when challenged with *Vibrio anguillarum*. The fish were fed purifed diets with supplemented vitamin E at 60 mg/kg (designated e) or 300 mg/kg (designated E) combination with selenium at 0 mg/kg (designated s) or 3 mg/kg (designated S). Each diet was fed to triplicate groups of flounder with initial body weight of 38.5±0.15 g. After the final weighing, 10 fish were randomly taken from each aquarium and injected with *Vibrio anguillarum*. Mortality was recorded daily for 7 days and nonspecific immune parameters were monitored in the remaining fish. At the end of the experiment, weight gain and phagocytic activity in blood was significantly greater in those fish fed the E diets compared with those fed the e diets. Glutathione peroxidase activity in blood plasma was significantly higher in fish fed the S diets compared with those fed the s diets. High cumulative mortality was in fish fed the s/e diet than other dietary groups. These results indicated that no synergistic effect of excessive dietary vitamin E and selenium on growth performance, nonspecific immune responses and disease resistance to *V. anguillarum* in japanese flounder.

Keywords: vitamin E; selenium; nonspecific immune response; japanese flounder

*E-mail address:* changqing@ysffri.ac.cn (Chang Qing).
The protection of vitamin C on trichlorfon-stress in *Carassius auratus gibelio*

Wei-na Xu*, Wen-bin Liu*, Xing-xing Fang, Xian-ping Shao, Guang-zhen Jiang
College of Animal Science and Technology, Nanjing Agricultural University, Nanjing, 210095, China

Abstract

To investigate the anti-oxidative function of vitamin C on primary cultured hepatocytes in vitro and plasma immunity of *C. auratus gibelio* in vivo. The hepatocytes were cultured with media contained 0, 50, 100, 200, 400 and 800µM concentration ascorbic acid. Cell viability, the changes in hepatocytes lactate dehydrogenase (LDH) activity and vitamin C concentration and antioxidative status with trichlorfon stress were assayed. In addition, with oral administration of ascorbic acid (0, 4.5 and 9 mg·kg BW⁻¹) in vivo, plasma immune NO, (immunoglobulin M) IgM and lysozyme were determined. The results showed that compared with free-ascorbic acid group, vitamin C could increase hepatocytes viability, enhance intracellular total antioxidant capacity (T-AOC), glutathione-S-epoxide transferase (GST) and butyrylcholinesterase (B-CHE) activities and cytochrome P450 (cyt P450) concentration, when cell were cultured with 50 to 200 µM concentrations vitamin C in vitro. And immunologic function of fish was increased when oral administration of ascorbic acid was 9 mg·kg BW⁻¹.

Keywords: vitamin C; anti-oxidative stress; immunity; *Carassius auratus gibelio*

*E-mail address:2007213003@njau.edu.cn (W.N, Xu).wbliu@njau.edu.cn (W.B. Liu)
Influence of dietary probiotic Bacillus TC22 and fructooligosaccharide on growth, immune capacity, microflora and disease resistance in sea cucumber Apostichopus japonicus

Yancui Zhao*, Hongming Ma, Kangsen Mai, Wenbing Zhang, Wei Xu
The Key Laboratory of Mariculture (Ministry of Education), Ocean University of China, 5 Yushan Road, Qingdao 266003, P.R. China

Abstract
The effects of probiotic Bacillus TC22 (isolated from intestine of infected sea cucumber) and the synergistic effects of TC22 and fructooligosaccharide (FOS) on growth, immune capacity, microflora and disease resistance in sea cucumber Apostichopus japonicus were studied. Animals were fed on diet with TC22 at doses of 0, 10^7, and 10^9 CFU/g feed with or without 0.5% FOS for 56d. At the end of the feeding trial, six sea cucumbers per tank were sampled for bacterial quantification and immune indices measurement. Then all the sea cucumbers left were challenged by injecting Vibrio splendidus. The results revealed that different doses of TC22 with or without FOS had no significant influence on sea cucumber growth (P>0.05). However, along with the increasing of TC22 dosage, the phagocytosis activity, respiratory burst activity and phenoloxidase activity of sea cucumber were improved significantly (P<0.05). The counts of total viable bacteria enhanced significantly in the group fed with TC22 at dose of 10^9 CFU/g feed (P<0.05). But there was no significant difference between trial treatments and control group (P>0.05). The cumulative mortality after V. splendidus challenge decreased significantly in the groups fed with TC22 at dose of 10^9 CFU/g feed (P<0.05). Animals fed with 0.5% FOS showed higher immune response and lower cumulative mortality than the control group (P<0.05). The combination of 0.5% FOS with TC22 at dose of 10^9 CFU/g feed produced significantly positive synergistic effects on sea cucumber immune responses and disease resistance compared with control group (P<0.05). Our results confirmed the potential of TC22 as dietary probiotic and the synergistic effects of TC22 and FOS in sea cucumber.

Keywords: Apostichopus japonicus; Probiotics; Prebiotics; Immunity; Microflora

* E-mail address: yancuizh@yahoo.com.cn.
P-213

The effects of dietary glycyrrhizic acid on growth, survival and immune response of large yellow croaker, *Pseudosciaena crocea*

Houguo Xu, Qinghui Ai*, Kangsen Mai, Wenbing Zhang, Wei Xu, Xiaojie Wang, Hongming Ma, Zhiguo Liu, Jun Wang, Rantao Zuo

The Key Laboratory of Mariculture (Education Ministry of China), Ocean University of China, Qingdao, 266003

Abstract

A 70 days feeding experiment was conducted to investigate the effects of dietary glycyrrhizic acid on growth, survival and immune response of large yellow croaker *Pseudosciaena crocea* (juveniles with 9.79g mean initial body weight), in seawater floating net cages (1.0 × 1.0 × 1.5 m). Triplicate groups of 60 fish were fed to apparent satiation by hand twice daily for 10 weeks. The water temperature ranged from 22.5 to 31.5 °C, the salinity from 28‰ to 33‰ and the dissolved oxygen content was approximately 6 mg l⁻¹ during the experimental period. Glycyrrhizic acid was supplemented into the basal diet to formulate four isonitrogenous and isoenergetic practical diets containing 0.00% (the control group), 0.01%, 0.02% and 0.04% glycyrrhizic acid of dry weight, respectively. The results showed that the specific growth rate, survival rate and feed efficiency ratio showed no significant differences among dietary treatments (*P* > 0.05). The phagocytosis index of head kidney macrophage was significantly increased by the supplementation of 0.04% dietary glycyrrhizic acid compared to the control group (*P* < 0.05) and fish fed 0.04% dietary glycyrrhizic acid also showed significantly higher serum lysozyme (LYZ) activity than fish fed the control diet and diet with 0.01% dietary glycyrrhizic acid (*P* < 0.05). However, respiratory burst activity of head kidney macrophage and serum superoxide dismutase (SOD) activity were not significantly influenced by the addition of glycyrrhizic acid to the diets. The challenge experiment showed that no significant differences were observed in 7-day cumulative mortality between fish fed glycyrrhizic acid-supplemented diets and fish fed the control diet. These results suggested that dietary glycyrrhizic acid improved certain nonspecific immune responses of juvenile large yellow croaker.

Key words: large yellow croaker; *Pseudosciaena crocea*; glycyrrhizic acid; growth; immune response

*E-mail address*: qhai@ouc.edu.cn (Qinghui Ai).
Effects of inulin and Lactobacillus plantarum on growth, bacterial quantity in hepatopancreas and intestine and resistance to Vibrio harveyi of Pacific white shrimp (Litopenaeus vannamei)

Boonkob Viriyapongsutee*, Kiron Viswanath, Naraid Suanyuk, Kidchakan Supamattaya, Wutiporn Phromkunthong and Chutima Tantikitti

1Aquatic Animal Health Research Center (AAHRC), Department of Aquatic Science, Faculty of Natural Resources, Prince of Songkla University, Hat Yai, Songkhla, 90112, Thailand
2Department of Fisheries and Natural Sciences, Bodø University College 8049, Bodø, Norway

Abstract

This study was carried out to evaluate inulin in shrimp feed in order to determine the effect on growth performance as well as growth enhancement of normal flora in hepatopancreas and intestine of shrimp (Litopenaeus vannamei). Juvenile L. vannamei (4.71±0.00 g) were fed with four isonitrogenous and isocaloric diets containing inulin at levels of 0, 0.22, 0.55 and 2.22% inulin in five replicate aquaria four times daily to apparent satiation for 6 weeks. The result showed that inulin had minimal effect on growth of L. vannamei. At termination of growth studied, shrimp from each treatment were fed with the same diet and supplemented with Lactobacillus plantarum (LP) feed for 7 days. Persistence and growth of LP and Vibrio spp. were monitored at 1, 5 and 10 days. The results from fluorescence in situ hybridization (FISH) technique using DNA gene probes showed an increase of LP and a decrease of Vibrio spp. in hepatopancreas and intestine of shrimp fed with high levels of inulin supplemented feed (2.22%). After injection with Vibrio harveyi suspension, it was found that the shrimp in treatment groups showed a higher resistance to pathogenic bacteria than the shrimp in control group, and therefore a higher survival rate.

Key words: Inulin; Lactobacillus plantarum; Litopenaeus vannamei; Fluorescence in situ hybridization

* E-mail address: boonkob.v@psu.ac.th
Long-term effect of a severe n-3 polyunsaturated fatty acid-restricted diet during the juvenile period on the efficacy of a linseed oil finishing strategy and on apparent in vivo Δ-6 desaturase activity in rainbow trout (Oncorhynchus mykiss)

Xavier Rollin1*, Jonathan Brel1, Junio Dort1, Mathieu Haerinck1, Tarik Abboudi1, Yvon Carpentier2, Yvan Larondelle1

1Laboratoire de Pisciculture Huet, Institut des Sciences de la Vie, UCLouvain, route de Bloery, 2, B-1348 Louvain-la-Neuve, Belgium
2L. Deloyers Laboratory for Experimental Surgery, Erasme Hospital, Université Libre de Bruxelles, Brussels, Belgium

Abstract

The aim of the present study was to determine the effect of a 60-day severe n-3 polyunsaturated fatty acid (PUFA)-deficient diet (0.09 %) during the juvenile period on (1) the fatty acid metabolism deduced by the whole-body fatty acid balance method (Turchini et al., 2007, 2009) and on (2) the efficacy of a linseed oil finishing strategy in rainbow trout. It was hypothesized that, if fish fed a n-3 PUFA-deficient diet for a long period of time during the juvenile period, prior to the dietary shift to a finishing 18:3n-3-rich linseed-based diet, an increase in the bioconversion of 18:3n-3 and in the n-3 highly unsaturated fatty acids (HUFA) deposition should occur. Two groups of fish (initial body weight 0.70±0.01(SE) g) were reared at 11±1 °C and fed for a 60-day conditioning period on a sunflower oil diet (SO) or a linseed oil diet (LO). The SO group was then shifted to the LO diet for a 36-day finishing period. During the conditioning period, rainbow trout increased their mean weight by up to 18-fold in the LO group (Daily growth coefficient (DGC) of 2.4%/day) and only by 12-fold in the severe n-3 PUFA-restricted diet (DGC of 1.9%/day). At the end of this 60-day period, fatty acid composition of the diet was mirrored in fish whole-body tissues and fish fed the SO diet were deeply depleted in n-3 PUFA (<2% of total identified fatty acids). During the finishing period, trout increased their mean body weight up to 3.7-fold. The LO group grew at significantly higher rate than the SO group (DGC of 3.61 and 3.37%/day, respectively; P<0.01). At the end of this 36-day finishing period, the n-3 PUFA content of the fish fed the SO diet during the conditioning period was only slightly (but significantly, P<0.01) inferior (86%) to the one observed in the LO group. However, no effects of the long term n-3 PUFA deficiency during the conditioning period on desaturation/elongation pathways for n-3 HUFA synthesis, on n-3 HUFA deposition (on a weight gain basis) or on the apparent in vivo Δ-6 desaturase, Δ-5 desaturase and elongase activities were recorded. In conclusion, (1) the tested n-3 PUFA deficiency period did not stimulate the further apparent in vivo Δ-6 desaturase, Δ-5 desaturase and elongase activities and (2) the n-3 PUFA deficiency approach to stimulate the HUFA deposition from 18:3n-3 linseed oil during the finishing period has to be considered unsuccessful.

Keywords: Rainbow trout; fatty acid metabolism; linseed oil finishing diet; Δ-6 desaturase; elongase

* E-mail address: xavier.rollin@uclouvain.be (X. Rollin).
Effects of different phosphorus sources on the growth performance of juvenile black sea bream, *Sparus macrocephalus*

Ying Hua1,*, Guan Yun Zhong1, Qing Jun Shao1, Fan Zhou1, Bergo Owari Ngandzali1, Yuan Jian Xu2 and Jun Zhuo Xu2

1College of Animal Sciences, Zhejiang University, Hangzhou 310029, P.R. China
2Marine Fisheries Research Institute of Zhejiang Province, Zhoushan 316100, P.R. China

Abstract

A feeding trial was conducted to investigate the effective phosphorus source and total phosphorus levels in diet for juvenile black sea bream *Sparus macrocephalus*. Fish (initial mean weight 13.32±0.11g) were in indoors flow-through circular fiberglass tanks (350L), fed with seven isoenergetic and isonitrogenous diets containing three inorganic phosphorus sources (monosodium phosphate (NaH2PO4), disodium phosphate (Na2HPO4) and trisodium phosphate (Na3PO4)) with 2 total phosphorus levels (1.4% for T2-T4 and 1.8% for T5-T7) respectively, as well as the basal diet (0.72% total phosphorus without inorganic phosphorus) for 8 weeks. Each diet was randomly allotted to triplicate groups of 25 juvenile fish twice daily (8:00 am and 16:00 pm) to apparent satiation. The results showed that survival rate were lower in T1 group. Weight gain was improved by inorganic phosphorus supplementary except for fish in T4, while there was no significant difference for condition factor or protein efficiency ratio among treatments. Feed efficiency ratio was significantly lower in fish fed T1, T4 and T7. Intraperitoneal fat of T1 and T4 were significantly higher, and significant higher hepatosomatic index were found in fish fed T1, T3 and T4. There was no significant difference on apparent digestibility coefficients (ADCs) of dry matter among all treatments, while ADCs of crude protein and crude lipid were significantly lower in T4 and T1, respectively. Dietary treatments had significant effect on phosphorus content in whole fish body and higher value and lower value was found in fish fed T5 and T1, respectively. Phosphorus content in vertebrae, skin and scale were improved by dietary inorganic phosphorus sources supplementary, and fish fed diets with NaH2PO4 showed relatively higher values in those tissues. There was a similar change trend of mineralization and Ca/P ratios. Regarding serum characteristics, fish fed diets supplied inorganic phosphorus showed significantly decreased in glucose and triacylglycerol concentration compared with fish fed basal diet, while total cholesterol content was reduced significantly except for fish fed T3 and T4. Fish fed T1 had significantly higher alkaline phosphatase activity, while no statistically differences were observed among other groups. Based on the results in present study, NaH2PO4 was more effective phosphorus source with 1.4% total phosphorus content in diet for juvenile black sea bream.

Keywords: Phosphorus sources; Juvenile black sea bream; Growth performance; Feed utilization; Nutrient apparent digestibility

*E-mail address: yinghua324015@163.com (Ying Hua).*
Effects of dietary copper levels on growth, hematological parameters and tissue copper accumulation in juvenile olive flounder (Paralichthys olivaceus)

Mohseni, M. 1, Park, G.H. 1, Lee, J. H. 1, Lee, S.H. 1, Hwang, N.Y. 1, Okorie, O. E. 1, Liu, Y. 1, Browdy, C. 2, Shishechyan, F. 2, and Bai, S. C. 1

1Department of Aquaculture/Feeds & Foods Nutrition Research Centre, College of Fisheries Biology, Pukyong National University, Nam-gu, Busan 608-737 Korea. 2Novus International, 20 Research Park Drive, St. Charles, MO 63304 USA

Abstract

A feeding trial was conducted to evaluate the effects of dietary copper (Cu) on growth, survival, tissue copper contents and hematological parameters in juvenile olive flounder, Paralichthys olivaceus. MINTREX®, a chelate of copper and 2-hydroxy-4-methylthiobutanoic acid, as the copper source was added to the basal diet (control) and nine semi-purified diets containing graded levels of 7 (control), 10.4, 15.8, 24.9, 43.4, 82.1, 158.1, 308.1, 657.5 and 1267.2 mg Cu/kg diet (Cu7, Cu10, Cu15, Cu25, Cu42, Cu65, Cu158, Cu308, Cu655 and Cu1267, respectively). Each diet was fed to fish (initial body weight: 3.84±0.13g) in triplicate groups for 12 weeks in the semi-recirculation system. Weight gain (WG) and specific growth rate (SGR) of fish from Cu10 and Cu16 were significantly higher than those of fish from Cu82, Cu158, Cu308 and Cu1267. However there were no significant differences in WG and SGR among fish from Cu7 (control), Cu10, Cu25 and Cu43, and fish from Cu25, Cu43, Cu82 and Cu158, and among fish from Cu43, Cu82, Cu158, Cu308, Cu655 and Cu1267. Feed efficiency (FE) of fish fed Cu7, Cu10 and Cu16 were significantly higher than those of fish from Cu82, Cu158, Cu308, Cu655 and Cu1267. Mortality of fish from Cu658 and Cu1267 were significantly higher than those of fish from the other groups. There were no significant differences in mortality between fish from Cu7 (control), Cu10, Cu25, Cu43, Cu82 and Cu158. The profile of Cu accumulation among tissue in olive flounder was dependent on the exposure periods and the Cu concentrations of diets. The liver of olive flounder was the most important storage tissue, and the order of Cu accumulation in tissue was liver > kidney >gill > muscle. Based on the one-way ANOVA test and the second order polynomial analysis, the optimum dietary MINTREX® Cu level could be between 12 and 13 mg Cu/kg diet for maximum WG and survival in juvenile olive flounder. Also these results indicated that MINTREX® copper concentrations of 308 mg Cu kg⁻¹ and above caused toxic responses as evidenced by the antagonistic interaction between Fe and Zn, changes in hematological parameters, and reduced growth and whole-body energy source.

Corresponding author: Sungchul C. Bai; scbai@pknu.ac.kr
Effects of dietary carbohydrate source and level on growth, feed utilization and body composition of the humpback grouper, *Cromileptes altivelis*

Rossita Shapawi¹*, Saleem Mustafa¹, Ng Wing-Keong²

¹Borneo Marine Research Institute, Universiti Malaysia Sabah, Locked Bag 2073, 88999 Kota Kinabalu, Sabah Malaysia
²Fish Nutrition Laboratory, School of Biological Sciences, Universiti Sains Malaysia, Penang 11800, Malaysia

Abstract

The effects of dietary carbohydrate source and level on growth, feed utilization and body composition of humpback grouper (*Cromileptes altivelis*) were determined. Six isonitrogenous experimental diets were formulated to contain corn starch, tapioca starch or dextrin at 10% and 20% inclusion levels, respectively. Final weight, percentage weight gain, and specific growth rate of humpback grouper were not affected by dietary carbohydrate source and level (P>0.05). However, slightly higher growth was observed in grouper fingerlings fed 20% dietary carbohydrate compared to the fish fed 10% carbohydrate irrespective of carbohydrate source. Similarly, feed conversion ratio, protein efficiency ratio, and net protein utilization were independent of dietary treatments. Whole body proximate composition did not differ among fish fed different diets. However, muscle lipid of fish fed diets with 7.6% lipid and 20% carbohydrate was significantly lower (P<0.05) than that of fish fed diets with 12% lipid and 10% carbohydrate. In conclusion, tapioca starch performed as well as corn starch and dextrin in the diets formulated for the humpback grouper. In view of the lower price and local availability of tapioca starch compared to corn starch and dextrin, tapioca starch is the preferred source of starch in practical diets for humpback grouper.
P-219

Study on Dietary L-Lysine Requirements for Juvenile *Pelteobagrus fulvidraco*

JunMing Cao¹*, Yan Chen¹, XuanZhu¹, Yan-huaHuang¹, Hong-xia Zhao¹, Guo-li Li¹, Han-bing Lan¹, Bing Chen¹, Qing Pan²

¹Institute of Animal Science, Guangdong Academy of Agricultural Science, Guangzhou, Guangdong, China, 510642
²College of Animal Science, South China Agricultural University, Guangzhou, China

Abstract

An 8-week feeding trial was conducted to determine lysine requirement for juvenile *Pelteobagrus fulvidraco* by feeding a formulated diets supplemented with crystal L-lysine. Six iso-nitrogenous and iso-energetic diets (40.5% protein, 18MJ/kg) with fish meal and soybean protein concentrate as protein resources and fish oil and soybean oil as lipid resources were formulated and supplemented with six graded levels of crystalline L-lysine. Analyzed dietary lysine concentrations were 1.73, 2.18, 2.60, 3.13, 3.55, 4.19g/100g dry diets, respectively. Crystalline amino acid mixture was supplemented to simulate the amino acid profile found in the whole body protein of *Pelteobagrus fulvidraco*. Each diet was randomly assigned to four replicate tanks with 40 fish each tank (initial weight of 1.48±0.01g) in freshwater (28.4±0.1°C) for 8 weeks in indoor flow-through culture system. The fish were fed by hand to apparent satiation two times daily. The results indicated that final body weight (FBM), weight gain (WG), specific growth rate (SGR), feed efficiency (FE), protein efficiency (PE) increased with increasing of dietary lysine levels, and then decreased beyond the requirement level. No significant differences in survival rate were found among dietary treatments. Condition factor, crude lipid and moisture content in muscle, main composition in whole body were significantly affected by dietary lysine level, however hepatosomatic index, intraperitoneal fat weight, viscerosomatic index, lipid content in whole body, crude lipid and ash content in muscle and proximate viscera composition were not significantly influenced by dietary lysine. Total protein level in plasma was significantly affected by dietary lysine level and cholesterol, triacylglycerol, glucose content in plasma showed no significant differences for fish fed diets with different dietary lysine level, but showed the trend that protein, triacylglycerol and glucose increased with increasing dietary lysine levels, and then decreased beyond the dietary lysine level of 3.45%. The amino acid profile in the muscle except for serine and glutamic acid showed no significant differences among treatments. Isoleucine, leucine, threonine, methionine, lysine, valine, glutamic acid, tyrosine, cystine and alanine contents in plasma were significantly affected by dietary lysine level, while any other EAA and NEAA showed no significant differences in fish fed diets with different dietary lysine level.

Second-order polynomial regression model analysis on the basis of specific growth rate showed that the dietary L-lysine requirement of juvenile *Pelteobagrus fulvidraco* was 3.45% of dry diet (8.52% dietary protein).

Key words: Juvenile *Pelteobagrus fulvidraco*; Growth performance; L-lysine Requirement

E-mail: qpan@scau.edu.cn
P-220

Dietary leucine requirement of juvenile Grass carp *Ctenopharyngodon idella*

Li Luo¹, Shi Chen¹, Yun Li¹, Shi-mei Lin¹, Fu-bao Wang¹, Ya-ge Wang¹, Hua Wen²

¹ College of Animal Science and Technology of Southwest University, Chongqing, China
² The key Lab of Freshwater Ecology and Healthy Aquaculture, Chinese Academy of Fishery Sciences, Yangtze River Fisheries Research Institute, Jingzhou, China
³ Chongqing Fishery Science Research Institute, Chongqing, China

Abstract

A 60-day feeding experiment was conducted to evaluate the dietary leucine requirement of juvenile grass carp *Ctenopharyngodon idella* (10.28 ± 0.06g, mean ± S.E) using amino acid test diets (32% crude protein) containing casein and gelatin as intact protein sources and L-crystalline amino acids. Growth performance and biochemical parameters were assessed by feeding six amino acid test diets supplemented with graded concentrations of leucine (1.15%, 1.55%, 1.95%, 2.35%, 2.75% and 3.15%) to triplicate groups of juveniles to apparent satiation divided over three feedings. The fed experimental diets are at the rate of 2~4% of their body weight/day. Performance of the fish was evaluated on the basis of live weight gain (WG), feed conversion ratio (FCR), protein retention efficiency (PRE) and specific growth rate (SGR) data. Maximum live weight gain (204.64%), best FCR (1.39), highest PRE (42.70) and SGR (1.59) were recorded at 2.35g per 100g dietary leucine. Statistical analysis of live weight gain, FCR, PRE and SGR data reflected significant differences (P<0.05) among treatments. WG, FCR, PRE and SGR data were also analyzed using second-degree polynomial regression analysis to obtain more accurate leucine requirement estimate which was found to be at 2.30, 2.32, 2.34 and 2.31 g per 100 g of dry diet, corresponding to 7.18, 7.25, 7.31 and 7.22 g per 100 g of dietary protein respectively. Based on the quadratic regression analysis of the live weight gain, FCR, PRE and SGR data, the optimum requirement of juvenile grass carp for leucine is estimated to be in the range of 2.30-2.34 g per100 g of the dry diet, corresponding to 7.18-7.31 g per100 g of dietary protein.

Keywords: grass carp *Ctenopharyngodon idella*; leucine; requirement
Dietary valine requirement of juvenile grass carp *Ctenopharyngodon idella*

Ya-ge Wang, Li Luo, Hua Wen, Fu-bao Wang, Shi Chen

1 College of Animal Science and Technology of Southwest University, Chongqing, China
2 The key Lab of Freshwater Ecology and Healthy Aquaculture, Chinese Academy of Fishery Sciences, Yangtze River Fisheries Research Institute, Jingzhou, China
3 Chongqing Fishery Science Research Institute, Chongqing, China

Abstract

A 70-day feeding trial was conducted to estimate the optimum requirement of dietary valine for juvenile grass carp in fresh water cages (1.0×1.0×1.0 m) using six isonitrogenous (32% protein) with Peanut meal–casein–gelatin as protein resource supplemented with six graded levels of coated valine (from 7.3 g kg\(^{-1}\) to 22.3 g kg\(^{-1}\) of dry diet). Coated amino acid mixtures were supplemented to simulate the amino acid pattern found in the whole-body protein of grass carp except for valine. Each diet was randomly assigned to triplicate groups of 20 fish each (9.50 ± 0.3 g, mean±S.E.M.) and fish were fed three times daily at 3% body weight per day. The results showed that weight gain (WG), specific growth rate (SGR), protein efficiency ratio (PER), body composition, RNA/DNA in muscle, digestive enzyme activity, AST, ALT and GDH activity in liver and hematology were all significantly (P <0.05) affected by the dietary valine concentrations. WG, SGR, PER and RNA/DNA significantly increased with increasing valine level from 7.3 g kg\(^{-1}\) to 16.3 g kg\(^{-1}\) of diet (P <0.05), and then leveled off. Second-degree polynomial regression analysis based on WG, PER and RNA/DNA indicated the optimum requirements of grass carp for dietary valine were 15.6, 15.1 and 16.0 g kg\(^{-1}\) of diet (48.8, 47.2 and 50.0 g kg\(^{-1}\) of dietary protein), respectively.

Keywords: Grass carp *Ctenopharyngodon idella*; Growth; Requirement; Valine
Effect of dietary lipid level on growth performance, intestine digestive enzymes activity and lipid metabolism of juvenile Chinese sucker (*Myxocyprinus asiaticus*)

Chao-ming Wang¹, Li Luo¹, Shi-mei Lin¹, Gui-zhong Zhang¹, Wei-min Shang¹, Zhong Yan², Ben-xiang Liu²

1 College of Animal Science and Technology, Southwest University, Beibei, Chongqing, China 400715
2 Wanzhou Fisheries Research Institute, Wanzhou, Chongqing China  404020

Abstract

540 Chinese sucker (initial body weight 6.73±0.21g) were randomly divided into 6 treatments (90 for each treatment, 30 for each replicate) to investigate the effect of dietary lipid level on growth performance, intestine digestive enzymes and lipid metabolism of juvenile Chinese sucker by feeding diet with six graded levels of Soybean oil (2.04%, 4.43%, 6.88%, 9.02%, 11.98%, 13.39 %). After an 56-day feeding trial, the results showed that: (1) Dietary lipid level had remarkably affected the growth performance of Chinese sucker (P<0.05). At 6.88% level, the WG, SGR and RNA/DNA got the maximum, while the FCR got the minimum; (2) Dietary lipid level had remarkably affected the intestine digestive enzymes of Chinese sucker (P<0.05). With the increase of dietary lipid level, protease activity was decreased, lipase activity increased at first and then maintained at a steady state, amylase activity was observed with lipase activity (3) TL, TG, TC of serum increased and MDH, LPL decreased with increasing dietary lipid (P<0.05). The optimal dietary lipid level of juvenile Chinese sucker is 6.62%-7.02%. Protease and amylase activity were negatively correlated with lipid level. Serum lipid increased, enzyme of lipogenic and lipase of lipolysis decreased with increasing dietary lipid.

Key words: Chinese sucker; growth performance; digestive enzymes of intestine; lipid metabolism.
**P-223**

**Effects of variation in dietary protein content on growth performance and feed utilization of mature Chinese softshell turtle, *Pelodiscus sinensis***

Zhanhui Qi 1, Jun Wang 2, Zhencai Yang 3*, Jinliang Hou 3, Shifeng Zhang 3

1. South China Sea Fisheries Research Institute, Chinese Academy of Fisheries Sciences, Guangzhou 510300, China
2. Key Laboratory of Mariculture (Ministry of Education), Ocean University of China, Qingdao 266003, P.R. China
3. Hebei Normal University, Shijiazhuang, 050016, P.R. China

**Abstract**

The effects of variation in dietary protein concentration on growth performance and feed utilization of Chinese softshell turtle, *Pelodiscus sinensis*, were investigated. Turtles with an initial mean body weight of about 3 g were divided into two treatment groups and were fed with diets containing 42.69% (D43) and 32.54% (D33) crude protein, respectively, (called Group T43 and T33). The experimental turtle was subjected to a long term of acclimation. In the first phase the experiment which last for 40 days (days 1-40), the feeding regime was the same with the acclimation. In the second phase (days 41-98), T43 was randomly divided into two groups and were fed with diets D43 and D33, respectively (referred to Group T43-43 and T43-33). Similarly, T33 were divided into two groups and were fed with D43 and D33, respectively (called T33-43 and Group T33-33). The body weight and specific growth rate of turtles in T33 were significantly lower than that in T43. During 41-68 days of the second phase, turtles in Group T33-43 showed significantly higher SGR than the other groups, indicating that a compensatory growth response occurred. The feed intake, feed conversion efficiencies and apparent digestibility of protein and energy were lower for turtles in T33-43 than those in T43-43. However, during the days 41-68, T33-43 maintained the higher protein efficiency ratio, which was higher than that in T43-43. Results of the present experiment indicated that the matured *P. sinensis* possess the capability of compensatory growth. The compensatory growth was mainly due to the maintenance of a higher protein efficiency ratio.

*E-mail address: qizhanhui006@163.com; yangzc18i@hotmail.com*
Effect of dietary carbohydrate level on growth performance, body composition and selected physiology parameters of juvenile cobia (*Rachycentron canadum*)

Mingchun Ren*, Qinghui Ai, Kangsen Mai, Wei Xu, Wenbing Zhang, Xiaojie Wang, Zhiguo Liufu
The Key Laboratory of Mariculture (Ministry Education of China), Ocean University of China, Qingdao 266003, PR China

Abstract

A 9-week feeding trial was conducted to investigate the effect of dietary carbohydrate level on the growth performance, body composition and selected physiology parameters of juvenile cobia (3.38 ± 0.04g) in indoor flow-through and aerated aquaria. Six isonitrogenous and isolipidic diets containing graded levels of gelatinized cornstarch (2.5%, 6.5%, 12.5%, 18.4%, 24.2% and 30.4% of dry weight) were fed to juvenile cobia. Specific growth rate (SGR) and feed efficiency ratio (FER) both increased with increasing dietary starch level till up to 18.4% diet (*P*<0.05), and thereafter SGR declined but FER remained nearly the same. Apparent digestibility coefficient of starch reduced significantly when dietary gelatinized cornstarch up to 30.4% compared with lower starch level. Fish fed the diets with starch from 18.4% to 30.4% showed higher amylase activities in intestinal tract than those fed diets containing starch 2.5% and 6.5% (*P*<0.05). Protease activity in both liver and intestinal tract showed no significant difference (*P*>0.05) among dietary treatments. Significantly higher whole-body lipid contents were observed in fish fed the diets containing higher starch. Whole-body moisture content was inversely correlated with the whole-body lipid content, while protein and ash showed no significant differences. Plasma glucose, hepatosomatic index (HSI), liver glycogen and liver lipid increased with increasing dietary starch (*P*<0.05). On the basis of SGR and FER, the appropriate dietary starch supplementations of juvenile cobia were estimated to be 20.9% of diet and 17.2% of diet, respectively.

Keywords: Carbohydrates utilization; Gelatinized starch; Cobia; Growth performance; Feeding and nutrition

*E-mail address: chuntian1981@sohu.com*
Comparison of morphometrical parameters, nutritional composition and A/E ratios between *Pelteobagrus fulvidraco* and *Erythroculter dabryi*

XY. Tan, Z. Luo, WJ. Ye, YD. Chen  
Key Lab of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education,  
Fishery College, Huazhong Agricultural University, Wuhan 430070, China

**Abstract**

The present experiment was undertaken to compare the differences of morphometrical parameters, nutritional composition and A/E ratios between *Pelteobagrus fulvidraco* and *Erythroculter dabryi*. *P. fulvidraco* was characterized by high viscerosomatic index, hepatosomatic index, intraperitoneal ratio and gonadosomatic index than in *E. dabryi* (*P* < 0.05). The fat contents of whole body, muscle, ovary for *P. fulvidraco* were higher than that of *E. dabryi* (*P* < 0.05); protein contents of whole body and ovary in *E. dabryi* were higher than in *P. fulvidraco* (*P* < 0.05). Some differences existed in fatty acid profiles of muscle and ovary of two fish species (*P* < 0.05). Amino acid compositions of whole body and muscle were similar between *P. fulvidraco* and *E. dabryi*. However, relative variation existed in amino acid profiles in ovary for the two fish (*P* < 0.05). The A/E ratios of whole body, muscle were similar between the two species and there were wide variations in A/E ratios based on ovarian amino acid profiles. The present study provided important information of nutritional composition and the estimated requirements for essential amino acids calculated from A/E ratios of whole body, muscle and ovary for the two fish species examined.
Effects of dietary lipid levels on growth performance, nutrient digestibility and blood biochemical indices of gift tilapia (*Oreochromis niloticus*)

WANG Ai-Min1,2,3 HAN Guang-Ming1 LV Fu2 FENG Gong-Neng2 YU Ye-Bing YANG Wen-Ping2 GUO Jia-Hao2 WANG Tian1 X U P a o1,3*
1. Wuxi College of Fisheries; Nanjing Agriculture University, Wuxi 214081; 2. Department of Ocean Technology, Yancheng Institute of Technology, Yancheng 224051; 3. Key Laboratory of Genetic Breeding and Aquaculture Biology of Freshwater Fishes, Ministry of Agriculture, Wuxi 214081

Abstract

In order to determine the optimal levels of the lipid of GIFT strain Nile tilapia (*Oreochromis niloticus*), 630 GIFT with average weight 2.63±0.16g were randomly divided into six groups (1.73% lipid, 3.71% lipid, 5.69% lipid, 7.67% lipid, 9.64% lipid and 16.55% lipid) randomly. One with triplication was the control group fed with basal diet (1.73% lipid), the others with triplication were the treated groups fed with basal diet (1.73% lipid) supplemented with 2%, 4%, 6%, 8%, 15% fish oil, respectively. After rearing 90d, the growth, feed conversion ratio, nutrient apparent digestibility and blood biochemical parameter were determined. Results showed, along with dietary lipid level raising, weight gain rate and specific growth rate had a trend of going up first then falling down (*P*<0.05), protein efficiency ratio improved (*P*<0.01) and feed conversion ratio reduced (*P*<0.01). Second-order regression of weight gain rate on concentrations of dietary lipid indicates that the optimal dietary lipid for maximal growth of GIFT tilapia is about 9.34%. Crude protein and dry-matter digestibility had no significant effected by dietary lipid levels (*P*>0.05). Crude lipid and phosphorus digestibility raised significantly with dietary lipid level increasing (*P*<0.05). Albumin and albumin/globulin ratio in 1.73% group (this group did not added fish oil) had significantly higher than other group (*P*<0.05). Along with dietary lipid level raising, cholesterol and alkaline phosphatase of fish serum had raised significantly (*P*<0.01). Dietary lipid level had significantly influence on blood glucose (*P*<0.05), and no significantly influence on the content of triglyceride and the activities of glutamic-pyruvic transaminase and glutamic-oxaloacetic transaminase (*P*>0.05). It indicated that the level of lipid in the dietary could improve the growth, the apparent digestibilities of crude lipid and phosphorus to some degree, but too high lipid of the dietary may be negative effects of fish growth and immune ability in GIFT. So 7.67%—9.34% lipid of the dietary are the optimal levels of GIFT Seedlings in aquaculture.

Key words: Genetic improvement of farmed tilapia (GIFT); Lipid requirement; Growth performance; Apparent digestibility; Serum biochemical parameter
Effect of dietary iron levels on growth, body composition and intestinal enzyme activities of juvenile Jian carp (*Cyprinus carpio* var. Jian)

Lin Feng¹, ², Juan Ling¹, Yang Liu¹, ², Jun Jiang¹, ², Wei-Dan Jiang¹, ², Kai Hu¹, ², Shu-Hong Li¹, Xiao-Qiu Zhou¹, ²*

¹Animal Nutrition Institute, Sichuan Agricultural University, Sichuan, Ya’an, 625014, China
²Key Laboratory for Animal Disease-Resistance Nutrition of China Ministry of Education, Sichuan Agricultural University, Sichuan, Ya’an, 625014, China
*Correspondence: Xiao-Qiu Zhou, Animal Nutrition Institute, Sichuan Agricultural University, Ya’an 625014, China.

Abstract

A sixty-day feeding trial was carried out to investigate the effect of iron on growth, body composition and digestive enzyme activities. Diets with seven levels of iron (53.9, 90.0, 115.6, 146.1, 176.0, 215.8 and 266.0 mg iron kg⁻¹ diet) were fed to Jian carp (initial weight 11.4 ± 0.0 g). Percent weight gain (PWG), feed efficiency ratio (FER) and protein efficiency ratio (PER) were the lowest in fish fed the basal diet (*P* < 0.05). Body protein content increased with the increasing iron levels (*P* < 0.05), while moisture, lipid and ash of fish were not significantly affected by dietary iron levels (*P* > 0.05). Activities of trypsin, lipase, α-amylase, Na⁺, K⁺-ATPase, alkaline phosphatase (AKP) and gamma-glutamyl transpeptidase (γ-GT) were improved with increasing dietary iron levels. Serum iron were significantly enhanced with dietary iron levels up to 146.1 mg iron kg⁻¹ diet, and plateaued thereafter. In conclusion, iron improved digestive enzyme activities of juvenile Jian carp and the dietary iron requirement for serum iron of juvenile Jian carp (11.4 - 64.0 g) was 147.4 mg iron kg⁻¹ diet with ferrous fumarate as the iron source.

Keywords: iron, juvenile Jian carp (*Cyprinus carpio* var. Jian), growth, digestive enzyme activities

Tel: +86-835-2885157; Fax: +86-835-2885968;
E-mail: xqzhouqq@tom.com
Study on the specific growth rate and changes of digestive enzyme activities of the juvenile soft-shelled turtle, *trionyx sinensis*

Yurong Zou¹², Kangsen Mai¹*, Qinghui Ai¹
¹ The Key Laboratory of Mariculture, Education Ministry of China, Ocean University of China, Qingdao 266003, China
² Hangzhou Wensli Biology Science and Technology Stock Co., Ltd, Hangzhou 311100, China

Abstract

The present study was conducted to determine the specific growth rate and changes of digestive enzyme activities of the juvenile soft-shelled turtle (T. japonicus) from hatching to 30 d of age. The results showed that the activity of pepsin was detected in the stomach and the amylase and lipase were detected in the intestinal tract in the 1st day after hatching. The juvenile soft-shelled turtle obtained rapid growth and weight gain during the development. The digestive function was gradual improvement with the physical growth, and the main digestive enzyme activities such as pepsin, amylase and lipase changed significantly.

Keywords: soft-shelled turtle, amylase, lipase, protease, growth

*E-mail address: Kmai@ouc.edu.cn*
Effect of wheat on the growth performance of grass carp (Ctenopharyngodon idellus)

Zhang Jun¹, Ye Yuan-tu*, Cai Chun-fang, Yin Xiao-jiang, Jin Su-ya, Yin Yong-feng
1 Preclinical Medicine and Biological Science College of Soochow University, Suzhou 215123

Abstract:
The present study was to compare and investigate the growth performance and liver glycogen of grass carp with dietary of wheat and expanded wheat, different addition of wheat or expanded wheat, the levels of 32% wheat or expanded wheat supplements of the 2% soybean oil or not. Grass carps (the initial average weight was 22.29 g) were randomly divided into 6 treatments which contained 3 replicates and 15 grass carps for each replicate. And six dietaries which contained 16% wheat, 32% wheat with not oil, 32% wheat, 16% expanded wheat, 32% expanded wheat with not oil and 32% expanded wheat were designed. The cultural time was 60 days. The result showed that: ① under the conditions of 16%, 32% and 32% oil-free, the SGR of the wheat groups were 6.34%, 3.55% and 5.56% higher (P>0.05), respectively, relative to the expanded wheat groups; the FCR of the wheat groups were 7.80%, 9.27% lower, and the PER were 8.51%, 7.93% higher, respectively, relative to the expanded wheat groups, under the two conditions of 32% and 32% oil-free (P>0.05); the body indexes and nutrient composition of grass carp had no significant difference; under the conditions of 32%, the liver glycogen of wheat group was 29.13 mg/g lower (P<0.05) , relative to the expanded wheat group. ② the SGR, FCR, PER, body indexes, nutrient composition and liver glycogen of grass carp had no significant difference between the 32% and 32% oil-free groups of wheat or expanded wheat. ③ when the wheat and expanded wheat were increased from 16% to 32% in the dietaries: the SGR of grass carp were 3.42%, 0.71% lower, respectively (P>0.05); the fullness of grass carp had a falling trend and the weight of the Viscus and Liver had a increasing trend; the body protein content was significantly lower and body fat content was significantly higher (P<0.05); the liver glycogen of grass carp was 30.45 mg/g higher significantly(P <0.05), when the expanded wheat was increased from 16% to 32% in the dietaries. The results suggest that: ① in the grass carp feed, utilization effect of wheat is better than expanded wheat. ② The suitable application amount of wheat and expanded wheat in grass carp feed was 16%, and the excessive use of the wheat or expanded wheat could reduce the culture efficiency of grass carp. ③ grass carp could transform wheat starch into fat better, and no fat added in the 32% level of wheat or expanded wheat dietary had no significant effect on the growth performance of grass carp.

Keywords: Wheat; Expanded wheat; Growth; Grass carp (Ctenopharyngodon idellus)

*E-mail address: yeyuant@pub.sz.jsinfo.net
Effects of Corn, Wheat and Cassava on Growth of *Ctenopharyngodon idella*

Ying Xiao-jing\(^1\)  Ye Yuan-tu\(^1\)  Jin Su-ya\(^1\)  Xiang Chao-lin\(^1\)  Qiu Yan\(^1\)  Zang Jun\(^1\)

1 Preclinical Medicine and Biological Science College of Soochow University, Suzhou 215123, China.

Abstract

(Abjective)The ability of *Ctenopharyngodon idella* to use corn, wheat and cassava was evaluated in terms of growth indices. (Method)Triplicate groups of fish (initial weight, \((4.62\pm0.35)\) g) were hand fed visual satiety one of eight diets containing 15% corn, 30% corn, 30% corn (lack of soybean oil), 15% wheat, 30% wheat, 30% wheat (lack of soybean oil), 15% cassava, and 20% cassava for 76 days in flush-out aquarium system. The results showed that: (Results) ⑴ There was no significant difference in specific growth rate, feed efficiency between corn and wheat with the uniform level; fish fed the 15% cassava diets had significantly \((P<0.05)\) worse specific growth rate compared to those fed the 15% corn diets, fish fed the 15% cassava diets had significantly lower \((P<0.05)\) feed efficiency and higher \((P<0.05)\) blood suger compared to those fed the 15% corn or wheat diets. ⑵ Compared with the 15% groups, specific growth rate of 30% corn and wheat significantly decreased by 3.57% \((P<0.05)\) and 3.64% \((P<0.05)\), respectively; protein deposit rate decreased by 4.68% and 0.47%, respectively; lipid deposit rate significantly increased by 30.83% \((P<0.05)\) and 13.54% \((P<0.05)\), respectively; glycogen significantly increased by 173.97% \((P<0.05)\) and 117.42% \((P<0.05)\), respectively. Compared with the 15% cassava, specific growth rate of 20% cassava group increased by 1.85%, feed conversion ratio decreased by 7.89% \((P<0.05)\), protein efficiency ratio, lipid deposit rate and glycogen significantly increased by 7.23% \((P<0.05)\), 37.62% \((P<0.05)\) and 96.18% \((P<0.05)\), respectively. ⑶ Compared with 30% normal groups, specific growth rate of 30% corn and wheat groups which lack of soybean oil significantly decreased by 4.17% \((P<0.05)\) and 1.89% \((P>0.05)\), respectively; feed conversion ratio increased by 12.68% \((P<0.05)\) and 4.73%, respectively; protein efficiency ratio decreased by 12.62% \((P<0.05)\) and 5.11%; protein deposit rate decreased by 13.04% \((P<0.05)\) and 7.08%, respectively; glycogen significantly decreased by 16.87% \((P<0.05)\), 23.87% \((P<0.05)\), respectively. (Conclusion)These results indicated that: ⑴ There was no significant difference in growth indices between corn and wheat, both of them were better than cassava. ⑵ The level of corn and wheat should be lower than 30%, while the cassava can be higher than 20%. ⑶ Corn and wheat can spare some lipid, however, they can not instead of lipid.

Keywords: Corn; Wheat; Cassava; *Ctenopharyngodon idella*

*E-mail address: yeyuant@pub.sz.jsinfo.net*
**P-231**

**Effect of Different Protein Level on Growth and Survival of the *Catla catla* (Hamilton) Reared in glass aquaria**

Department of Fresh Water Biology and Fisheries, University of Sindh, Jamshoro.

**Abstract**

To study the effect of different protein levels on growth and survival of *Catla catla* (Hamilton) reared in glass aquaria during May to August 2009. Three iso-caloric feeds were formulated with locally available ingredients (rice protein, rice bran and wheat bran) of different protein levels such as 30%, 35% and 40% (dietary protein levels) of 2 mm dia were prepared with the help of manually operated pellet machine. The feed ingredients were tested for proximate (bio-chemical composition) analysis according to the methods given in AOAC (1980) and found 13%, 12% and 40% protein respectively. Each feed was supplied at a rate of 8% of the body weight of fish twice a day. The results of the various growth parameters like suitability of protein level requirement, specific growth rate, mean total weight gain, percentage weight gain, feed conversion ratio, survival rate and production of the experimental fish showed significantly (p<0.05) highest growth and production was observed in feed B followed by feed C while significantly (p<0.05) lowest growth and production was recorded from feed A. It is therefore concluded that the feed with 35% gross protein found to be suitable for the better growth and production of major carps.

**Key words:** Growth and Survival, Suitability of Protein, *Catla catla* and Glass aquaria

*E-mail address:* bushraannya@yahoo.com
P-232

Effect of dietary protein and energy levels on growth and feed performance of juvenile orange-spotted grouper, *Epinephelus coioides*

B. Ahmadi¹*, J. G. Marammazi², P. Kochanian¹, V. Yavari¹, and M. Kazem

¹Department of Fisheries, Khoramshahr Marine Science and Technology University, Khoramshahr, Iran
²South Iranian Aquaculture Research Center, Ahvaz, Iran

Abstract

In order to evaluate the protein and energy requirement of orange-spotted grouper, *Epinephelus coioides* fingerlings, nine semi-purified experimental diets containing 3 levels of crude protein (40%, 50% and 60%) and 3 levels of digestible energy (DE, 14, 16 and 18 KJ/gr), in a flow-through system (2 Lit/min) at 24.32±1.11°C were tested. For 8-weeks, Triplicate groups of 20 fish (16.79±.4 gr) were each stocked in 300 L circular polyethylene tanks and were fed the experimental diets to approximate satiation, twice daily. Results showed that the survival rate, hepatosomatic index (HSI) and condition factor (CF) were independent of the dietary treatments, but final weight, weight gain, specific growth rate (SGR), feed efficiency (FE), feed conversion ratio (FCR), protein efficiency ratio (PER) and daily feed intake (DFI) were significantly affected by protein and digestible energy levels. Diet 5 (50%CP and 16 KJ/gr DE) was the preferred diet and resulted in the best growth performance, feed and protein efficiency and nutrient utilization among the examined diets. DFI was the lowest for diet with the highest protein (60%) and energy (18 KJ/gr). Apparent net protein utilization (ANPU) varied insignificantly (P>0.05) between the treatments. Viscerosomatic index (VSI) increased significantly with increasing dietary energy concentrations.

Key words: *Epinephelus coioides*; Protein; Energy; Growth; Feed utilization

*E-mail address:banafsheahmadi19@gmail.com*
In vitro pH-stat determination of protein digestibility in fish species: recovery and standardization of enzyme extracts for acid and alkaline hydrolysis assays in juvenile rainbow trout and Nile tilapia

Daniel Lemos¹*, Fanny Yasumaru¹
¹Marine Aquaculture Laboratory, Department of Biological Oceanography, University of São Paulo, São Paulo, Brazil.

Abstract

Almost half of aquaculture production is represented by finfish. Nutrition of most fish species is partially or totally dependent on compound aqua feeds manufactured under cost-efficiency principles. The challenging predicted scenario of accessibility and cost of feed ingredients suggests the need for strategic use of different raw materials and flexible feed formulations taking into account nutrient content and availability. Thus, the manufacturing of aqua feeds may depend on assessing nutritional information at industry convenience. In vitro methods based on the digestion of feedstuffs by standardized enzymes extracted from the target species has shown applicable by the livestock industry and promising in the prediction of nutrient digestibility in fish and crustacean. The present study reports the development of an in vitro pH-stat routine to quantify protein digestion in stomached fish species, using carnivorous (rainbow trout, Onchorhynchus mykiss) and omnivorous (Nile tilapia, Oreochromis niloticus) species as models. It aims to set the basis for application of enzyme technology in the quality control of feedstuffs for fish culture. Crude enzyme extracts were recovered from stomach, pyloric caeca and intestine of individuals at different weights (100, 300, 400, 700g), nutritional status (fed or unfed) and rearing systems (trout: clear water tank; tilapia: fertilized pond or cage in reservoir). The pH of stomachs, pyloric caeca and intestines was determined prior to excision. Organs were cleaned from residual food and enzyme extracts were obtained after homogenization in distilled water (1:2 or 1:4, w/v). The degree of protein hydrolysis (DH, %) was determined in a pH-stat reaction (25°C, 60 min.) at pH 2.0 (stomach enzymes) and 8.0 (pyloric caeca and intestine enzymes) with haemoglobin and casein as substrates (80 mg protein), respectively. Since protein hydrolysis implies in pH shift, DH is proportional to the volume of 0.1 N acid (HCl) or alkali (NaOH) expended to maintain the reaction at established pH. Different enzyme:substrate ratios were tested both for acid and alkaline hydrolysis: 0.625, 2.5, 7.5 and 12.5 µL/mg protein. Stomach mean pH corresponded to 3.2 and 3.8 in trout and tilapia, respectively. No clear pH difference was found in pond versus cage culture tilapia. Stomach pH showed higher in fed compared to fasted individuals either for trout or tilapia. In contrast, no effect of nutritional status upon pH was detected in pyloric caeca and intestine. Both stomach and pyloric caeca/intestine enzyme extracts produced significant DH. Regressions of DH versus enzyme:substrate ratios were logarithmically adjusted for both acid and alkaline assays, suggesting DH near maximum at 7.5 µL/mg protein, either for trout or tilapia. No major effect of fish weight was verified upon DH. The DH was slightly higher in pond compared to cage reared tilapia for stomach (fed and unfed) and intestine (unfed) hydrolysis. On the other hand, fed individuals from cages showed increased DH compared to pond tilapia. Overall, unfed trout and tilapia tended to show higher DH than fed individuals in both acid and alkaline media. Further development of the in vitro procedure include DH analysis with practical feed ingredients including stomach, pyloric caeca/intestine enzymes as well as a simplified simulation of live animal digestion by alkaline DH determination in samples pre-hydrolyzed with stomach enzymes. These data could then be related to in vivo outputs of fecal digestibility under standardized experimental protocols

Keywords: fish; In vitro; Protein; Digestibility; Development

* E-mail address: dellemos@usp.br (D. Lemos).
P-234

The effects of total dietary C_{18} PUFA on growth performance and fillet fatty acid composition of rainbow trout (*Oncorhynchus mykiss*) and subsequent fatty acid wash-out using a finishing period

Thanongsak Thanuthong\(^1\), David S. Francis\(^1\), Paul L. Jones, Shyamalie P. S. D. Giovanni M. Turchini\(^1\)

\(^1\)School of Life and Environmental Sciences, P.O. Box 423, Deakin University, Warrnambool, Victoria 3280, Australia

Abstract

Due to environmental and economic concerns, fish oil replacement with readily available terrestrial alternatives is an increasingly investigated area of research. Readily available alternative terrestrial oils are characterised by different fatty acid compositions, and it is envisaged that more effective utilisation practices of such raw materials can be obtained by a better understanding of the effects of different dietary fatty acid classes. In particular, C\(_{18}\) polyunsaturated fatty acids (PUFA), the two most important of which are \(\alpha\)-linolenic acid (ALA, 18:3n-3) and linoleic acid (LA, 18:2n-6), play crucial roles in fish fatty acid metabolism, being essential and precursors of the more unsaturated and biologically active long chain PUFA (LC-PUFA). In this study, the effects of total dietary C\(_{18}\) PUFA on growth performance, tissue fatty acid composition and fatty acid profile restoration were investigated in rainbow trout (*Oncorhynchus mykiss*). Fish were fed with one of five diets containing different amounts of dietary C\(_{18}\) PUFA, but all formulated to have a constant ratio of ALA/LA (1/1) for 91 days, and then shifted to a fish oil diet for 35 days. A fish oil-based diet was also included as a control treatment. The diets: PUFA15, PUFA30, PUFA40, PUFA55 and PUFA65 contained a blend of sunflower oil, linseed oil and beef tallow and 15, 30, 40, 55 and 65\% of C\(_{18}\) PUFA, respectively. The C\(_{18}\) PUFA level did not affect the growth performance or proximate composition of rainbow trout. Fish receiving the experimental diets had significantly lower levels of the health promoting n-3 LC-PUFA (EPA and DHA) and higher levels of less beneficial C\(_{18}\) PUFA (ALA and LA) in their tissues. With respect to the fatty acid wash-out period, rainbow trout fed higher inclusion rates of C\(_{18}\) PUFA required a longer period of time for fatty acid profiles to return to levels similar to fish continuously fed FO based diets, suggesting that excessive dietary level of C\(_{18}\) PUFA can be detrimental to the overall quality of the final product. The percentage of recovery of EPA and DHA after fish fed wash-out FO diet for 35 days was up to 52.46 and 74.58, respectively.

Keywords: Fish oil replacement; \(\alpha\)-linolenic acid; ALA; 18:3n-3; Linoleic acid; LA; 18:2n-6; finishing

* E-mail address: df francis@deakin.edu.au (D. S. Francis).
Efficacy of ALA/LA ratio on growth performance and fillet fatty acid profile of Murray cod and subsequent fatty acid restoration by a finishing diet

Shyamalie D. Senadheera*1, David S. Francis1, Thanongsak Thanuthong1, Giovanni M. Turchini1
1School of Life and Environmental Sciences, P.O. Box 423, Deakin University, Warrnambool, Victoria 3280, Australia

Abstract

Effective fish oil replacement in aquaculture continues to prove problematic and research in this area remains a high priority. Alternative terrestrial oils are typically characterised by different overall fatty acid compositions, and, more specifically, different C18 polyunsaturated fatty acid (PUFA) contents. These C18 PUFA are commonly referred to as essential fatty acids (EFA), namely α-linolenic acid (ALA, 18:3n-3) and linoleic acid (LA, 18:2n-6). ALA and LA are precursors of the longer chain and more biologically active unsaturated fatty acids, such as EPA (20:5n-3), DHA (22:6n-3) and ARA (20:4n-6), which greatly impact on fish metabolism and final product quality. The present study evaluated the effects of varying ALA/LA ratios on the growth performance and fillet fatty acid composition of Murray cod (Maccullochella peelii peelii), and subsequent fatty acid restoration during finishing period on a fish oil-based diet. Fish were subjected to five diets containing specifically formulated blends of sunflower oil, linseed oil and beef tallow to obtain graded ALA/LA ratios of 0.31 (T1), 0.58 (T2), 0.97 (T3), 1.60 (T4), 2.87 (T5) and a fish oil control diet (CD) for a 74 day period. Subsequently, fish were reverted to CD for 42 days. No significant differences in growth were observed between treatments although Murray cod receiving alternative oil diets were smaller than fish reared on the CD (P > 0.05). There were notable reductions (P < 0.05) recorded in EPA and DHA fillet composition in treatments T1 - T5 and the general fatty acid make-up reflected that of the dietary treatment. By implementing the whole-body fatty acid balance method, the overall fatty acid metabolism was assessed, and higher fatty acid β-oxidation activity was recorded in T5 compared to T1 and CD, while in general, with the exclusion of CD treatment, the Δ-6 desaturase activity was unaffected amongst treatments with different ALA/LA ratios. During the finishing period, fish reared on diets containing high ALA/LA ratios exhibited higher rates of compensatory growth and, in particular, treatments T3-T5 demonstrated a more effective recovery rate of EPA, DHA and ARA during the course of the finishing period, compared to others.

Keywords: ALA/LA ratio; finishing; Vegetable oil; EPA; DHA

* E-mail address: dfrancis@deakin.edu.au (D. S. Francis).
P-236

Reduction of protein in diets for hybrid catfish (Clarias macrocephalus x Clarias gariepinus) with special focus on amino acid supplementation

Dhanapong Sangsue1*, Orapint Jintasataporn2, Oratai Triwutanon3, and Andreas Lemme4

1 Evonik Degussa (SEA) Pte. Ltd., 3 International Business Park, #07-18 Nordic European Centre, Singapore 609927
2 Kasetsart University, Faculty of Fisheries, Department of Aquaculture, Bangkok, Thailand
3 Kasetsart University Kampangsan Campus, Faculty of Agriculture at Kampangsan, Department of Animal Science, Nakornprathom, Thailand
4 Evonik Degussa GmbH, Rodenbacher Chaussee 4, 63457 Hanau, Germany

Abstract

The aim of the catfish experiment was to investigate the impact of dietary protein reduction when amino acids were balanced with supplementation of DL-Methionine, L-Lysine HCl and L-Threonine according to a target amino acid profile. In addition, the effect of an extra dose of supplemental DL-Methionine to the low protein diet was examined. In the 90 d-experiment 240 hybrid catfish (Clarias macrocephalus x Clarias gariepinus) with an average body weight of 30.7g were equally distributed to 16 tanks containing 1m3 water. Water temperature was 27.0±2.0°C and dissolved oxygen was >6.0mg/l in the closed system. Fish were fed until satiation twice a day. The experiment comprised four dietary treatments fed to four tanks each. Treatments included A) a control with 31.4% crude protein (1.99% Lys, 0.59% Met, 1.04% Met+Cys, 1.29% Thr; analysed), B) a treatment with 28.2% crude protein supplemented with amino acids (1.80% Lys, 0.56% Met, 0.96% Met+Cys, 1.20% Thr), C) a treatment with 26.0% crude protein supplemented with amino acids (1.86% Lys, 0.60% Met, 0.95% Met+Cys, 1.24% Thr) and D) diet C with an extra dose of 0.3% supplemental DL-Methionine (1.78% Lys, 0.83% Met, 1.18% Met+Cys, 1.20% Thr). Diets consisted mainly of soybean meal, rice bran, rapeseed meal and tapioca but also 7.5% fishmeal and 5.0% bloodmeal were used. Significant differences (p<0.05) were identified by Duncan’s multiple range test and differences are indicated by different superscripts. Final body weights (108.2a, 105.5a, 93.6b, 100.9ab g/fish of treatments A, B, C, D) suggested that protein reduction to 26.0% resulted in decreased performance. A similar pattern applied for feed conversion ratio (1.2a, 1.2a, 1.4c, 1.3b) whereas protein efficiency (g weight gain/g protein intake) was highest in treatments B and D (2.7b, 2.9a, 2.7b, 3.1a). Survival rate decreased at lowest dietary protein level (diet C) but an extra dose of DL-Methionine recovered survival to that of treatments A and B (95.0ab, 98.3a, 85.0b, 96.7a %). This might indicate that metabolic processes were severely disturbed with diet C which could be alleviated by extra methionine. Dietary treatments did not affect carcass composition. As dietary methionine levels were similar in diets A, B, and C it might be speculated whether relatively low methionine and cystine levels partly accounted for the observed performance depression. Data suggest that dietary protein can be reduced by 3 percentage points without impacting hybrid catfish performance. However, further reduction in dietary protein requires special consideration of dietary methionine supply.

* Email address: dhanapong.sangsue@evonik.com (D. Sangsue).
Fishmeal reduction in diets for sex-reversed red hybrid tilapia (Oreochromis spp.) with focus on amino acid supply

Orapint Jintasataporn¹, Prathak Tabthipwon¹, Dhanapong Sangsue²” and Andreas Lemme³
¹ Kasetsart University, Faculty of Fisheries, Department of Aquaculture, Bangkok, Thailand
² Evonik Degussa (SEA) Pte. Ltd., 3 International Business Park, #07-18 Nordic European Centre, Singapore 609927
³ Evonik Degussa GmbH, Rodenbacher Chaussee 4, 63457 Hanau, Germany

Abstract

Diets reduced in fishmeal are well established in sex-reversed red hybrid tilapia for certain production systems including pond housing. However, for the more intensive cage system, rather high fishmeal inclusions are still used. The aim of the present 90d-experiment was to investigate the effects of a dietary fishmeal reduction from 10% to 3% in sex-reversed red tilapia (Oreochromis spp.). A total of 160 tilapia with an average initial weight of 138 g/fish was equally distributed to 16 tanks of 700 L each (10 fish/tank). Temperature was 27.0 ± 2 °C and dissolved oxygen was >5.0 mg/L. Four replicate tanks were assigned to one of four dietary treatments comprising A) a positive control containing 10% fishmeal (0.67% apparent digestible methionine, ADMet), B) the negative control, a diet in which fishmeal was reduced to 3% but soybean meal was increased from 42 to 53% (0.44% ADMet) C) the diet B with 0.25% supplemental DL-Methionine (0.67% ADMet) and D) the negative control with 0.55% supplemental DL-Methionine (0.97% ADMet). Floating extruded feed was fed three times a day in a closed system. According to a feeding plan, daily quantities ranged between 3 and 4% of body weight. If necessary, uneaten feed was collected one hour after feeding. Quantities were adjusted every two weeks after weighing. No significant (p<0.05) effects were observed among the performance criteria and overall survival was between 98 and 100%. However, over the entire experimental period average daily feed intake of treatments A, B, C, D (4.01, 3.51, 3.48, 4.09 g/fish/d) suggested that high DL-Methionine supplementation allowed for similar feed intake compared to positive control. This effect was reflected in average gain (241.3, 218.8, 218.4, and 258.3 g/fish), and specific growth rate (1.12, 1.05, 1.06, 1.18 %), respectively. Feed conversion ratios were 1.49, 1.45, 1.44, and 1.42 in treatments A, B, C, and D, respectively. Carcass analysis (fillet %) did not reveal treatment effects. Examination of blood samples taken at termination of the experiment showed a significant reduction of blood protein (3.6ab, 3.3bc, 3.0f, 3.8g/dL) and IgM levels (0.07a, 0.04b, 0.03b, 0.06a g/L) in treatments B and C compared to positive control (A). However, in treatment D these levels were recovered to levels of positive control. From results of this experiment it is concluded that supplementation of 0.55% DL-Methionine to the fishmeal reduced diet allowed for similar performance and immune status as sex-reversed red hybrid tilapia fed 10% fishmeal.

* E-mail address: dhanapong.sangsue@evonik.com (D. Sangsue).
Dietary lysine requirement of juvenile Pacific threadfin (*Polydactylus sexfilis*)

Dong-Fang Deng1*, Warren Dominy1, Zhi Yong Ju1, Shunsuke Koshio2, Ryan Murashige3
1 Aquatic Feeds and Nutrition Department, Oceanic Institute, Waimanalo, Hawaii, 96795, USA
2 Laboratory of Aquatic Animal Nutrition, Faculty of Fisheries, Kagoshima University, Kagoshima, Kagoshima 890-0056,
3 Grove Farms Fish & Poi LLC, Lihue, HI 96766, USA

Abstract

The Pacific threadfin, a candidate species for aquaculture in Hawaii, is also called Moi: Fish of Kings in Hawaii. The current culture of this specie depends on commercial feeds developed for salmonids or other marine species. No information is available on the nutritional requirements of this fish and thus no optimal diet has been developed for the Moi. The objectives of this study were to 1) develop an amino acid test diet for this species; and 2) estimate its lysine (Lys) requirement based on growth performance. A basal test diet was formulated using Moi muscle and a mixture of precoated crystalline amino acids as the protein source to achieve the same amino acid profile as in Moi muscle. An 8 week grow trial was conducted with juvenile Moi (initial body weight, 3.0±0.1g) fed 8 isonitrogenous (35% protein) and isoenergetic (19.1 MJ/kg diet) semi purified diets containing 1.29 to 2.91% Lys. The fish were cultured in an indoor flow-through system with 31‰ sea water at 25 ºC. There were three replicates per dietary treatment. No mortalities occurred during the growth trial. Fish fed diets containing 2.23-2.43% Lys had higher specific growth rate, feed efficiency and protein efficiency ratio than those fed diets containing less than 1.74% Lys. Based on broken-line analysis of the growth performance, data indicates the Lys requirement is 2.01% diet (5.7% protein) under current culture conditions. The effects of dietary treatment on body composition will also be presented.

Keywords: Lysine; Pacific Threadfin; Amino acid requirement

*E-mail address: dfdeng@oceanicinstitute.org  (D.F. Deng).
P-239

Dietary L-methionine requirement of juvenile black sea bream Sparus macrocephalus at a constant dietary cystine level

Fan Zhou1,*, Qing Jun Shao1, Jin Xing Xiao1, Bergo Owari Ngandzali1, Yuan Jian Xu2 and Jun Zhuo Xu2

1College of Animal Sciences, Zhejiang University, Hangzhou 310029, P.R. China
2Marine Fisheries Research Institute of Zhejiang Province, Zhoushan 316100, P.R. China

Abstract

An 8-week feeding trial was conducted to determine the methionine requirement and the effect of dietary methionine levels on growth, feed utilization and body composition in juvenile black sea bream Sparus macrocephalus. Fish (initial body weight 14.21 ± 0.24 g) were reared in eighteen 350-L indoors flow-through circular fibreglass tanks (20 fish per tank) at 28 ± 1°C, salinity and dissolved oxygen concentrations were 24-28 mg L⁻¹ and above 5.0 mg L⁻¹, respectively. Crystalline L-methionine was added to the basal diet at six graded levels in a 0.30% increment at a constant dietary cystine level of 0.31%, and the methionine contents of six experimental diets were 0.75%, 1.09%, 1.41%, 1.72%, 2.06% and 2.35%, respectively. The results showed survival rate of experimental fish was generally high (91.67%-100.00%), and independent of the dietary treatments. Growth performance and feed utilization were poorest in 0.75% methionine diet. Maximum weight gain (WG), feed efficiency ratio (FER), protein efficiency ratio (PER) and protein productive value (PPV) occurred at 1.72% dietary methionine, beyond which these index declined. Apparent digestibility coefficients (ADCs) of dry matter was significantly lower in 1.85% methionine diet than those of date in 2.86% and 3.46% methionine diets, ADCs of crude protein was lower in 1.85% methionine diet than the other group. ADCs of gross energy increased significantly to 2.86% methionine diet and remained nearly the same thereafter. However, ADCs of crude lipid were not affected by dietary treatments. Protein content in whole fish body and dorsal muscle were positively correlated with dietary methionine level, while lipid content in dorsal muscle showed an opposite trend to body protein content. Fish fed the grade level of methionine exhibited a significant improvement of free methionine content, total essential amino acids (ΣEAA), total non-essential amino acids (ΣNEAAs) and ΣEAA/ΣNEAA ratio in whole fish body. Hepatosomatic index (HSI) was higher in 2.35% methionine diet than that of in 0.75% methionine diet, while condition factor (CF) of fish was independent by different dietary methionine level. Regarding serum characteristics, significant differences were observed in total cholesterol, glucose and free methionine concentration, while total protein level and triacylglycerol concentration kept relatively constant among treatments. Analysis of dose-response with second order polynomial regression on the basis of either WG or FER, the optimum dietary methionine requirements of juvenile black sea bream were estimated to be 1.80% of diet (corresponding to 4.72% dietary protein) and 1.84% of diet (corresponding to 4.84% dietary protein) in the presence of 0.31% cystine, respectively.

Keywords: Black sea bream juvenile Sparus macrocephalus; Methionine requirement; Growth performance; Feed efficiency ratio; Digestibility of nutrients.

* E-mail address: zhoufan0302@126.com (Fan Zhou)
Dynamic and integrated production biology of farmed salmon in sea. Effects of dietary supplementation of the bioactive fatty acid TTA.

Magny S. Thomassen\textsuperscript{1*}, Henriette Alne\textsuperscript{1,2} Maike Oehme\textsuperscript{1,3}, Rørvik K-A\textsuperscript{1,2}

1 Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, P.O.Box 5003, 1432 Aas-UMB, Norway
2 NOFIMA, P.O.Box 5010, 1432 Aas, Norway
3 NOFIMA, N-6600 Sunndalsora, Norway

Abstract:

The salmon farming industry demands optimal production throughout the entire life-cycle. This demand is currently not realized due to seasonal environmental changes that dramatically influence on diseases, feed intake, lipid accumulation and growth rate during the sea stage. To improve the predictability and cost-efficiency of salmon farming, there is a need to compose dynamic diets that are optimized according to the major seasonal changes. The objectives of our research are to develop a new and integrated understanding of how growth and diseases relates to the physiological state of the fish and how this interacts with seasonal and climatic conditions. Our main focus is to study how this may be manipulated by diet composition and strategic supplements to optimize the performance by the fish, thus achieving a more sustainable salmon production. In particular, we try to characterize physiological and underlying biochemical responses to feed supplements in order to boost and regulate the metabolism of salmon in advance of or during critical periods of the production cycle.

In this presentation we focus on the use of dietary supplementation of the bioactive fatty acid Tetradecylthioacetic acid (TTA) during critical period for S1 smolt in the weeks after sea transfer and on S0 salmon in the spring after one sea winter. Data will be presented that show significant effects on natural outbreaks of Infectious Pancreas Necrosis (IPN) in S1 and of Heart and Skeletal Muscle Inflammation (HSMI) in S0. Further, we show that the problem with increasing early sexual maturation in S0 in the autumn after one sea winter can be significantly reduced. Changes in protein and lipid retention as well as in production data will be discussed.

Keywords: Atlantic salmon, smolt, TTA, IPN, HSMI, sexual maturation

*E-mail address: magnth@umb.no
P-241

Effect of different dietary carbohydrate to lipid ratios on growth and feed performance of juvenile benni (*Barbus sharpeyi*)

M. Kazemi1*, J. G. Marammazi2, P. Kochanian1, V. Yavari1, E. Rajabzadeh1 and B. Ahmadi1

1 Department of Fisheries, Khoramshahr Marine Science and Technology University, Khoramshahr, Iran
2 South Iranian Aquaculture Research Center, Ahvaz, Iran

Abstract

A study was undertaken to determine the effects of various dietary carbohydrate to lipid ratios on growth and feed performance of benni (*Barbus sharpeyi*) juveniles. Nine iso-nitrogenous (25% crude protein) and iso-caloric (3.5 kcal/g digestible energy) semipurified diets with varying carbohydrate to lipid (CHO/L) ratios (0.8 to 8.8) were tested for 8 weeks with three replicates. Each replicate was stocked with 20 fish (initial mean weight: 16.47 ± 0.25 g) that were fed to satiation thrice daily. Twenty seven fiberglass tanks of 300 L capacity with a flow rate of 1.5 L min⁻¹ were used for rearing the experimental fish in a flow-through system. Weight gain (WG), weight gain rate (WGR), specific growth rate (SGR), feed conversion ratio (FCR), feed efficiency ratio (FER), protein efficiency ratio (PER) and apparent net protein utilization (ANPU) were studied in this experiment. During the experiment the survival rate did not vary among the treatments. CHO/L ratios lower than 3.8 did not seem to affect (P > 0.05) growth and feeding performances of *B. sharpeyi* juveniles. Growth and feed performance increased significantly (P < 0.05) at dietary CHO/L ratios of 3.8 (diet D4) and 4.8 (diet D5). The growth indices such as WG (118.44 ± 7.87), WGR (35.94 ± 2.58), SGR (0.54 ± 0.03) were the highest at a CHO/L ratio of 4.8. Similarly improved FCR (2.78 ± 0.04), FER (0.36 ± 0.01), PER (1.43 ± 0.02) and ANPU (30.93 ± 6.36) were also recorded in fish fed the same CHO/L ratio. Growth and feed performances were poor when dietary CHO/L ratio was further increased to 5.8 (diet D6). Based on the results of the present study it could be concluded that the optimal dietary CHO/L ratio for optimal growth and feeding performance of *Barbus sharpeyi* juveniles, ranged between 3.8 and 4.8

Key words: *Barbus sharpeyi*, Carbohydrate, Lipid, Growth, Feeding

*E-mail address: mahtab_kzm86@yahoo.com
Effects of feeding non-marine raw materials on performance, gut microbiota and intestinal histology in Atlantic salmon (Salmo salar L.) – New Microbial Insights

Mali Bjerkhaug Hartviksen1*, Jose L. González Vecino1, Simon Wadsworth1, Åshild Krogdahl2, Anu Kettunen3, Einar Ringø4

1 EWOS Innovation, 4335 Dirdal, Norway.
2 Aquaculture Protein Centre, Norwegian School of Veterinary Science, Department of Basic Science and Aquatic Medicine, Post Office Box 8146 Dep, NO-0033 Oslo, Norway
3 Alimetrics Ltd, Koskelontie 19B, FIN-02920 Espoo, Finland
4 Norwegian College of Fisheries Science, Faculty for Bioscience, Fisheries and Economics, University of Tromsø, 9037 Tromsø, Norway

Abstract

In order to cater to the increasing demands for marine protein from a growing world population, alternative raw materials have become a focus for the aquaculture feed industry in order to alleviate the demands for such protein. Feeding alternative raw materials have proved challenging with possible negative effects such as enteritis of the intestine, reduced digestibility and growth. It is generally accepted a clear link between performance, diet components and the gut microbiota in endothermic animals, but equivalent knowledge is lacking in fish. Based on this fact, the aim of the present study was therefore to evaluate how protein alterations affect; 1) performance parameters (growth and feed conversion ratio FCR), 2) the gut microbial community, 3) intestinal histology and 4) can the effects observed on performance, microbiota and histology be combined to gain knowledge on the usefulness of a raw material as a feed component in diets for salmonids? Twenty percent of the fish meal (FM) fraction was replaced by either extracted sunflower meal (ESF), poultry by-product meal (PBY) or pea protein concentrate (PPC). The four experimental diets were fed to Atlantic salmon (∼305 g Salmo salar L.) in a 12-week feeding trial. Feed intake was recorded daily, and weight every 4 weeks. At the start and end of the feeding period samples for gut microbiota and histology were taken. Intestinal microbiota was analyzed by 16S-DNA technology using qPCR, while histological changes were investigated by light microscopy. Based on growth and feed intake parameters, pea protein concentrate performed better than fishmeal, poultry by-product performed at level with fishmeal, and extracted sunflower was the worst performing diet. Analysis of microbial samples revealed that pea protein concentrate did not modulate the microbial community as compared to fishmeal and that poultry by-product showed few differences in the allochthonous microbial community. Extracted sunflower however modulated the microbial community compared to fishmeal. Preliminary histological analysis of the intestine reveals that extracted sunflower may induce enteritis like symptoms, conditions which are not evident in the other diet groups. Our results indicate a clear link between diet, performance and the gut microbiota and the results will be discussed in a larger perspective.

Key words: Atlantic salmon; diet; performance; 16S Q-PCR; microbiota; histology

*E-mail adress: Mali.Hartviksen@ewos.com
Supplemental effects of some crude ingredients in improving feed intake and performances of red sea bream, *Pagrus major* fed high soy protein concentrate diet

Md. Abdul Kader1*, Saichiro Yokoyama2, Manabu Ishikawa2, Shunsuke Koshio1, 2

1Applied Sciences of Marine Resources, The United Graduate School of Agricultural Sciences, Kagoshima University, 1-21-24 Korimoto, Kagoshima 890-0056, Japan;
2Laboratory of Aquatic Animal Nutrition, Faculty of Fisheries, Kagoshima University, Shimoarata 4-50-20, Kagoshima 890-0056, Japan;

Abstract

The inclusion of higher levels of plant protein in diets of carnivorous fish resulted in lower feed intake (FI) and growth performances because of imbalance amino acids and decrease palatability of the diets. Therefore, a feeding trial was conducted to comparatively study some crude ingredients at minimum inclusion level in high soy protein concentrate (SPC) based diets for red sea bream, whether they are effective in recovering feed intake and performance in fish. Seven isonitrogenous (50% crude protein), isolipidic (14% total lipid) and isocaloric (22 KJ/g gross energy) diets were formulated, where diet 1 was a fishmeal (FM) based diet. Diets 2 to 7 were prepared as follows by replacing 60% fishmeal protein with: SPC alone (diet 2); SPC and 2.5% crystalline amino acids (CAA) (diet 3); SPC and 10% fish soluble (FS) (diet 4); SPC and 10% krill meal (KM) (diet 5); SPC and 10% squid meal (SM) (diet 6) and SPC and a mixture (total 15%) of FS, KM and SM 5% each (diet 7) respectively. Triplicate groups of fish (average initial weight 0.82 ± 0.01 g) were randomly stocked in twenty-one 100L polycarbonate tanks at a stocking density of 15 fish per tank. The fish were fed to apparent satiation for 50 days. Results of the study showed that FI was significantly (*P* <0.05) depleted in fish fed diet 2 which was compensated with the supplementation of FS, KM and SM to the extent of being approximately equal (*P* >0.05) in diets 5 and 6 or higher (*P* <0.05) in diets 4 and 7 than in fish fed the diets 1 (control) and 3. Growth performance and feed utilization were directly influenced by FI and followed the same trend as FI. Weight gain (%) and specific growth rate (SGR) of fish were both significantly lower in fish fed diet 2, but returned in fish fed all the supplemented SPC based diets compared to the control. As with FI, these values were significantly higher in fish fed diets 4 and 7; while feed efficiency ratio and protein efficiency ratio were comparatively lower in these groups. No difference was found in the digestive tract and liver protease activity among treatments. Blood parameters showed that the physiological condition of fish fed diet 2 was significantly depleted which was recovered with the supplementation of either CAA or crude ingredients. Based on the overall performances of fish, it can be concluded that supplementation of fish soluble, krill meal and squid meal are as effective as CAA to keep amino acids balance and can act as attractant in high soy protein concentrate diet for maintaining normal feeding behavior, growth performance and health or welfare of juvenile red sea bream.

Key words: crude ingredient, feeding stimulants, palatability, feed intake and red sea bream.

*E-mail address: abdulkader_fc@yahoo.com (M.A. Kader)*
Study of dietary protein and energy requirements of rainbow trout (Oncorhynchus mykiss) in inland brackish water condition

Alizadeh, M
Saltwater Fishes Research Station, Iranian Fisheries Research Organization, POBOX 89715-1123, Bafgh, Iran.

Abstract
A completely randomized design, with 3×3 factorial arrangement, was conducted to test various ratios of dietary protein to energy (P/F) for rainbow trout reared in brackish water. Three crude protein levels (35, 40 and 45%) and three energy levels (370, 400 and 430 Kcal/100g) at each protein level were utilized. Semi-purified diets containing fish meal, casein and gelatin as the sources of protein and dextrin, starch and oil as the sources of energy were used. Each experimental diet was fed to triplicate groups of 20 fish with average weight of 81.5g. In nine 2000lit flow trough fiberglass tanks, each partitioned into three sectors. Average water temperature, dissolved oxygen, pH and EC were between 15±2°C, 6.5-8.1 mg/L, 7.7-8.6 and 25400µm/s, respectively. Fishes were fed for 84 days at a rate between 1.6-2% of body weight per day. In three equal amount, adjusted bi-weekly. At each protein level, weight gain (WG%), average daily growth (ADG%), protein efficiency ratio (PER), apparent net protein utilization (ANPU%), specific growth rate (SGR) and condition factor (CF) increased, but feed conversion ratio (FCR) decreased with increasing energy level from 370 to 430 Kcal/100g. The highest growth performance was obtained by fishes fed by 35% protein, 430 Kcal/100g energy diet with P/E ratio of 81.4 mg protein/Kcal energy. Carcass fat and moisture were affected by dietary protein and energy levels, whereas, carcass protein and ash levels were similar between dietary treatments.

Key words: rainbow trout, Oncorhynchus mykiss, brackish water, protein and energy requirements

* Email: m_alizadeh47@yahoo.com (M.Alizadeh)
Protein requirement of Arctic charr (*Salvelinus alpinus*).

Ólafur Ingi Sigurgeirsson¹*, Jón Árnason², and Aðalheiður Ólafsdóttir²

¹University Collage, Hólar, Iceland
²Matis ohf, Iceland

Abstract.

The protein requirement of Arctic charr (cold water salmonid species) of different size from first feeding to 1 kg was studied in six separated growth trials. All size groups of the charr were fed six isoenergetic feed formulations containing 34.7-49.2% crude protein (CP). Growth rate, feed conversion ratio (where possible), protein efficiency and chemical composition were compared among groups of fish fed different formulations.

The results suggest that the CP in feed must be 45-49% CP to support maximum growth of fish from start feeding up to 10g. For larger fish the protein requirements are progressively reduced. Fish that are 90 g fish grow equally well on feed containing either 34.7% or 49.2% CP. Reduced CP in feed had minimal effects on feed conversion ratio but results in increased protein efficiency and conservation of protein in muscles of larger fish. It was not possible to determine exactly the minimum CP required to maintain maximum growth for fish of all sizes since the lowest CP levels tested did not result in significantly reduced growth rate in larger fish.

In following experiment with substitution of marine protein with different plant protein sources the lower protein requirement was established. The results also highlighted the possibilities in the substitution level of marine raw material in feed for this carnivore fish species. The results from these projects will be presented and discussed.

Keywords: Protein; requirement; Arctic charr

*E-mail address: olisig@holar.is
Effects of dietary carbohydrate sources on growth performance and hepatic carbohydrate metabolic enzyme activities of juvenile cobia
(*Rachycentron canadum*)

Xian-Jun Cui1*, Qi-Cun Zhou1, Hai-Ou Liang2, Jun Yang1, Li-Mei Zhao2

1 Laboratory of Aquatic Economic Animal Nutrition and Feed, College of Fisheries, Guangdong Ocean University, Zhanjiang 524025, People’s Republic of China
2 Aquatic Animal Feed Research and Development Center, Guangdong Evergreen Group Corp. Zhanjiang 524094, People’s Republic of China

Abstract

An 8-week feeding trial was conducted to investigate the effect of dietary carbohydrate sources on growth performance and hepatic carbohydrate metabolic enzyme activities of juvenile cobia. Six experimental diets were formulated to contain 20% glucose, sucrose, maltose, dextrin, corn starch, and wheat starch, respectively. The results indicated that fish fed the wheat starch and dextrin diets had significantly better weight gain, specific growth rate and protein efficiency ratio compared to those fed the other diets. However, fish fed the glucose diet had the significantly lower survival and condition factor than those fed the other diets. There were significant differences in total plasma glucose and triglyceride concentration in fish fed diets with different dietary carbohydrate sources. Haematocrit, hemoglobin, red blood cell and leukocyte were significantly affected by the dietary carbohydrate sources. The activities of glucose-6-phosphate dehydrogenase (G6PD), 6-phosphofructokinase (PFK) and fructose-1,6-bisphosphatase (FBPase) were significantly affected by the dietary carbohydrate sources, while fish fed the glucose diet had higher G6PD, PFK and FBPase activities than those fed the other diets. These data indicated that dextrin and wheat starch were the optimal carbohydrate sources for juvenile cobia.

Keywords: Cobia (*Rachycentron canadum*); Carbohydrate sources; Growth performance; Hepatic metabolic enzyme activities

*E-mail addresses: qicunzhou@21cn.com (Q. C. Zhou)
Vitamin and mineral status in young rainbow trout fed extruded feeds with graded inclusions of a vitamin and mineral premix

Rune Waagbo*1, Eldar Á. Bendiksen2, Kim S. Ekmann3, Arne Guttvik2, Gro-Ingunn Hemre1
1NIFES, PO Box 2029 Nordnes, N-5817 Bergen, Norway
2BioMar AS, Trondheim, Norway
3BioMar FTU Hirtshals, Denmark

Abstract

In the present experiment rainbow trout (Oncorhynchus mykiss) of 28 g start weight was fed graded levels of a revised vitamin and mineral premix that should meet the requirement at 0.300% inclusion level in feed (NRC 1993). Five experimental feeds were obtained by adding the same pre-defined vitamin and mineral premix to the basal feed recipe at 0% (PM0), 0.075% (PM25), 0.150% (PM50), 0.300% (PM100) or 0.600% (PM200) inclusion. Consequently the experimental feeds differed systematically in vitamins (C, E, K3, B1, B2, niacin, B5, biotin, folic acid and B12) and minerals (Zn, I, Cu, Co and Mn), while keeping the dietary ratio between the premix micronutrients constant across diets. The feeds were commercial-like high-energy feeds produced at BioMar TechCenter (Brande, Denmark) by using the same feed recipe containing equal amounts of marine and vegetable oil and protein raw materials, and commercial levels of binder, antioxidants and inorganic phosphorus. In addition all trial feeds were balanced with crystalline amino acids and were added 1% lecithin. A feeding experiment was conducted in freshwater at stable light (LD12:12) and temperature (16°C±1°C) conditions for 72 days in triplicate. Levels of all vitamins and minerals in experimental diets mainly confirmed the supplementation levels of premix. Growth performance and biochemical markers of all added vitamins and minerals were recorded at the end of the 12 weeks feeding experiment when mean fish weights had increased more than 5-fold (SGR; 2.56±0.04 % day⁻¹). Samples of whole fish, fish tissues (liver, muscle and gills) and plasma were collected for analyses of vitamin and mineral status. No vitamin and mineral supplementation (PM0) caused reduced growth, gill pathology, and reduced tissue status of riboflavin, pyridoxin, pantothenic acid, folate, vitamin C and zinc. No increase in status indicators for thiamine, biotin, niacin, vitamin B12 and the minerals Co, Cu, I and Mn with increasing premix supplementation indicating sufficient supplies of these constituents from the feed ingredients. The present data on micronutrient feed retention, digestibility, body status, and corresponding functional markers in rainbow trout are valuable in the reassessment of practical requirements for salmonids and by use of novel feed ingredients.

Keywords: Vitamin, mineral, practical requirement, micronutrient status, rainbow trout

* E-mail address: rwa@nifes.no (R. Waagbo)
Use of low-phosphorus feed for Biwa salmon aquaculture

Shozo Sugiura¹*, Takashi Taguchi¹, Akira Yamaoka², Hidetomo Tanaka³, Kunihiko Kuwamura³

¹Department of Bioresources Management, University of Shiga Prefecture, Hikone, Shiga, 522-8533, Japan
²Matsuya Co., Ltd., Kinomoto, Shiga, Japan
³Shiga Fisheries Experiment Station, Hikone, Shiga, Japan

Abstract

Biwa salmon, Oncorhynchus masou rhodurus, is endemic to Lake Biwa, and is considered one of the tastiest fishes among salmonids. However, aquaculture of this species is still in its infancy. Thus, we conducted the present study to develop eco-friendly feed for Biwa salmon aquaculture. Biwa salmon (1.5 year-old, Samegai strain, initial mean body wt. 277g) were reared in FRP tanks (1mt ton) and also in concrete ponds for 3 months. Fish were fed either LP feed (low-phosphorus (P), fish meal-free diet; total P 0.73%, CP 39%, fat 16%) or control feed (commercial feed for Masu salmon; total P 1.61%, CP 45%, fat 6%) once daily to apparent satiation. Availability of dietary P was determined in vivo in separate trials. Both groups of fish showed comparable feed consumption on an energy basis; however, toward the end of feeding duration feed intake decreased due to sexual maturation. Final mean body wt was 370g and 392g for LP and control groups, respectively (p>0.05). Both groups of fish showed similar feed efficiency (48-61%). Carcass P content was similar for both groups of fish, and was within a normal range. Fat content of fish fillets was 8.9% (LP) and 7.6% (control)(p<0.05). Both EPA and DHA contents were significantly higher in LP than control fish. Gonad- somatic index (GSI: 3.2-3.6%) and Visero- somatic index (VSI: 4.8-5.2%) were similar for both groups of fish (p>0.05). The mRNA expression of phosphate-transporter (NaPi2b) in the colon was higher in control than LP fish (p<0.05). Fish fillets were evaluated by young panels (n=68) and senior panels (n=43) using sensory and hedonic ratings. Both panels tended to give higher ratings for LP fish on taste, favor, texture and overall quality, though not significant (p>0.05). Effluent P concentration was 10.7-18.2ppb in LP tanks, and 23.8-35.9ppb in control tanks (p<0.05). However, using a stagnant water system, net P excretion over 6 hours became 0ppb in LP and 118.3ppb in control tanks (p<0.01). Apparent availability of dietary P was 50.8% (LP) and 28.8% (control). Estimated P excretion per kg of feed consumed was 3.5g (LP) and 12.0g (control). When the LP feed was pre-treated with phytase, fecal P excretion decreased by 33%. These results indicate several merits of using LP feed in Biwa salmon aquaculture.

Keywords: Phosphorus; Low-pollution; Biwa salmon; Sustainable feeds

* E-mail address: sugiura@ses.usp.ac.jp (S.Sugiura).
P-249

Gastrointestinal evacuation time in European sea bass (*Dicentrarchus labrax*) fed diets containing mixture of vegetable oils at high temperature

Eroldoğan O. Tufan*, Çiçek C. İşil, Yılmaz H. Asuman, Dedeler H., Türkmen, S.
Department of Aquaculture, Faculty of Fisheries, Çukurova University, Adana 01330, Turkey

Abstract
The rate of gastric emptying is positively related with the return of appetite and food consumption in fish. Gastric evacuation is affected by various factors, including water temperature and diet composition. Thus, we hypothesized that increasing levels of vegetable oil could have effect gastrointestinal evacuation time (GET) at high temperature. Triplicate groups of European sea bass (initial wt. 39.7 g) were fed three diets in which the added lipid was 100% fish oil (FO), 70% FO + 30% vegetable oil mix (15% canola:15% cottonseed oil; VO30) and 40% FO + 60% vegetable oil mix (30% canola:30% cottonseed oil; VO60) for a period of 120 days. Fish were held at optimum (24°C) and maximum (30°C) water temperature. At the end of experiment, fish were fasted for 72 h before being fed to make sure that gastrointestinal tract was empty. Six fish per treatment were sacrificed at 0, 8, 16, 24, 32 and 48 h after a single satiation feeding. The digestive tract was separated into three parts: stomach, foregut and hindgut. The mathematical models giving the best fit were found to tested diets being exponential for the stomach and linear for the foregut and the hindgut. The control diet (FO) required 9.0 h to evacuate half of the initial feed content in stomach, followed by VO30, 10.5 h and VO60 with 8.6 h at 24°C. However, GET for control, VO30 and VO60 was 8.0, 9.1 and 9.8 h at 30°C, respectively. While increasing levels of vegetable oil in the diet increased GET in stomach of fish held at 30°C, there was no dietary effect on GET at 24°C. The whole meal cleared from the foregut within 60.7-61.1 h for all dietary groups at 30°C. With regards to the effects of dietary vegetable oil level, it was found that while evacuation rates in foregut decreased in fish fed VO60 at 24°C, there was no significant dietary effect on GET at 24°C. The whole meal cleared from the foregut within 60.7-61.1 h for all dietary groups at 30°C. With regards to the effects of dietary vegetable oil level, it was found that while evacuation rates in foregut decreased in fish fed VO60 at 24°C, there was no significant dietary effect on GET at 24°C. However, GET in hindgut was significantly higher in fish held at 30°C compared to fish held at 24°C. The GET computed for hindgut significantly longer in groups fed FO compared to the VO30 and VO60. The findings of this study suggest that 30% or 60% vegetable oil mixture could increase GET in the stomach; however, GET could decrease through digestive track for European sea bass fed with those diets at both temperature.

Keywords: Vegetable oil; European sea bass; Gastric evacuation; Temperature

*E-mail address: mtufan@cu.edu.tr
Dietary ratio of (n-3)/(n-6) Polyunsaturated Fatty Acids (PUFAs) during the Artemia feeding period affects the growth of Senegal sole (*Solea senegalensis*) larvae but not larval morphogenesis

Anaïs Boglino*, Maria J. Darias, Filiz Ozcan, Karl B. Andree, Alicia Estévez, Enric Gisbert
Centro de Acuicultura-IRTA, Ctra. Poble Nou km 5,5, 43540 Sant Carles de la Ràpita, Tarragona, España

Abstract

The development of skeletal malformations in finfish larvae remains an important bottleneck in aquaculture. Larval nutrition has been shown to be one of the key factors affecting morphogenesis and skeletogenesis processes. Some nutrients such as vitamins, lipids and fatty acids are responsible for the apparition of skeletal deformations when they are not provided to the fish larvae in sufficient amount or adequate form in the diet. Because most of marine fish are not able to synthesize essential fatty acids, dietary lipids represent the main source of n-3 and n-6 PUFAs, required for normal larval growth and bone formation. Although the effect of these two kinds of PUFAs on Senegal sole (*Solea senegalensis*) larvae development has previously been studied separately, the interaction between them should be considered since they share the same pathway of biosynthesis.

Available commercial enrichments for live prey present considerable differences in their nutritional composition, especially in the amount of PUFAs and their ratios. Thus, the objective of this work was first to determine whether any of these products was more suitable for Senegal sole larval development and secondly, to analyze the influence of their different dietary (n-3)/(n-6) PUFAs ratios on larval growth and performance. Senegal sole larvae were fed from 8 to 38 days post hatching (dph) with *Artemia* nauplii previously enriched with the following six commercial enrichments containing different (n-3)/(n-6) PUFAs ratios: Easy Selco® (INVE, 4.06), Easy Selco® (INVE) half diluted with olive oil (3.39), Multigain® (BioMar, 3.83), Red Pepper® (Bernaqua, 3.16), Aquagrow Gold® (ABN, 4.33) and Aquagrow DHA® (ABN, 5.68).

The dietary treatments significantly affected larval growth; larvae fed *Artemia* enriched with Aquagrow Gold presenting the highest standard length and dry weight at 22 and 38dph, and those of the Easy Selco group showing the lower ones. The maturation of the digestive system was also affected by the diet, larvae of Aquagrow Gold and Aquagrow DHA groups showing a better development of their digestive capacities. No significant effect of the dietary treatments was detected in final survival, metamorphosis progress and incidence of skeletal deformities.

Ongoing studies of the expression profiles of genes known to be implicated in intracellular transport of fatty acids (FABPs), larval growth (IGFs) and skeletogenesis (osteocalcin, PPARs, RXRs, RARs) will bring to light an eventual effect of the dietary treatments at molecular level. Moreover, since the six enrichments contain graded concentrations of Vitamin A (VA), the expression of genes markers for VA metabolism will be also analysed. The obtained results would be helpful to understand physiological changes occasioned by different dietary VA and PUFAs levels, whose molecular signalling pathways strongly interact with each other.

Keywords: Senegal sole; Larvae; Skeletal malformations; Lipids; Fatty acids; Morphogenesis

* E-mail address: anais.boglino@irta.cat
P-251

Assessment of dietary lysine requirement in largemouth bass, *Micropterus salmoides*

Naisong Chen*, Hengyong Zhou, Jianzhong Ma, Jie Zhou
Shanghai Ocean University, 999 Huchen Guan Road, Shanghai 201306, PR China

Abstract

An 89-day feeding trial was conducted to evaluate the quantitative requirement of dietary lysine in largemouth bass (initial weight 32±1g). Eight isonitrogenous and isoenergetic diets (diets 1-8) were formulated to contain crude protein 45.36% and gross energy 19.73 MJ/kg of dry diet. Diets 1-7 were formulated using plant protein and crystalline amino acids as a primary protein source to mold the EAA profile of largemouth bass body except for lysine under investigation. Seven levels of dietary lysine ranging from 1.88% to 4.18% of dry diet at an about 0.4% increment were achieved by adding zein-coated crystalline lysine at the expense of zein-coated crystalline glycine. Diet 8 which was formulated using 48% white fishmeal as a major protein source without adding any crystalline amino acids was served as a positive control to examine whether feed intake and resulting growth of fish fed diets 1-7 would be affected by incorporating crystalline amino acids. Each diet was randomly assigned to triplicate tanks connected to an indoor flow-through and aerated freshwater system. In each tank, 22 fish were fed by hand to apparent satiation twice daily (09:00 and 15:00). Water temperature was maintained at 25±1℃ and light cycle 12:12 h.

At the end of the trial, data analysis showed that specific growth rate (SGR), feed efficiency ratio (FER), protein retention rate (PRR) and survival rate were significantly affected by dietary lysine levels (*P*<0.05). However, proximate composition of fish body was not significantly affected by dietary lysine levels (*P*≥0.05). Diets supplemented with crystalline amino acids were accepted as well as the control diet by test fish. Fish fed diet 1 with lower lysine level (1.88% lysine) suffered ventral fin erosion with higher mortality compared to fish fed the other diets (*P*<0.05). Serum non-special immunity parameters (lysozyme activity, classical complement pathway activity and protein content) of fish fed diet 1 or diet 2 were significantly lower than those of fish fed the other diets (*P*<0.05). Those findings indicated that dietary lysine level affected not only growth performance but also immunity in largemouth bass.

Based on a broken-line model used to fit SGR, FER, and PRR against dietary lysine level, dietary lysine requirements in largemouth bass were 3.08%, 2.65% and 2.73% of dry diet (6.79%, 5.84% and 6.02% of dietary protein), respectively.

Keywords: Largemouth bass; Lysine requirement; none-special immunity

*E-mail address: nschen@shou.edu.cn (N. Chen)
Effects of dietary different carbohydrate to lipid ratios on the growth performance, body composition and non-specific immunity of largemouth bass (*Micropterus salmoides*)

Xiaojie Qiu*, Naisong Chen, Hengyong Zhou, Jianzhong Ma
Shanghai Ocean University, 999 Hucheng Huan Road, Shanghai 201306, PR China

Abstract

An 8-week feeding trial was conducted to investigate the effect of dietary different carbohydrate to lipid ratios on the growth performance, body composition and non-specific immunity in largemouth bass. Six isonitrogenous (46% crude protein) and isoenergetic (19 MJ/kg gross energy) fishmeal based diets were formulated with varying carbohydrate to lipid (CHO/LIP, w/w) ratios from 0.51 to 5.18 for diets 1-6 respectively. Each diet was randomly assigned to four tanks (800L/tank) connected to an indoor flow-through and aerated freshwater system. In each tank, 35 fish were fed by hand to apparent satiation, twice daily, at 08:00 and 16:00 h. Water temperature was maintained at 28±1°C, and the natural light cycle was adapted.

Results showed that dietary varying CHO/LIP ratios caused different effects on specific growth rate (SGR), protein efficiency rate (PER), feed efficiency ratio (FER) and body composition. SGR of fish fed diet 1 was higher than that of fish fed the others \( (P<0.05) \). Fish fed diet 1 has a significantly higher PER than fish fed diet 4, but there was no significant difference between fish fed the other diets \( (P>0.05) \). Diet 2 leaded to a significantly higher FER than diet 4 \( (P<0.05) \), but no significant difference were observed between the other treatments. As for fish body composition, significant difference was found in body lipid and moisture content \( (P<0.05) \), however, crude protein and ash content in fish body were not affected by varying CHO:LIP ratio. HIS increased with CHO/LIP ratios increasing from 0.51 to 5.18, significantly higher in fish fed diet 1-2 than that in fish of the other treatments \( (P<0.05) \). In the non-specific immunity parameters, no significant difference were observed in respiratory burst activity of head kidney leucocytes and classical complement pathway activity (CH50), but plasma lysozyme activity of fish fed diet 1 was significantly higher than that of fish fed the other diets \( (P<0.05) \).

The present study indicated that there was a difference in the ability for largemouth bass to utilize non-protein energy. Lipid can be better utilized by largemouth bass as energy source than carbohydrate.

Keywords: Largemouth bass; Carbohydrate to lipid ratio, Growth, Body composition, Non-specific immunity

*E-mail address: phoenix03@163.com*
Effect of dietary carbohydrate-to-lipid ratios on growth performance, body composition, nutrient utilization and hepatic enzymes activities of omnivorous tilapia (*Oreochromis niloticus* × *O. aureus*)

Wen Gao¹*, Yong-Jian Liu¹, Li-Xia Tian¹, Kang-Sen Mai²

¹ Nutrition Laboratory, Institute of Aquatic Economic Animals, School of Life Science, Sun Yat-sen University, Guangzhou 510275, P.R. China
² Laboratory of Aquaculture Nutrition, College of Fisheries, Ocean University of China, Qingdao 266003, P.R. China

Abstract

Six isonitrogenous (39% crude protein) and isoenergetic (16.2 KJ g⁻¹) diets with varying carbohydrate: lipid (CHO: L) ratios (202.5 to 1.7), were fed to triplicate groups of 25 hybrid tilapia in indoor recirculation system. Over 8-week-growth trial, growth rates in fish differed \( (P < 0.05) \) with CHO: L ratios, producing a quadratic pattern. Best weight gain (WG) and specific growth rate (SGR) were observed in fish fed a 31% carbohydrate and 4% lipid diet, corresponding to a CHO: L ratio of 7.5, which were not significantly different from those of 4.4. FCR value was the highest and PER and PR values were the lowest in the diet containing 40% carbohydrate level without lipid supplementation and no significant differences were observed for fish diets containing lipid levels higher than 2%. The values of hepatosomatic index (HSI) and intraperitoneal fat ratio (IPF) increased as dietary CHO: L ratios decreased. There were no significant differences in whole body and liver moisture and crude protein among dietary treatments. Whole body and liver lipid deposition increased as CHO: L ratios decreased. Plasma total cholesterol and triacylglyceride levels increased linearly as dietary CHO: L ratios decreased. Data on the dietary regulation of hepatic enzymes were also generated. Activities of glucokinase (GK) and pyruvate kinase (PK) were stimulated by elevated levels of dietary carbohydrate and activities of lipase (LPS) and alkaline phosphatase (AKP) were stimulated by elevated levels of dietary lipid. Based on second-order polynomial regression analysis of WG against dietary carbohydrate and lipid levels, 27.4% of carbohydrate and 5.9% of lipid, corresponding to a CHO: L ratio of 4.64, in a diet holding 39% crude protein and 16.3 KJ g⁻¹ of gross energy, proved to be optimal for juvenile hybrid tilapia.

Keywords: Tilapia; Carbohydrate: lipid; Weight gain; Lipid deposition

*E-mail address: gaokaijian@yahoo.cn*
Comparisons of carbohydrate utilization of herbivorous grass carp (Ctenopharyngodon idella) and omnivorous tilapia (Oreochromis niloticus×O.aureus)

Wen Gao¹*, Yong-Jian Liu ¹, Li-Xia Tian¹, Kang-Sen Mai²

¹ Nutrition Laboratory, Institute of Aquatic Economic Animals, School of Life Science, Sun Yat-sen University, Guangzhou 510275, P.R. China
² Laboratory of Aquaculture Nutrition, College of Fisheries, Ocean University of China, Qingdao 266000, P.R. China

Abstract

Two 10-week growth trials were conducted to compare carbohydrate utilization of 4.35-g herbivorous grass carp and 8.37-g omnivorous tilapia. Utilizing a 2×3 factorial design, experimental diets containing two levels of crude protein (CP; 38 and 25%) and three levels of carbohydrate (40, 20 and 0%) were formulated for use in both feeding trials. At the end of the trial, Weight gain (WG) and specific growth ratio (SGR) increased as dietary carbohydrate level increased in tilapia while the protein level was the same (P<0.05) except SGR in 25/20 and 25/40 groups. However, in grass carp diets, when the protein level was the same, WG and SGR of fish fed 20% carbohydrate diet were the highest (P<0.05) and those fed 40% carbohydrate diet were the lowest (P<0.05). The growth of tilapia was significantly (P<0.05) higher in 25/40 group than those of 25/20 and 38/0 groups, while in grass carp, the growth was significantly higher (P<0.05) in 38/0 group than those of 25/40 and 25/20 groups. Protein retention efficiency (PR) significantly (P<0.05) decreased with the increasing dietary protein level when the carbohydrate level was the same in both grass carp and tilapia. When the dietary carbohydrate level was the same, there was no significant difference in whole body moisture between fish fed 38% protein diets and 25% protein group in both grass carp and tilapia. Higher levels of dietary carbohydrate level increases the whole body, muscle and liver lipid content both in grass carp and tilapia(P<0.05). Grass carp fed 40% and 20% carbohydrate diets were hyperlipidemic(P<0.05) and there was no difference in all treatments at each dietary protein level in tilapia. The activities of hexokinase HK and pyruvate kinase PK significantly (P<0.05) increased with increasing dietary carbohydrate level at the same protein level and they were higher in tilapia than in grass carp. These data suggested that high carbohydrate diets promote tilapia growth and protein-sparing effect by carbohydrate was observed in tilapia and there was none in grass carp.

Keywords: Grass carp; Tilapia; Carbohydrate utilization

* E-mail address: gaokaijian@yahoo.cn
Effects of dietary vitamin C on growth, tissue ascorbic acid concentration and immune response of *Monopterus Albus*

Hu Yi¹, He Lanbo², Xiao Tiaoyi¹, Zhou Dinggang

¹College of Animal Technology, Hunan Agriculture, ChangSha 410128  
²College of Animal Technology, Sichuan Agriculture, YaAn 625000

Abstract

A 12-week feeding experiment was conducted to investigate the effects of dietary VC on the growth, tissue ascorbic acid concentration, stress resistance and immunity of juvenile *Monopterus Albus* (initial average weight 32.60±0.30g). Six test diets containing various levels of dietary vitamin C were formulated by supplementing ascorbic acid-2-phosphate (0 mg.kg⁻¹, 50 mg.kg⁻¹, 100 mg.kg⁻¹, 200 mg.kg⁻¹, 400 mg.kg⁻¹ and 800 mg.kg⁻¹ diet). Each diet was randomly fed to triplicate groups of 50 fishes per net cages (1.5m ×2.0m ×1.0m). The results showed that fish fed vitamin C with 50-200 mg.kg⁻¹ showed relatively higher growth compared with the control group \((P<0.05)\), the highest growth among the dietary treatments was observed when fishes fed the dietary vitamin C with 200 mg.kg⁻¹. The survival ranged from 96.67% to 100.0% and was not significantly different among the dietary treatments. Muscle, serum and hepatopancreas ascorbic acid level increased with increasing of dietary vitamin C \((P<0.05)\). Glucose and MDA in the serum of fish decreased with increasing dietary vitamin C level, fish showed relatively lower Glucose and MDA concentration compared with the control group when dietary vitamin C level more than 100 mg.kg⁻¹ and 400 mg.kg⁻¹, respectively \((P<0.05)\). The highest activity of CAT and GSH-px was observed in the fish fed diet of VC level reach 100-200 mg.kg⁻¹. The activity of lysozyme,C3,C4 and IGM in serum increased with increasing of dietary vitamin C, the activity of lysozyme,C3,C4 and IGM were significantly higher than that of control group when supplemental VC was more than 200 mg.kg⁻¹ diet, 50 mg/kg diet, 50 mg/kg diet and 100 mg.kg⁻¹ diet, respectively. The challenge experiment with *Aeromonas hydrophila* showed that the cumulative mortality in fish with 200 mg.kg⁻¹ ascorbic acids was significantly lower than that in fish with 0 mg.kg⁻¹ ascorbic acid. These results suggested that based on the WG and SGR of juvenile *Monopterus Albus*, it is proposed that the optimal vitamin C enrichment in the form of ascorbic acid-2-phosphate is about 200 mg.kg⁻¹, and vitamin C significantly influenced the stress resistance, the immune response and disease resistance of *Monopterus Albus*.

Keyword: *Monopterus Albus*; Vitamin C; Growth; Immunity; Tissue ascorbic acid concentration

*E-mail address: huyi740322@163.com*
Effects of different dietary protein, lipid and digestible energy levels on growth, nutriments utilization and body composition of Juvenile Channel Catfish (*Ictalurus punctatus*)

Guang-zhen Jiang*, Wen-bin Liu*, Xing-xing Fang, Xue-jiao Jiang, Dan-ni Liang, Su Zhuang
Laboratory of Aquatic Nutrition and Ecology, College of Animal Science and Technology, Nanjing Agricultural University, Weigang Road 1, Nanjing 210095, China

Abstract

A 60-day feeding trial of two protein levels (22%, 28%), two lipid levels (10.0%, 14.0%) and two digestible energy levels (3.4 Kcal/g, 3.0 Kcal/g) designed with three replications was conducted to investigate the growth, nutriments utilization and body composition of Juvenile Channel Catfish (141.5±1.0g initial weight). Fish were randomly distributed into 24 tanks (3m×0.8m×0.8m) at a rate of 20 fish per tank and fed three times daily. Fish were fed respectively to apparent satiation by experimental diets named as P28L10h, P28L14h, P22L10h, P22L14h, P28L10l, P28L14l, P22L10l and P22L14l. Weight gains was significantly influenced (P<0.05) by protein levels with the highest found in fish fed diet P28L10h and P28L14h in high digestible energy (3.4 Kcal/g); however, in low digestible energy (3.0 Kcal/g), P28L14l and P22L10l got highest weight gain (P<0.05), P28L10l got lowest weight gain (P<0.05). Growth were significantly (P<0.05) affect by dietary protein and lipid levels when digestible energy decreased from 3.4 Kcal/g to 3.0 Kcal/g. With the protein/energy radio increasing, it took an increasing trend to weight gain and specific growth rates (P<0.05). The digestibility of dietary protein, lipid, energy and dry matter is increasing by dietary digestible energy decreased (P<0.05); With the dietary protein decreasing, it took a decreasing trend to the digestibility of dietary protein, energy and dry matter (P<0.05), but no significantly in digestibility of lipid (P>0.05); The digestibility of dietary protein, lipid, energy and dry matter is increasing by dietary lipid levels (P<0.05). Nitrogen retention was significantly increased as dietary protein levels decreased (P<0.05), lipid retention decreased significantly with dietary digestible energy and lipid levels decreasing (P<0.05), protein efficiency radio was significantly increased as dietary digestible energy decreased (P<0.05). Carcass lipid levels was significantly increased as dietary lipid levels in low dietary digestible energy (P<0.05), but no significantly in high dietary digestible energy (P>0.05). The results of this study indicated that the diet containing 28% protein with 14% lipid and 3.0Kcal/g digestible energy is optimal for growth of Juvenile Channel Catfish. However, group P28L10h and P22L10l got second best weight, highest nutriments utilization and best body composition of Juvenile Channel Catfish.

Keywords: dietary protein; dietary lipid; digestible energy; growth; nutriments utilization; body composition; Channel Catfish.

* E-mail address: 2009205013@njau.edu.cn or jiangzhe1n@126.com (Guang-zhen Jiang)
Dietary manganese requirement for Juvenile cobia, *Rachycentron canadum*

Kang Liu*, Xiaojie Wang, Qinghui Ai, Kangsen Mai, Wenbing Zhang
The Key Laboratory of Mariculture (Education Ministry of China), Ocean University of China, Qingdao, China

Abstract
A 10-wk feeding trial was conducted to estimate the optimum dietary manganese requirement for juvenile cobia, *Rachycentron canadum* L. The basal diet was formulated to contain 50.1 % from vitamin-free casein, gelatin, fish protein concentrate. Manganese sulfate was added to the basal diet at 0.00 (control group), 6, 12, 18, 24 and 36 mg Mn kg⁻¹ diet providing 5.98, 7.23, 16.05, 23.87, 28.87 and 41.29 mg Mn kg⁻¹ diet, respectively. Each diet was fed to three replicate groups of cobia for 9 weeks, and each tank was stocked with 30 fish (initial weight: 6.27±0.01g). The Manganese concentration in rearing water was monitored during the feeding period, and was <0.01 mg/L. Dietary manganese level significantly influenced survival (SR), specific growth rate (SGR), feed efficiency ratio (FER) and the manganese concentrations in the whole body and vertebra of cobia. Base on broke-line regression of SGR, manganese concentration in whole body and vertebra, the manganese requirements of juvenile cobia were 21.72mg/kg, 22.38mg/kg and 24.93mg/kg diet in the form of Manganese sulfate, respectively. The manganese-Superoxide dismutase (Mn-SOD) activity of liver and serum increased with the increase of dietary manganese level from 5.98mg/kg to 23.87 mg/kg (P<0.05) and then had no significantly difference for the groups fed diets containing higher levels of manganese.

Keywords: *Rachycentron canadum*; manganese; requirement; fish nutrition

*E-mail address: oursmn@163.com. (Liu Kang)*
Dietary magnesium requirements of juvenile grass carp, *Ctenopharyngodon idella*

Fu-bao Wang, Li Luo, Shi-mei Lin, Yun Li, Shi Chen, Yage Wang, Hua Wen, Chongjiang Hu

1 The key Laboratory of Freshwater Fish Reproduction and Development (Ministry of Education), College of Animal Science and Technology of Southwest University, Chongqing, China
2 The key Lab of Freshwater Ecology and Healthy Aquaculture, Chinese Academy of Fishery Sciences, Yangtze River Fisheries Research Institute, Jingzhou, China
3 Chongqing Fishery Science Research Institute, Chongqing, China

Abstract

A 76-day feeding trial was conducted to determine the dietary magnesium (Mg) requirement and physiological responses of juvenile grass carp, *Ctenopharyngodon idella*. Purified casein-gelatin-based diets with six levels of supplemental Mg (0, 150, 300, 600, 1200, 2400 mg kg⁻¹) were fed to juvenile grass carp (initial weight: 7.69 ± 0.13 g). Each diet was fed to three replicate groups of fish in a closed, recirculating rearing system. The rearing water contained 5.6 mg Mg l⁻¹. No mortality or nutritional deficiency signs were observed except for growth depression in fish fed Mg-deficiency diet. Grass carp fed diet supplemented with 600 mg Mg kg⁻¹ had significantly (*P* < 0.05) highest growth performance and activities of superoxide dismutase (SOD), glutathione peroxidase (GPx) and lysozyme (LSZ). The Mg concentrations of whole-body and vertebrae of grass carp were promoted first, then tended to steady values when fed diets supplemented with ≥ 300 mg Mg kg⁻¹. Analysis by polynomial regression of WG, by broken-line regression of vertebrae Mg concentration and by linear regression of whole-body Mg retention of grass carp, indicated that the adequate dietary Mg concentration for juvenile grass carp was 618.7, 627.7 and 469.8 mg kg⁻¹ diet, respectively, supplied as magnesium sulphate.

Keywords: *Ctenopharyngodon idella*; magnesium; growth; requirement; oxidative stress
Growth and postprandial plasma profiles as affected by dietary lipid level and source in rainbow trout (*Oncorhynchus mykiss*)

Lin Luo1*, M Xue2, C. Vachot3 S. Kaushik3, I. Geurden3
1. Beijing Fisheries Research Institute, Beijing 100068, China
2. Feed Research Institute, the Chinese Academy of Agricultural Sciences, Beijing, 100081, China
3. Nutrition Aquaculture and Genomics Research Unit, UMR 1067, INRA, Pôle d’Hydrobiologie, 64310 St-Pée-sur-Nivelle, France

Abstract

The study was conducted to examine the effect of dietary medium chain triglycerides supplied by coconut oil on growth performance and on postprandial plasma lipid profiles in rainbow trout. The fish (initial body weight 71.3±0.3g, 18°C) were fed for 3 weeks one of four practical diets formulated to contain either 5% fish oil (fish oil low fat, FL), 15% fish oil (fish oil high fat, FH), 5% coconut oil (coconut oil low fat, CL) or 15% coconut (oil high fat, CH), respectively. At the end of the trial, the fish were weighed and postprandial plasma were sampled to determine glucose, triglyceride (TG), cholesterol, high density lipoprotein-cholesterol (HDL-cholesterol) and free fatty acids (FFA) at 3, 6, 9, 12, 15 and 24h, respectively. Plasma total ketone bodies (KB) were determined at 6, 12 and 24h, respectively. Plasma nitroblue tetrazolium (NBT) and myeloperoxidase (MPO) tests were also performed in plasma samples 24h after meal.

There were no significant differences in growth performance, but lipid source and level affected postprandial plasma profiles. Plasma glucose peak was found at 9-12h. Plasma glucose was higher in fish fed the low compared to high lipid level. Fish fed CH showed higher plasma TG than CL at 3h after meal, and there was no significant difference in plasma TG at the other sampling times. The peak of TG appeared 12h after the meal. No clear plateau was found for cholesterol and HDL-cholesterol in any of the groups. However, fish of FH showed highest postprandial plasma HDL-cholesterol and ratio of HDL-cholesterol/cholesterol. The peak of FFA was observed at 12-15h after meal and plasma FFA of fish fed CH was the highest. Total KB of fish decreased with postprandial time, and fish of FH groups showed higher KB than that of CL group at 6h. Besides, OD value of NBT for fish fed FH was significantly higher than that of CH, but there were no differences in MPO between groups. In summary, time-course changes in plasma profiles related to dietary lipid level were as expected whereas those related to dietary fat source were relatively small.

Key words: rainbow trout (*Oncorhynchus mykiss*); coconut oil; fish oil; growth; plasma lipids

* E-mail address: luo_lin666@sina.com
Effect of different diets on weight gain, hepatic lipase and antioxidant enzyme of juvenile silver pomfret (*Pampus argenteus*)

Peng Shi-ming*, Yin Fei, Sun Peng, Shi Zhao-hong, Wang Jian-gang
East China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Shanghai, 200090

Abstract

A 9-week feeding experiment was carried out on juvenile silver pomfret *Pampus argenteus* (initial weight, 4.80 ± 0.11g) to evaluated the effects of feed composition on weight gain, hepatic lipase and antioxidant enzyme activities. Four diets were formulated and named as diet 1 (fresh fish meat), diet 2 (fresh fish meat mixed with formulated feed), diet 3 (fresh fish meat mixed with formulated feed and solen meat) and diet 4 (fresh fish meat mixed with formulated feed, solen meat and copepods). Each diet was assigned to triplicate groups. The results showed that the weight gain of silver pomfret could be affected significantly by the feed composition. The weight gain of diet 1 was the lowest among the four diets, and was significantly lower than those of other three diets. The weight gain of diet 4 was the highest, and was significantly higher than those of diet 2 and diet 3 (*P* < 0.05). Hepatic lipase and total lipase activities of diet 1 were significantly lower than those of other diets (*P* < 0.05), but no significant difference was found in hepatic lipase, lipoprotein lipase and total lipase activities among diet 2, diet 3 and diet 4 (*P* > 0.05). There was no significant difference in superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX) activities among all diets (*P* > 0.05). However, the lowest SOD and GSH-PX activities were both in diet 1 and highest were in diet 4 (*P* > 0.05). In conclusion, high lipid content in diet could improve the growth performance of silver pomfret. Copepods could be also used in the diet to improve the growth and antioxidant ability of silver pomfret.

Keywords: Silver pomfret (*Pampus argenteus*); Diet; Growth; Lipase; Antioxidant enzyme

* E-mail address: shiming.peng@163.com (S.M. Peng)
Optimum dietary level of protein for sea cucumber, *Apostichopus japonicus*

T. Ren*, L. He, Zh-Q. Jiang, Y. Han, B. Jiang
Key Laboratory of Mariculture, Ministry of Agriculture, Dalian Fisheries University, Dalian, 116023 China

Abstract

The sea cucumber is a very important culture species in China, because of its high market value (US$800/ kg dried sea cucumber) and recent shortage of supply. A feeding experiment was conducted to determine the optimum dietary level of protein for sea cucumber juveniles based on growth and several immunological parameters. Test diets with 7 levels of protein (5, 10, 15, 20, 25, 30, and 35%) were fed to juvenile sea cucumber (1.05 ± 0.03g) once a day for 100 days. High survival rates (> 70%) were observed among all dietary treatment groups. The specific growth rates of the sea cucumber fed diets containing 10 and 15% protein were significantly ($P < 0.05$) higher than those of sea cucumber fed diets containing 35% protein. The lysozyme activity of the sea cucumber fed diets containing 15% protein was significantly ($P < 0.05$) higher than those of sea cucumber fed diets containing 5, 30, and 35% protein. The superoxide dismutase level of the sea cucumber fed diets containing 10 and 15% protein were significantly ($P < 0.05$) higher than those of sea cucumber fed diets containing 5% protein. The acid phosphatase of the sea cucumber fed diets containing 10 and 15% protein were significantly ($P < 0.05$) lower than those of sea cucumber fed diets containing more than 20% protein. Above results suggests that optimum dietary level of protein for the sea cucumber juveniles was estimated to be 10-15%.

Keywords: sea cucumber; *Apostichopus japonicus*; Protein ; Immune response

*E-mail address: sea_bream@hotmail.com*
Effects of different protein levels on blood biochemical parameters of carp (Cyprinus carpio L. minor) at different temperatures

Tang Ling ¹,², Xu Qi-You ¹, Wang Chang-An ¹, Yin Jia-sheng ¹
¹. Heilongjiang River Fisheries Research Institute of Chinese Academy of Fishery Sciences, Harbin 150070, China
². Fisheries and Life Science College of Shanghai Ocean University, Shanghai 201306, China

Abstract

The trial has been conducted to study the effects of basal diets with different temperature (18°C, 22°C, 26°C) and dietary protein levels (29%, 31%, 34%, 38%, 40%) on blood biochemical parameters of carp respectively. The experimental carps were randomly assigned to 15 groups (with 3 replicates and 14 fish in each replicate) for 8 weeks. The results showed CREA and CK were decreased with increasing temperature, at 22°C and 26°C were lower than 18°C significantly (P<0.05). TG, LDHP, AST, and AST / ALT was slightly lower at 22°C than at 18°C and 26°C (P>0.05), and CHOL was decreased significantly at 22°C than at 18°C and 26°C (P<0.05). GLU, TP, GLB, ALB were increased with rising protein levels. At 18°C, LDH and CK were increased as the protein gradually decreased, the 38% protein group was significantly reduced than 29% group (P<0.05). At 22°C, CHOL was decreased as protein levels increased, 40% group was significantly lower than other protein groups (P<0.05). At 26°C, ALT was significantly higher in 40% group than 34% and 38% group (P<0.05). Conclusion: As serum biochemical analysis, 22°C is better than 18°C and 26°C for carp life activities. The carp fed with 38%, 40% protein is appropriate at 18°C, 22°C respectively. At 26°C the high protein level is harmful to carp, so more research should be further studied for exact protein additive amount.

Keywords: carp; temperature circumstance; protein levels; serum biochemical parameters

*E-mail address: tangling2008v@yahoo.cn
Estimating the Requirements of dietary essential amino acid pattern for Young Amur Sturgeon *Acipenser schrenckii*

QiYou Xu¹, JunLing Yang¹,², ChangAn Wang ¹, Hong Xu¹, DaJiang Sun¹
¹ Heilongjiang River Fisheries Research Institute of Chinese Academy of Fishery Sciences, Harbin 150070, China.
² College of Aqua-life Science and Technology, Shanghai Ocean University, Shanghai 200090, China.

Abstract

A feeding experiments was conducted in aquaria to determine the quantitative requirements of the essential amino acids (EAA) pattern for young amur sturgeon (*Acipenser schrenckii*). Sturgeon (initial mean weight: 35.3 ± 0.6 g) were fed protein diets (PD), protein-free diets (FPD) or starved for 4 weeks. The experiment was conducted in triplicates for each of the test trials. The amino acid compositions of muscle, gastrointestinal tract, and the free amino acids in plasma of fish fed PD, FPD or starved were determined on the basis of the amino acid composition of the whole fish, daily essential amino acid requirements were estimated, the pattern of the dietary essential amino acid expressed as daily increment for young growing amur sturgeon was compared to the pattern expressed as daily increment plus daily maintenance requirement. The results were as follows: (a) The amino acid compositions of all the tissues were not influenced significantly in the starved fish and in fish fed protein-free diets; (b) the plasma free amino acids were significantly (*P*<0.05) decreased in starved fish compared to fish fed protein-free diets; (c) The estimated dietary essential amino acid pattern for young Amur sturgeon expressed as daily increment plus daily maintenance requirement (expressed in g/100g diet protein) was determined for arginine with 8.38; for histidine with 3.06; for isoleucine with 4.77; for leucine with 8.84; for lysine with 8.99; for methionine (plus cystine) with 5.78; for phenylalanine (plus tyrosine) with 8.01; for threonine with 5.11; for tryptophan with 0.83; for valine with 5.18, and for the pattern expressed as daily increment (expressed in g/100g diet protein) for arginine with 7.37; for histidine with 2.40; for isoleucine with 3.86; for leucine with 7.31; for lysine with 7.31; for methionine (plus cystine) with 5.77; for phenylalanine (plus tyrosine) with 6.57; for threonine with 4.27; for tryptophan with 0.85; and for valine with 4.28.

Keywords: essential amino acid, requirements, *Acipenser schrenckii*

* E-mail address: xuqiyou@sina.com
Effects of dietary pantothenic acid on growth, body composition and hepatic pantothnate content for Japanese seabass

Chun-xiao Zhang¹,², Kang-sen Mai¹, Qing-hu Ai¹, Lu Zhang¹
¹Key Laboratory of Mariculture, Education Ministry of China, Ocean University of China, Qingdao 266003, China
²The Key Laboratory of Science and Technology for Aquaculture and Food Safety, Fisheries College, Jimei University, Xiamen 361021, China

Abstract
A feeding trial was conducted to estimate the optimum requirement of dietary pantothenic acid for juvenile Japanese seabass, and to assess the effects of dietary pantothenic acid on growth, body composition and hepatic pantothnate content. Six purified diets were formulated to contain graded levels (0.03, 4.92, 9.80, 18.51, 35.16 and 72.08 mg pantothnate/kg dry diet) of pantothnate. Each diet was randomly assigned to triplicate groups of fish, and each group was stocked with 20 fish (initial body weight, 2.18 ± 0.26 g) in flow-through aquaria (300 l/tank). Fish were fed twice daily (06:00 and 16:30) to satiation for 9 weeks. During the experimental period, the water temperature fluctuated from 24 to 27°C and salinity from 28 to 29.5‰. Specific growth rate (SGR), feed efficiency ratio (FER), protein efficiency ratio (PER), hemoglobin and hepatic pantothnate concentration were significantly affected by the different dietary treatments. Fish fed the pantothnate-free diet performed poorly in terms of growth parameters and exhibited high mortality, clubbed gill, gill pale, dark body color, lethargy and skin hemorrhages. No deficiency signs were observed in fish fed the pantothnate-supplemented diets. On the basis of SGR or hepatic total pantothenate concentration (TP), the optimum dietary pantothenic acid requirements of juvenile Japanese seabass were estimated using broken-line regression analysis to be 11.64 or 12.09 mg/kg, respectively.

Keywords: Fish; Japanese seabass; Lateolabrax japonicus; Pantothenic acid; Feeding and nutrition

*E-mail address: cxzhang@jmu.edu.cn.
Assessment of arginine requirement for largemouth bass, Micropterus salmoides

Hengyong Zhou1*, Naisong Chen2, Xiaojie Qiu, Jianzhong Ma
Shanghai Ocean University, 999 Hucheng Huan Road, Shanghai 201306, P R China

Abstract

An 8-week feeding test was conducted to quantify the dietary arginine requirement of juvenile largemouth bass (initial weight 25g) reared in indoor flow-through and aerated aquaria. Six isonitrogenous and isolipidic (45.90% crude protein and 12.24% crude lipid of dry diet) diets were formulated to contain graded levels of arginine ranging from 1.76% to 2.92% (diets 1-6) at about 0.27% increment by adding zein-coated crystalline arginine. Equal amino acid nitrogen was maintained by replacing arginine with zein-coated nonessential amino acids. Zein-coated crystalline amino acid mixtures were supplemented in test diets to simulate the amino acid pattern of the muscle protein of largemouth bass except for arginine. Each diet was randomly assigned to quadruple groups of 35 juvenile fish. Fish were fed by hand to apparent satiation twice daily (08:00 and 16:00). During the experimental period, water temperature was maintained at 28±1℃ and the natural light cycle was adapted.

At the end of the trial, the results showed that specific growth rate (SGR) and protein retention ratio (PRR) were significantly affected by dietary arginine levels (p<0.05). The whole body crude protein content was significantly (p<0.05) affected by dietary arginine levels, while crude lipid content, moisture and ash showed no significant differences(p>0.05). The condition factor (CF) was significantly lower (p<0.05) with fish fed diet 4, but Hepatosomatic index and viscerosomatic index were not affected by dietary arginine levels (p>0.05). The activities of serum lysozyme and classical complement pathway (CH50) and the respiratory burst activity of head kidney leukocytes were significantly lower (p<0.05) with fish fed diet 1 or diet 2, compared to those with fish fed the other diets. Broken-line analysis based on SGR and PRR indicated that the optimum requirements of largemouth bass for dietary arginine were 2.28 and 2.33% of diet (4.97 and 5.08 % of dietary protein), respectively.

Keywords: largemouth bass; arginine requirement; non-special immunity

1 E-mail address: yonghengzhou@163.com
2 Corresponding author: Tel.: +86 21 61900465. E-mail address: nschen@shou.edu.cn
Effect of pre-digestion on absorption, retention and metabolism of lipids in Atlantic cod (Gadus morhua) larvae

Kristin Hamre1*, Ingochouba M. Lukram, I.E.1,2, Ivar Rønnestad3, Andreas Nordgreen1,4, Øystein Sæle1
1NIFES, PO Box 2029, 5817 Bergen, Norway
2Aqua Research Lab, Department of Zoology, University of Delhi, Delhi-110007, India
3Department of Biology, University of Bergen, Bergen, Norway
4Present address: Nofima Ingredients, Kjerreidviken 16, 5141 Fyllingsdalen, Norway

Abstract

The digestive tract of cod larvae is not fully developed and earlier reports have described a limitation in digestion of protein in early larvae. With respect to lipid digestion gene expression of bile activated lipase (BAL) and phospholipase A2 (PLA2) is low until approximately 50 days post hatch (dph), after which the expression increases dramatically until about 80 dph. However, lipase enzyme activities in cod did not change in parallel with lipase gene expression. Based on this the hypothesis of the present study was that cod larvae have a limitation in lipid digestion and that absorption of lipids would increase by pre-hydrolysation. The diets used contained 15% lipid, of which 40% was phospholipid (PL) and 60% was triacylglycerol (TAG). Cod larvae (40 dph) were fed a single meal where either PL or TAG was radioactively labeled and the labeled PL or TAG was either intact or hydrolyzed (pre-digested). The larvae were then incubated individually in chambers with collection of CO2 for 10 hours. The following fractions were analyzed for radioactivity: the incubation water (evacuated feed), the intestine, the body and the CO2 trap. The larvae ate 15-30 μg diet corresponding to 3-5.5% of dry body weight. 0-16% of the lipid was evacuated and larvae which had eaten less than 20 μg diet absorbed close to 100% of the lipid. In these larvae close to 100% of the absorbed lipid was catabolized. In larvae which ingested more than 20 μg diet, there was a linear reduction in lipid absorption to minimum 60% at the highest feed intakes. These larvae also retained an increasing amount of the lipid in the carcass in response to increased feed intake. There were only minor differences in digestion, absorption, retention and metabolism of lipid between larvae fed the different diets. This shows that 40 dph old Atlantic cod larvae have an efficient digestion and utilization of dietary lipids supplied as intact PL and TAG.

Keywords: Cod larvae; Phospholipids; Triacylglycerol; Lipid digestion; Lipid absorption; Lipid catabolism

*Email address: kha@nifes.no (K. Hamre)
P-267

The optimal lysine level of large yellow croaker (*Pseudosciaena crocea*, Richardson) larvae

Fengjun Xie, Qinghui Ai*, Kangsen Mai, Xiaojie Wang, Wei Xu, Wenbin Zhang, Hongming Ma

Key Laboratory of Mariculture, Education Ministry of China, Ocean University of China Qingdao 266003, P.R. China

Abstract

A 30-day feeding experiment was conducted to determine the quantitative requirement of lysine in large yellow croaker larvae (13 days after hatch, DAH) with initial weight of 4.08 ± 0.1mg. Six isonitrogenous and and isoenergetic micro-diets (MD), containing six levels lysine ranging from 2.48% to 4.10% (dry weight) at about 0.3% increment (diet 1-6), were used in this experiment. Triplicate groups of 3,000 fish larvae were fed to apparent satiation by hand eighth daily in floating blue tanks (188L). Both specific growth rate (SGR) and survival in fish larvae first increased and then decreased with increasing lysine levels, and treatments with 3.06% and 3.35% lysine had better results in SGR and survival. The special activities of several selected digestive enzymes were significantly influenced by different lysine levels. The ratio of specific activity of trypsin between intestinal and pancreatic segments in 3.35% lysine treatment was significant higher than that in 2.48% and 4.10% lysine treatments (p<0.05), but had no significant differences with that in 2.78%, 3.06% and 3.72% lysine treatments (p>0.05). The specific activity of Phosphatase Alkaline (PAL) in 3.35% and 3.72% lysine treatment were significant higher than that in 2.48%, 3.06% and 4.10% lysine treatment (p<0.05), and had no significant differences with that in 2.78% lysine treatments(p>0.05). The specific activity of Leusine Aminopeptidase (LAP) had a similar result with PAL. Results of this study suggest that the optimal lysine levels were 3.32% and 3.36% in MD according to survival and SGR, respectively.

Keywords: Large yellow croaker; *Pseudosciaena crocea*; Lysine; Survival; Growth; Digestive enzymes

Email: qhai@ouc.edu.cn (Q. Ai); qinghuiai@yahoo.com
Apparent digestibility of common and alternative ingredients in youth of silver arowana *Osteoglossum bicirrhosum*

**Jenny M. Moreno Poveda**, **Yolanda Ojeda Torres**, **Adriana P. Muñoz-Ramírez**


**Abstract**

The present study was performed in order to estimate the apparent digestibility coefficient (ADC) of protein and energy of 12 ingredients classified as alternatives (6) and common (6) in silver arowana (*Osteoglossum bicirrhosum*). Among the alternative use animal origin ingredients, banana and yam were outstanding that regardless their high content of soluble carbohydrates (88.88 and 84.94% respectively) presented the highest ADC for energy (96.64 and 86.36 respectively) and for protein (100% and 98.9% respectively). In the group of alternative use ingredients of animal origin, flour of piranha was outstanding both for energy (88.28%) and for protein (91.10%). Also, among the group of common ingredients of vegetable origin a behavior similar to alternative use was observed since maize regardless of having a high soluble carbohydrate level (67.83) presented the highest ADCs (energy 93.51% and protein 99.3%). Finally, among the group of common use ingredients of animal origin, ADC for energy of meat and bone flour (97.03%) was higher than the ADC for vegetable origin of common or alternative use; additionally this ingredient had the highest ADC of this group for protein (98.52%). Regardless of being classified as carnivorous specie, the high ADC of carbohydrate rich ingredients showed that arowana has capacity to digest these alternative feed sources. It is suggested that this specie can present omnivorous tendency.

**Key words:** ADC; Digestible energy; Vegetable origin; Animal origin; Digestible protein

*E-mail address: apmunozr@unal.edu.co (A.P. Muñoz-Ramírez).*
Effect of dietary protein and lipid levels on growth, survival and feed utilization of Mexican silverside (*Menidia estor*) juveniles

Jesús López-García¹, Carlos A. Martínez-Palacios¹*, M. Gisela Ríos-Durán¹

¹Instituto de Investigaciones Agropecuarias y Forestales, Universidad Michoacana de San Nicolás de Hidalgo, Av. San juanito Itzicuaro s/n, Col. San Juanito Itzicuaro, Morelia, México.

Abstract

An experiment was carried out in order to evaluate the effect of dietary protein and lipid levels on growth, survival and feeding efficiency in juveniles of Mexican silverside *Menidia estor*. Nine practical diets with increasing levels of protein (36, 43 and 50% DM) and 3 lipid levels (5, 10 and 15%) within each protein level were tested. P/E ratios of the diets ranged from 78 to 123 mg protein/kcal. Juvenile *M. estor* (560.74±74.13 mg initial weight) were fed with the diets to apparent satiation, four times a day, for 100 days. The growth, survival and feeding efficiency were evaluated every 20 days. Fish fed the diet containing 43% protein and 5% lipid (P/E ratio of 105 mg/kcal) showed the highest weight gain and specific growth rate. Those fed the other diets with 5% lipid (36 and 50% protein) (P/E ratio of 88.28 and 122 mg/kcal respectively), showed similar weight gain. Fish given diets containing higher lipid contents showed lower growth. Fish fed the diet containing 36% protein and 15% lipid (P/E ratio of 78 mg/Kcal) showed the poorest growth and survival. Body fat increased with fat content of the diet. The use of lipid levels higher than 5%, regardless of the protein content, reduces the growth, survival and performance of the juveniles. Under the experimental conditions used in the study, the diet containing 43% protein and 5% lipid with P/E ratio of 105 mg protein/kcal was found to be optimum for juvenile *M. estor*. However the data suggest that, when the energy level of the diet is maintained at 407.8 kcal/100g, the dietary protein level can be lowered from 43% to 36%, with a lipid content of 5%.

Keywords: Dietary protein and lipid levels; Mexican silverside; growth and survival

*E-mail address: fobospalacios@hotmail.com (C.A. Martinez-Palacios).
Effects of dietary lysine level on growth of soft-shelled turtle, *Pelodiscus sinensis*

Chih-Sheng Lu*, Chen-Huei Huang  
Department of Aquatic Biosciences, National Chiayi University, Taiwan

**Abstract**  
Soft-shelled turtles (*Pelodiscus sinensis*) are mainly farmed in China and other Asian countries. It is used as a dietary supplement in traditional Chinese medicine for human ailments such as hypertension, menopause, diabetes, anemia, etc. Although farming of soft-shelled turtle has been practiced for centuries, the dietary requirement of this species is not fully determined. A feeding trial was conducted to evaluate the lysine requirement of juvenile soft-shelled turtles when plant protein was the major protein source. A basal diet with 40% crude protein was prepared using corn gluten meal as the major protein. Soft-shelled turtles with initial mean body weight of 6.0 g were fed diets supplemented with 0, 2, 4, 8, 16, 32 and 64 g lysine/kg diet. The feeding trial was conducted for 8 weeks. The weight gain (WG) of turtles increased when dietary lysine increased up to 16 g/kg. Beyond this level, the WG reached plateau. Analyzed by polynomial regression, lysine supplementation level for optimal growth of soft-shelled turtles was approximately 23 g/kg based on the WG.

**Keywords:** Lysine requirement; Soft-shelled turtle

*E-mail address: s0960533@mail.nctu.edu.tw*
The present study evaluated different sources of carbohydrate, including locally available sources, on growth performance, feed utilization and key intermediary enzymes in Asian seabass, an economically important species in Thailand. A series of isonitrogenous (45%) and isolipidic (12%) diets was produced incorporating 17% glucose, dextrin, tapioca starch, corn starch, rice starch, wheat flour and rice bran. Diets were fed to apparent satiation to triplicate groups of juvenile seabass (initial weight 5.84±0.05 g) cultured in freshwater aquaria for 8 weeks. Fish fed the diet with tapioca starch had the highest (P<0.05) final weight, weight gain and specific growth rate but were not significantly different (P>0.05) from those fed the wheat flour diet. Feed intake, feed conversion efficiency and protein efficiency ratio were the highest (P<0.05) in fish fed tapioca starch and wheat flour. The fish fed the glucose containing diet had the poorest growth and feed utilization (P<0.05). Dietary carbohydrate source affected blood glucose levels such that fish fed diets containing tapioca starch, wheat flour, corn starch and rice starch had similarly high glucose levels of 106.83-122.88 mg/dl, dextrin and rice bran of 82.82-99.03 mg/dl and those fed the glucose diet 50.63 mg/dl. Fish fed diets with dextrin and wheat flour deposited higher intraperitoneal fat than other groups (P<0.05) and those fed the glucose containing diet deposited the least but exhibited the highest content of liver glycogen (P<0.05). Pyruvate kinase (PK, glycolysis), glucose-6-phosphatase (G6Pase, gluconeogenesis) and glucose-6-phosphatedehydrogenase (G6PDH, lipogenic enzyme) were affected by dietary carbohydrate source. Activities of PK were the highest in the dextrin groups and G6Pase activities in the glucose groups were distinctly elevated. Dietary dextrin and glucose increased hepatic G6PDH activity. This study indicates that tapioca starch and wheat flour are good carbohydrate sources for Asian seabass.

**Keywords:** Diet carbohydrate sources; Feed utilization; Intermediary enzymes; Asian seabass
Digestive enzyme activity in juvenile Nile tilapia (*Oreochromis niloticus* L.) submitted to different dietary levels of shrimp protein hydrolysate

Juliana F. Santos¹,², Patrícia F. Castro ³, Albino L. G. Leal ⁴, Daniel Lemos⁵*, Luiz B. Carvalho Jr¹, Ranilson S. Bezerra¹

¹ Departament of Biochemistry, Federal University of Pernambuco, Recife, Brazil.
² Serra Talhada Academic Unit, Federal University of Pernambuco, Serra Talhada, Brazil.
³ Embrapa, Parnaiba, Brazil.
⁴ CHESF, Recife, Brazil.
⁵ Department of Biological Oceanography, University of São Paulo, São Paulo, Brazil.

Abstract

The effect of different dietary inclusion levels of shrimp protein hydrolysate (SPH) on digestive enzyme activity of Nile tilapia juveniles was evaluated. SPH was included in diets at 0, 15, 30 and 60 g kg⁻¹; corresponding to treatments SPH0, SPH15, SPH30 and SPH60, respectively. A commercial diet was used as reference. Haemoglobin, azocasein, BApNA, SApNA, AA-β naphthylamide and starch were used as substrates for enzyme activity determinations. The activities of total alkaline protease, trypsin and chymotrypsin were significantly (P<0.05) higher in fish under SPH15 treatment (inclusion of 15 g kg⁻¹) than the control (SPH0). However, the effect was not dose dependent. Substrate-SDS-PAGE was also performed for the analysis of changes in the profile of Nile tilapia digestive proteases caused by the inclusion of SPH. Substrate-SDS-PAGE revealed 12 active proteolytic bands, eight of which responding to SPH dietary incorporation. Inhibition zymograms of substrate-SDS-PAGE indicated that there was a decrease in the activity of three enzymes with trypsin activity as SPH increases, whereas the opposite occurred for two aminopeptidase. Distinct protease profiles were also found for each treatment, suggesting possible adaptability of digestive proteases from Nile tilapia to the different diets.

Keywords: Nile tilapia; Shrimp processing waste; Shrimp protein hydrolysate; Digestive proteases and amylases; SDS-PAGE zymograms.

*E-mail address: dellemos@usp.br (D. Lemos).
Digestibility of alternative protein-rich feedstuffs for channel catfish, *Ictalurus punctatus*

Renato E. Kitagima and Débora M. Fracalossi*
Aquaculture Department, Federal University of Santa Catarina, Rodovia Admar Gonzaga 1346, 88034-001 Florianópolis, SC – Brazil.

Abstract

Apparent digestibility coefficients (ADC) of amino acids, crude protein, gross energy, and dry matter of canola meal, corn gluten feed, fish offal meal, shrimp and fish offal meal, poultry by-product meal, and hydrolyzed feather meal were determined for channel catfish, *Ictalurus punctatus*. Experimental diets contained 30% test-ingredient, 69.5% casein-based reference diet, and 0.5% chromic oxide. Groups of 20 fish (102.45 g) were fed the experimental diets twice a day at 3.5% body weight. Faecal samples were collected in triplicate daily at 0000 h and 0600 h after settlement into collection devices. Shrimp and fish offal meal and corn gluten feed presented significantly lower (p < 0.05) ADC for dry matter (59.5% and 39.3%) suggesting their low value as feedstuffs for catfish feeds. Although ADC values for crude protein were above 80% for all the test-ingredients, amino acid digestibility varied significantly, except for histidine whose ADC remained constant regardless the protein source. Lysine was the first limiting amino acid in most of the test-ingredients, except in fish offal meal and poultry by-product meal. Among the protein sources tested, only fish offal meal and poultry by-product meal met channel catfish amino acid requirements for a 28% digestible protein grow-out diet.

Keywords: Canola meal; Corn gluten feed, Fish offal meal; Shrimp and fish offal meal; Poultry by-product meal; Hydrolyzed feather meal

* E-mail address: deboraf@cca.ufsc.br
Lipid storage and liver size in Atlantic cod fed energy diluted diets

Gro-Ingunn Hemre¹, Ørjan Karlsen², Anette Lekva¹, Ann-Cecilie Hansen¹ and Grethe Rosenlund³
¹NIFES, National Institute of Nutrition and Seafood Research, Bergen, Norway
²Institute of Marine Research, Austevoll Aquaculture Research Station, Austevoll, Norway
³Skretting ARC, Stavanger, Norway

Abstract

Farmed Atlantic cod (*Gadus morhua* L.) is known to grow large livers. The liver index depends upon the protein to fat ratio in the diet, the leaner the lower liver index. In addition, restricted feeding will affect liver indexes, but unfortunately also reduce growth. The aim in the present project was to evaluate if liver size and lipid storage could be steered by giving energy diluted diets fed to satiation, and still obtain high growth rate. Our first experiment was designed with indigestible and inert alpha cellulose in a regression design from no added and up to 18%. All diets showed a similar protein to energy (P:E) ratio. Two basal diet mixtures were used, one was with soy protein concentrate, wheat gluten and fish meal as protein sources (Plant diet), and the other was based on fish meal only (FM diet). Major outcome from our study was that cod compensated by eating more when fed energy diluted diets, and that it adjusted its feed intake according to dietary content of protein relative to energy (P:E ratio), with no changes in liver or muscle relative sizes. Further, great health evaluated by means of hematology, and no mortality was seen even when diets held up to 18% α-cellulose, and independent of the diet being based on pure FM or a mix of plant proteins and FM. Would these results change if the agent used to dilute energy was of marine origin, e.g. crab shell or shrimp shell meal. Two new experiments, one with small, and one with larger cod were designed with low or high inclusion of these. In broad terms, the results were confirmed with crab shell meal while high inclusion shrimp shell meal resulted in reduced feed intake.

*E-mail address: ghe@nifes.no*
P-275

Effects of different diets on the digestive physiology of Norway lobster Nephrops norvegicus

G. Rotllant¹*, E. Gisbert¹, I.T. Karapanagiotidis², E. Gkolomazou² and E. Mente²

¹ IRTA. Carretera del Poble Nou km 5.5, 43540 Sant Carles de la Ràpita, Tarragona, Spain
² School of Agricultural Sciences, Department of Ichthyology and Aquatic Environment, University of Thessaly, Fytoko, 38446 Nea Ionia Magnesia’s, Greece

Abstract

The fisheries of the Norway lobster are economically important in Europe, but catches declined during the last decade. There is a need for conservation and enhancement measures. Rearing lobsters for re-stocking is a management option and then elaborating appropriated artificial diets for the species is necessary. The digestive physiology of Nephrops norvegicus was investigated in adults fed different diets (frozen mussel, fishmeal pellet diet and starvation). Thirty animals (CL=40.0±3.8 mm) were reared individually during 6 months. The histological organization of the hepatopancreas and intestine and the activity of digestive enzymes (total proteases, trypsin, chymotrypsin, pepsin, amylase, aminopeptidase-N and alkaline phosphatase) were analyzed to assess the effects of different treatments on the digestive performance of animals. The most remarkable anatomical differences among treatments were observed at the level of the hepatopancreas. The height of hepatopancreatic cells of animals fed the pellet diet were shorter than those of animals fed with mussels. In addition, hepatopancreatic tubules presented high level of lipid vacuoles whereas no signs of lipid inclusions were observed in lobster fed with mussels. Starvation dramatically affected the histological organization of the hepatopancreas showing severe signs of tissue degeneration (collapsed cytoplasm and picnotic nuclei). Lobsters fed with mussels increased their body weight in a 30.8% whereas those fed inert diets only grew a 14.5%. Starved specimens did not show any change in body weight and present the lowest enzyme activities. Trypsin activity in lobsters fed the inert diet doubled that of those animals fed fresh mussel, whereas no differences between them were detected in protease and chymotrypsin. Pepsin in the inert diet-fed group was higher than that of animals fed the fresh diet. Higher levels of amylase were detected in lobsters fed the inert diet while frozen mussels fed animals showed intermediate values. The amylase/trypsin was not affected by the diet. Knowledge on crustacean nutrition is hampered because of the experimental difficulties caused by moulting and by the feeding habits of crustaceans. Degradation of the hepatopancreas and different digestive enzyme patterns between diets were due to their different nutrient composition. These results indicated that Norway lobster is able to digest and assimilate inert diets formulated for fishes.

Keywords: Norway lobster; Compound diets; Digestive enzymes

* E-mail address: guiomar.rotllant@irta.cat (G. Rotllant).
Rainbow trout regulates lean body growth but does not adjust feed intake of diets non-limiting in protein with varying lipid levels

I. Geurden*1, J.W. Schrama2, F. Terrier1, F. Médale1, S.J. Kaushik1
1. INRA UMR 1067, NUAGE, Laboratory for Aquaculture, Genomics and Nutrition, 64310 St Pee sur Nivelle, France
2. Aquaculture and Fisheries Group, Wageningen University, PO Box 338, 6700 AH Wageningen, The Netherlands

Abstract

Feed intake (FI) of juvenile rainbow trout Oncorhynchus mykiss was assessed in relation to: 1) the nutritional history and 2) the dietary macro-nutrient composition of diets being non-limiting in protein content. Three diets were formulated: the LL (low lipid) diet with 57% CP, 11% fat and 16 kJ DE/g (on DM basis); the HL (high lipid) diet with 48% CP, 26% fat and 22 kJ DE/g, which was obtained by substituting 15% of the complete ingredient mixture of LL by 15% fish oil; and the L10 diet with 57% CP, 21% fat and 22 kJ DE/g. The L10 diet was formulated to have a similar CP level as diet LL and a similar digestible energy level as diet HL, by altering the starch to fat ratio in the LL ingredient mixture. Fish (90g, triplicates per treatment with each 100 fish) were fed to visual satiation during two consecutive 8-week phases and initial and final body composition was measured. During Phase 1, fish were fed either the LL or HP diet. At the start of Phase 2, both dietary groups of Phase 1 were split into three subgroups, which were fed diet HP, LL or L10 (triplicates per treatment with each 20 fish).

FI (in g/fish) during both Phases was not different between experimental diets. During both Phases, trout had equal protein gains irrespective of the diet, but fat deposition was higher depending on the fat level of the diets leading to differences in fish adiposity. FI during Phase 2 was unaffected by nutritional history (i.e. adiposity at end of Phase 1), but depended on the body weight at the start of Phase 2. In other words, trout of similar body mass had equal FI irrespective of the diet composition during Phase 1. Cumulated data over both Phases suggest that FI was not regulated by dietary lipid and/or DE content and that trout aimed for maximizing lean protein deposition. However, during the last week of Phase 2, trout with the highest adiposity (HL Phase1 and HL Phase2) showed a tendency for a reduced FI. This may be an indication that an increase in lipid energy is only sensed as satiating by trout when the lipid deposition capacities are exceeded.

Keywords: Feed intake; DP/DE, Dietary lipids

*E-mail address: geurden@st-pee.inra.fr (Dr. Inge Geurden)
Effect of different diets on growth performances and composition body at Red Pacu fish (Piaractus brachypomus)

M. Saedi1,*, B. Tizkar2, M.M. Sajjadi3
1Department of Fisheries, Agriculture Faculty, Hormozgan University of Iran
2Department of Aquaculture, The Mirza Koocheck Khan Higher Fisheries Education & Training Centre, Guilan, Iran
3Department of Biology, Science Faculty, Hormozgan University of Iran

Abstract
This study conducted for determine effect of plant protein (mainly, soybean meal) on growth performances and body composition in Red pacu (Piaractus brachypomus). Three isonitrogenous and isocaloric (33% protein and 2800 kcal/kg energy) diets formulated with 52.8% soybean meal (P1), 34.8% soybean meal with 20% fish meal (P2), and 15% soybean meal with 10% fish meal and 40% wheat meal (P3). 45 Fingerling Red Pacu (initial body weight of 9.12±1.81g) were equally distributed into nine aquariums (60×30×40 cm filled with 60 liters water) with stocking 5 fish in per aquarium. Fish were fed their respective diets for 8 weeks, four daily (8.00, 12.00, 16.00, and 22.00) till satiated. In this study Temperature, pH, and Dissolved Oxygen were measured daily, amount of this parameters was 25.44± 2.2(°C), 7.83± 0.22, and 7.99± 0.54(ppm), respectively. The fish readily accepted all three experimental diets and no fish died during the experimental. There were no significant differences in Weight Gain (WG), Feed Conversion Ratio (FCR), Specific Growth Rate (SGR), and Protein Efficiency Ratio (PER) in treatments, as fish fed the ingredient plant (P3) were cheaper and contain plant proteins, not significant difference to other treatments in growth performances. No significant differences in carcass protein content was found among the treatments, but fish fed the feeds in which 52.8% soybean meal had higher protein and lower lipid and ash content in carcass than those of fish fed the other diets. The results of the present study indicated that soybean meal can using to level 52.8% of fish meal protein in diets for Red Pacu with good growth and feed utilization. Also indicated high growth without reduce growth and protein composition body, when used of soybean meal with plant ingredient for source protein (e.g. wheat meal and bran meal) in diet(P3). Therefore Red pacu have high digestibility to plant ingredient and simply can using of plant ingredient in formulation Red pacu diet.

Keyword: soybean meal; Red pacu (Piaractus brachypomus); growth performances; plant ingredient

* E-mail address: saedi.majid@yahoo.com (M. Saedi)
Effects of different levels of dietary vitamin C (L-ascorbyl-2-polyphosphate) on liver total ascorbic acid concentrations and its changes under acute hypoxia in juvenile Mexican silverside (*Menidia estor*)

M. Gisela Ríos-Durán1*, Carlos A. Martínez-Palacios1

1Instituto de Investigaciones Agropecuarias y Forestales, Universidad Michoacana de San Nicolás de Hidalgo, Av. San juanito Itzícuaro s/n, Col. San Juanito Itzicuaro, Morelia, México.

Abstract

An experiment was conducted to study the effects of different levels of vitamin C, L-ascorbyl-2-polyphosphate (ASPP), on liver vitamin C concentrations and the effects of stress by acute hypoxia on such concentrations in juvenile Mexican silverside (*Menidia estor*). Four practical diets (≈2224 KJ/100g) with increasing levels of vitamin C (15, 80, 100 y 650 mg Kg⁻¹) were tested. Juvenile *M. estor* (1.3 ± 0.08 g initial weight) were fed with the diets to apparent satiation, six times a day, for 90 days. Then, the fish of all treatments were divided in two groups: a control group and a stress group. The control groups were submitted to normoxic conditions while the stress groups were submitted to acute hypoxia (≈40% of oxygen saturation) during one hour. After that, for the stress groups, the oxygen levels were returned to saturation conditions (normoxia) during 35 minutes, while control groups were maintained under normoxic conditions. The fish survival was registered during this process. At the beginning and at the end of the experiment, fish samples were taken to extract the liver and determine the Total ascorbic acid (TAA) levels and hepatosomatic index (HSI). Liver TAA concentrations significantly increased with dietary vitamin C levels. There were not significant differences in survival between control and stress groups (p>0.05) of the different treatments. When juveniles were submitted to acute hypoxia, there was a trend to increase liver TAA concentrations. There was a significant increase in HSI after the 90 days of feeding, but there were no differences in HSI values between fish fed the different diets neither between the control and stress groups. It can be concluded that liver TAA concentrations are directly related to dietary vitamin C and that one hour of acute hypoxia and the return to normoxic conditions during 35 minutes, have an influence on liver TAA concentrations in juvenile *M. estor*.

Keywords: Dietary vitamin C; Mexican silverside; liver total ascorbic acid concentrations; acute hypoxia; hepatosomatic index

*E-mail address: riosduran@gmail.com (M.G. Rios-Durán).
Is there a digestive maturation model in agastric fish species?

E. Mayra Toledo-Cuevas1*, Francisco J. Moyano López2, Carlos A. Strüssmann3, Manuel Diaz2, Carlos A. Martinez Palacios1.


3 Tokyo University of Marine Science and Technology, Faculty of Marine Science, Department of Marine Biosciences, Japan.

Abstract

Our research group has been working since several years ago in achieving the culture of a Mexican silverside known as Pez Blanco of Pátzcuaro (Menidia estor). This is an endemic freshwater species belonging to the Atherinopsidae family. Although a lot of progress has been done, it hasn’t been possible yet to develop a balanced diet that allows good growth and survival. In similar situation are “pejerreyes”, other freshwaters members of the family that are successfully cultured in Japan. Additionally, their weaning period is very long, about 5 months for M. estor and 2 months for pejerrey. It’s known that digestive physiology’s studies help to determine the most adequate weaning period that coincides with digestive maturation, and also to “design” the most appropriate feed according to their digestive capacities. However, preliminary studies in M. estor seem to show that because of being an agastric fish, they don’t follow the described digestive maturation model for marine gastric species. The aim of this work was to analysis more extensively the digestive development of two pejerrey species, Odontesthes bonariensis and O. hatcheri, also agastric species. They were reared at the Tokyo’s University of Marine Science and Technology Experimental facilities, since fertilized eggs up to 3 months post hatching, under standard culture and feeding conditions. Groups of 15 individuals were sampled each week, from the 1st post hatching week to the 9th, and then the 11th and 13th weeks. Because of their small size, hepatopancreas and intestine were extracted only from the 9th to 13th week’s fish. Whole individuals and organs were lyophilized during 48h. Enzyme extracts were prepared by homogenization in cold MilliQ water, followed by centrifugation (12000rpm, 20 min, 4°C). Sample extracts were assayed for the determination of trypsin, chimotrypsin, lipase and acid and alkaline phosphatases by fluorometric assays. Important variations in the digestive activity profiles were found during the development of each species and also among them. These findings can reflect the differences in feeding habits and their digestive abilities. Nevertheless, it seems that the described digestive maturation pattern couldn’t be found. For example, the acid phosphate activity was always lower than the alkaline one, but both increase during the ontogeny of the species.

Keywords: Digestive Maturation, stomachless, fish, Atherinopsidae.

* E-mail address: mayra.toledo@gmail.com
Comparative study of digestive enzymes in rainbow and brown trout

Renata Baric-Rafaj1*, Josipa Kules1, Emil Gjurcevic2, Zvonimir Kozaric3
1 Department of Chemistry and Biochemistry, Veterinary Faculty, University of Zagreb, Heinzelova 55, 10000 Zagreb, Croatia
2 Department of Biology and Pathology of Fishes and Bees, Veterinary Faculty, University of Zagreb, Heinzelova 55, 10000 Zagreb, Croatia
3 Department of Anatomy, Histology and Embriology, Veterinary Faculty, University of Zagreb, Heinzelova 55, 10000 Zagreb, Croatia

Abstract

The aim of this study was to build up informations and compare activities of digestive enzymes of rainbow trout (Oncorhynchus mykiss) and brown trout (Salmo trutta morpha fario). From the digestive enzyme profile it is possible to predict the ability of a species to use different nutrients. The ability to transform and use the dietary nutrients depends on the enzyme distribution over the digestive tract.

Specific activities of alkaline phosphatase (AP), amylase (AMY), lipase (LIP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamine aminotransferase (GGT) in the tissue samples (liver; anterior, middle and posterior intestine) were determinated using a automatized biochemical analyser Olympus (AU 6000).

Overall, the enzymatic activity in the digestive tract of brown trout was greater than in rainbow trout. Brown trout showed significantly higher values of lipase, ALT and AST activity, while amylase activity and amylase/lipase ratio were significantly lower. These findings suggest greater and better fat digestibility in brown trout than in rainbow trout. Because of higher amylase activity rainbow trout can better use carbohydrates than brown. Enzymes activities was the highest in the anterior and middle intestine, where the most digestion and absorption processes in fish occurred. The study of the digestive biochemistry is a very important in respect of the diversification of the fish species cultivated. Enzyme contents and distribution over the digestive tract is crucial for fish farming and determining fish nutrition.

Keywords: rainbow trout, brown trout, digestive enzymes, amylase/lipase ratio

* E-mail address: rrafaj@vef.hr (R. Baric-Rafaj)
A 60-day severe n-3 polyunsaturated fatty acid-deficient diet during the juvenile period does not improve the efficacy of a fish oil finishing strategy in rainbow trout (*Oncorhynchus mykiss*)

Jonathan Brel¹, Junio Dort¹, Mathieu Haerinck¹, Tarik Abboudi¹, Yvon, Carpentier², Yvan Larondelle¹, Xavier Rollin¹*

¹Laboratoire de Pisciculture Huet, Institut des Sciences de la Vie, UCLouvain, route de Blocry, 2, B-1348 Louvain-la-Neuve, Belgium

²L. Deloyers Laboratory for Experimental Surgery, Erasme Hospital, Université Libre de Bruxelles, Brussels, Belgium

Abstract

The aim of the present study was to determine the effect of a 60-day severe n-3 polyunsaturated fatty acid (PUFA)-deficient diet (0.09 %) during the juvenile period on the fatty acid metabolism deduced by the whole-body fatty acid balance method (Turchini et al., 2007, 2009) and on the efficacy of a fish oil (FO) finishing strategy in rainbow trout. It was hypothesized that, if fish fed a severe n-3 PUFA-deficient diet for a long period of time during the juvenile period, prior to the dietary shift to a finishing FO-based diet, an increase in the n-3 highly unsaturated fatty acids (HUFA) deposition should occur. Two groups of fish (initial body weight $0.70 \pm 0.01$ (SE) g) were reared at $11 \pm 1$ °C and fed for a 60-day conditioning period on a sunflower oil diet (SO) or a cod liver oil diet (FO). The SO group was then shifted to the FO diet for a 36-day finishing period. During the conditioning period, rainbow trout increased their mean weight by up to 20-fold in the FO group (Daily growth coefficient (DGC) of 2.5%/day) and only by 12-fold in the n-3 PUFA-restricted diet (DGC of 1.9%/day; P<0.01). At the end of this 60-day conditioning period, fatty acid composition of the diet was mirrored in fish whole-body tissues. In particular, fish fed the SO diet were deeply depleted in n-3 PUFA (<2% of total identified fatty acids). During the finishing period, trout increased their mean body weight up to 4.5-fold and grew at similar rates in both fish groups (DGC of 3.7%/day; P>0.05). No compensatory growth was observed in the SO to FO shifted group. At the end of this 36-day period, the n-3 PUFA content of the fish fed the SO diet during the conditioning period was only slightly (but significantly, P<0.01) inferior (87%) to the one observed in the FO group, confirming the success of the FO finishing strategy. However, no effects of the n-3 PUFA deficiency during the 60-day conditioning period on desaturation/elongation pathways for n-3 HUFA synthesis, on n-3 HUFA deposition (on a weight gain basis) or on the apparent *in vivo* Δ-6 desaturase and elongase activities were recorded. Therefore, the tested n-3 PUFA deficiency approach to stimulate the HUFA deposition during the finishing period has to be considered unsuccessful.

Keywords: Rainbow trout; fatty acid metabolism; fish oil finishing diet; Δ-6 desaturase; elongase

*E-mail address: xavier.rollin@uclouvain.be (X. Rollin).
Effects of dietary zinc supplementation on growth, digestive enzyme activity and antioxidant ability in grouper, Epinephelus malabaricus

Chen Cheng-xun¹, Wang Qing-kui*, Xing Ke-zhi¹, Bai Dong-qing¹, Yu Wen-wen¹, Lv Zhi-min²
¹ Tianjin Key Laboratory of Aquaculture and Aquaculture, Fisheries Science Department, Tianjin Agricultural University, Tianjin, 300384 China
² Tianjin Seafood Industrial Development Co. Ltd, Tianjin, 300450 China

Abstract

This study investigated zinc (Zn) supplemented in diet on growth, digestive enzyme activity and antioxidant ability of grouper, Epinephelus malabaricus. Zinc carbonate was added to the basal diet at 0, 13, 39, 91 and 195 mg Zn/kg diet. Each diet was fed to triplicate groups of grouper (initial body weight 57.8±0.7 g) in a recirculated rearing system for 6 weeks. Fish were fed two times daily to near satiation. Weight gain rate (WGR), specific growth rate (SGR) and condition factor (CF) increased with the increase of Zn supplemented in diet. WGR, SGR and CF in fish fed Zn supplemented diets (Zn≥39 mg Zn/kg diet) were significantly higher than those in control. Protein efficiency ratio increased while feed coefficient decreased with the increase of Zn supplemented in diet, which showed significant difference between 195mg Zn/kg diet group and control. There was a positive linear correlation between WGR and RNA/DNA ratio in muscle and liver. Correlation equation between WGR (y) and RNA/DNA ratio (x) in muscle was y=21.417x+24.724 (R² = 0.9566). Correlation equation between WGR (y) and RNA/DNA ratio (x) in liver was y=47.877x-1.439 (R² = 0.7924). The activities of proteinase, lipase in stomach and pyloric caecum and amylase in pyloric caecum increased with the increase of Zn supplemented in diet, and reached peak value in 91 mg Zn/kg diet group. Superoxide dismutase (SOD) activity in serum, liver, head kidney and spleen and catalase (CAT) activity in liver and spleen were higher than that in control group. Activities of SOD and CAT were the highest in 91 mg Zn/kg diet group. Malonaldehyde (MDA) content in serum, head kidney, liver and spleen was decreased in Zn supplemented groups, which was the lowest in 91 mg Zn/kg diet group. The adequate dietary Zn supplementation in growing grouper was 91 mg/kg diet.

Keywords: Grouper; Zinc; Growth; Digestive enzyme activity; Antioxidant ability

* E-mail address: wqkmail@yahoo.com.cn (Wang Qing-kui).
Estimation of optimal dietary protein and lipid requirements for *Trachinotus ovatus*

Hong-Yan Kou*, Yu-Tao Miao, An-Li Wang¹, Shu-Ying Xu
Key Laboratory of Ecology and Environment Science in Guangdong Higher Education, Guangdong Provincial Key Laboratory for Healthy and Safe Aquaculture, College of Life Science, South China Normal University, Guangzhou 510631, People’s Republic of China

Abstract

Linnaeus (*Trachinotus ovatus*) is one of the most high-valued aquaculture in the world due to its commercial value. There was no special purified diet for them, so it was necessary to develop a nutritionally completed artificial diet which was well-balanced and cost-effective to replace the traditional feed. It had been reported that lipid had an effect on protein-sparing in other species. Considering the importance of knowledge of the protein and lipid requirements, the objective of this paper was to estimate the optimal dietary protein and lipid requirements for *Trachinotus ovatus* by conducting a growth experiment. A 8-week feeding trial with four dietary protein levels (35%, 40%, 45%, 50%) and four dietary protein levels (8%, 12%, 16%, 20%) was conducted to estimate the optimal dietary protein and lipid requirements for the growth of *Trachinotus ovatus*. Weight gain of the fish improved with the dietary protein increase up to 40.02%, then decreased with further increase in protein level. Specific growth rate of the fish fed the diets was higher with the dietary protein increasing up to 41.04%, then decreased with further increase of protein level. Protease activity improved with the increasing protein level of fish meal, whereas there were no significant differences between the different treatments at the same protein levels. Amylase activity improved with the increase of the flour in the diets. Lipase activity increased with the increasing lipid at the same protein levels. The value of CH50 in the treatment which contained 40%, 45% protein and 12% lipid was much higher than that of the fish in other treatments. The muscle of the fish contained 19.00% essential amino acid, 31.79% amino acid, the content of tasty amino acid was high. The results of the experiment revealed that lipid had an effect on protein-sparing. The diet containing 41.04%-45.17% protein with 10.55%-12.67% lipid would be more economical and optimal for the growth and effective feed utilization of the fish.

Key words: Linnaeus; *Trachinotus ovatus*; Protein; Lipid; Protein-sparing

*E-mail address: langfanghongyan@163.com (H. Y. Kou)*
Effects of dietary carbohydrate levels on growth performance and body composition of Turbot (*Scophthalmus maximus* Linnaeus)

Xiaoning Li, Kangsen Mai*, Qinghui Ai, Wei Xu, Wenbing Zhang, Xiaojie Wang
The key laboratory of Mariculture (Ministry Education of China), Ocean University of China, 5 Yushan Road, Qingdao, 266003, P.R. China

Abstract

Three 9-week growth trials were conducted to determine the effects of dietary carbohydrate on growth performance and body composition of Turbot (*Scophthalmus maximus* Linnaeus) with average initial body weights (4.30 ± 0.01) g (trial I), (89.57 ± 1.12) g (trial II) and (208.30 ± 0.29) g (trial III). Four isonitrogenous and isolipidic diets containing graded levels of wheat meal in order to obtain 15.5%, 19.0%, 22.4%, and 25.8% of carbohydrate (%/dry matter) were randomly fed to triplicate groups of Turbot. Fish were fed to apparent satiation twice daily (07:00 and 19:00). The survival, specific growth rate (SGR), feed efficiency ratio (FER), condition factor (CF), viscera index (VSI) and hepatosomatic index (HSI) were measured. In trial I, survival showed no significant difference among dietary treatments (*P* >0.05). The SGR of the fish fed the diet containing 15.5% carbohydrate was significantly higher than those of the fish fed other diets (*P* <0.05). The FER was significantly decreased with increasing dietary carbohydrate levels (*P* <0.05). The CF of the fish fed the diets containing 15.5% and 25.8% of carbohydrate was significantly lower than those of the fish fed other diets (*P* <0.05). The VSI of the fish fed the diet containing 15.5% of carbohydrate was significantly higher than those of the fish fed the diets containing 19.0%, 22.4%, and 25.8% of carbohydrate (*P* <0.01). Comparing with lower carbohydrate levels (15.5% and 19.0%), higher dietary carbohydrate levels (22.4%, and 25.8%) significantly decreased the HSI of the fish (*P* <0.05). There were no significant differences of the survival, SGR, FER, CF, HSI and VSI among dietary carbohydrate levels in trial II and III (*P* >0.05), however the SGR showed a decreasing trend with the increasing dietary carbohydrate levels. Results of the experiment suggest that the optimal carbohydrate level for juvenile turbot is 15.5% of the dry diet, and four levels of carbohydrate are appropriate for adult turbot (initial body weights 89.57 ± 1.12g and 208.30 ± 0.29 g).

Keywords: Turbot *Scophthalmus maximus* Linnaeus; Carbohydrate; Growth; Feeding and nutrition

*E-mail address: kmai@ouc.edu.cn (Kangsen Mai).*
P-285

Protein: energy ratio in practical diets for Nile tilapia

Yan Li¹, André Moreira Bordinhon², D. Allen Davis³*, Xuezhi Zhu⁴

¹The key laboratory of Mariculture (Ministry Education of China), Ocean University of China, Qingdao 266003, P. R. China
²Agriculture and Environment Institute, Federal University of Amazonas, Rua 29 de Agosto 786 – Centro – Humaitá – AM- Brasil, CEP 69080000
³Department of Fisheries and Allied Aquacultures, 203 Swingle Hall, Auburn University, Alabama 36849-5419, USA
⁴Institute of Aquatic Economic Animals, School of Life Science, Sun Yat-sen University, 135 Xingang West Guangzhou 510275, PR China

Abstract

The present study was conducted to evaluate the interaction of protein and energy ratios in low protein diets for juvenile Nile tilapia (*Oreochromis niloticus*) (Initial weight 7.44 ± 0.03g). Nine practical diets were formulated to contain three protein levels (20, 25 and 30 g/100g), each with three digestible energy levels (260, 280 and 300 kcal/100g), the Digestible Energy/Protein ranged from 9.4 to 14.8 kcal/g protein. Each diet was randomly assigned to four replicate groups of 15 fish in a recirculation system for 10 weeks. Growth was significantly (P<0.05) affected by dietary protein and energy. Weight gain generally increased with increasing dietary protein and energy. Fish fed diet 1 (20/260), which had the lowest dietary protein (20%) and lowest energy (260kcal/100g) were significantly smaller than fish maintained on the other diets, however there was no significant difference compared to Diet 2 (25/260). The shift in FCR values was in general the reverse to weight gain. There was no interaction between dietary protein and DE levels on growth, FCR and survival. The whole body moisture and protein level were significantly influenced by dietary protein and energy content. Whereas, whole body energy content was only influenced by energy content of the diet. Similarly, dietary digestible energy content significantly influenced energy retention but dietary protein level did not. Conversely, dietary protein level significantly influenced protein retention, but did not influence energy retention. Under the reported conditions, the response of tilapia to dietary energy or protein was on seen at the lower levels of inclusion, tilapia demonstrated limited protein sparing as dietary energy was increased. However, this did modify the carcass composition through higher dietary energy.

Key words: Tilapia; Protein; Energy; Growth; Feeding; Retention

*E-mail address: ddavis@acesag.auburn.edu (D.A. Davis)
Effects of protein to energy ratios in practical diets on growth and body composition of Half-Smooth Tongue-Sole, *Cynoglossus semilaevis* Gunther

Xingwang Liu, Kangsen Mai *, Qinghui Ai, Zhiguo Liu, Wei Xu, Wenbing Zhang, Xiaojie Wang

The Key Laboratory of Mariculture (Ministry of Education), Ocean University of China, Qingdao 266003, P.R. China

Abstract

A 9-week feeding experiment was conducted to estimate the optimal dietary protein to energy (P/E) ratio for *Cynoglossus semilaevis* Gunther (initial average weight of 43.78 ± 0.18 g, mean ± S.E). Six practical test diets were formulated to contain three protein levels (45%, 50% and 55%) at two lipid levels (12.0% and 16.0%) with P/E ratios ranging from 20.79 to 26.37 mg protein kJ⁻¹. Each diet was randomly fed to triplicate groups of 20 fish per tank (1000 l). Fish were fed twice daily (08:30 and 18:30) to apparent satiation. The water temperature fluctuated from 24.0 to 28.0 °C, the salinity from 28.5‰ to 32.0‰ and dissolved oxygen content was approximately 7 mg l⁻¹ during the experimental period. The results showed that the growth was significantly affected by dietary P/E ratio (P < 0.05). Fish fed the diets with 55% protein (12% lipid, P/E ratio of 26.37mg protein kJ⁻¹) had the highest specific growth rates (SGR), feed efficiency ratio (FER), protein productive value (PPV) and energy retention (ER). SGR significantly increased with increasing dietary protein from 45% to 55% irrespective of dietary lipid (P < 0.05). However, at each protein level, fish fed the diet with 16% lipid showed significant lower growth than fish fed the diet with 12% lipid. No significant differences in survival were found among dietary treatments. Carcass protein content was significantly reduced by diet with 45% protein and 16% lipid and inversely correlated with carcass moisture content. Carcass lipid and ash contents were generally not significantly affected by dietary treatments. These results suggest that the diet containing 55% protein and 12% lipid with P/E of 26.37 mg protein kJ⁻¹ is optimal for *Cynoglossus semilaevis* Gunther and increase of dietary lipid level has not efficient protein-sparing effect.

Keywords: Half-Smooth Tongue-Sole; *Cynoglossus semilaevis* Gunther; Protein/energy ratio; Growth; Feeding and nutrition

* E-mail address: kmai@ouc.edu.cn (K.Mai)
Effects of dietary protein to energy ratios on growth and body composition of Turbot, *Scophthalmus maximus* Linnaeus

Kangsen Mai *, Xingwang Liu, Qinghui Ai, Zhiguo Liufu, Wei Xu, Wenbing Zhang, Xiaojie Wang
The Key Laboratory of Mariculture (Ministry of Education), Ocean University of China, Qingdao 266003, P.R. China

Abstract

Three separate feeding trials were performed, at different growth stages, to determine the optimal dietary protein to energy (P/E) ratio for Turbot *Scophthalmus maximus* Linnaeus (initial average weight 4.50±0.01, 59.09±0.24, and 209.12±0.21 g respectively). Six practical test diets were formulated to contain three protein levels (45%, 50% and 55%), each with two lipid levels (12.0% and 16.0%), in order to produce a range of P/E ratios (from 20.79 to 26.37 mg protein kJ⁻¹). Each diet was randomly fed to triplicate groups of 20, 15 and 12 fish per tank in three stages. Fish were fed twice daily (08:30 and 18:30) to apparent satiation for 9 weeks. Results of three experiments suggested that the growth performance and feed conversion ratio generally was improved with increasing dietary protein (P<0.05). Fish fed the diets with 55% protein (12% and 16% lipid, P/E ratio of 26.37 and 25.45 mg protein kJ⁻¹) had the highest specific growth rates (SGR), feed efficiency ratio (FER) and energy retention (ER). However, fish fed the diet with 45% protein and 16% lipid showed comparable protein efficiency ratio (PER) and protein productive value (PPV), and had higher values than fish fed the diet with 45% or 50% protein and 12% lipid, indicating a protein sparing effect of lipid. No significant differences in survival were found among dietary treatments. Both carcass lipid and energy content were positively correlated with dietary energy level except in adult stage fish. Carcass protein and ash contents were generally not affected by diet. Results of the present showed that the proper dietary protein level for turbot is above 55% and the optimum P/E ratio should be furthermore studied.

Keywords: Turbot; *Scophthalmus maximus* Linnaeus; Protein/energy ratio; Growth; Feeding and nutrition

* E-mail address: kmai@ouc.edu.cn (K.Mai)
Effects of dietary copper supplementation on growth, digestive enzyme activity and antioxidant ability in grouper, *Epinephelus malabaricus*

Wang Qing-kui¹*, Chen Cheng-xun¹, Xing Ke-zhi², Bai Dong-qing¹, Yu Wen-wen¹, Yu Xue-quan²

¹ Tianjin Key Laboratory of Aquacology and Aquaculture, Fisheries Science Department, Tianjin Agricultural University, Tianjin, 300384 China
² Tianjin Seafood Industrial Development Co. Ltd, Tianjin, 300450 China

Abstract

This study investigated copper (Cu) supplemented in diet on growth, digestive enzyme activity and antioxidant ability of grouper, *Epinephelus malabaricus*. Copper sulfate was added to the basal diet at 0, 5, 13, 24 and 41 mg Cu/kg diet. Each diet was fed to triplicate groups of grouper (initial body weight 58.4±1.1 g) in a recirculated rearing system for 6 weeks. Fish were fed two times daily to near satiation. Weight gain rate, specific growth rate and condition factor increased with the increase of Cu supplemented in diet. There was significant difference between 41 mg/kg diet group and control group. Protein efficiency ratio increased while feed coefficient decreased with the increase of Cu supplemented in diet, which showed significant difference between ≥13 mg Cu/kg diet groups and control group. There was a positive linear correlation between WGR and RNA/DNA ratio in muscle and liver. Correlation equation between weight gain rate (y) and RNA/DNA ratio (x) in muscle was y=30.592x+17.637(R²=0.9323). Correlation equation between weight gain rate (y) and RNA/DNA ratio (x) in liver was y=14.374x+12.438(R²=0.8733). The activities of proteinase, lipase and amylase in stomach and pyloric caecum were highest in 13 mg Cu/kg diet, lowest in control and 41 mg Cu/kg diet group. Superoxide dismutase (SOD) activity in spleen, head kidney, liver and serum and catalase (CAT) activity in liver were higher than that in control group. Activities of SOD and CAT were the highest in 13 mg Cu/kg diet group. Malonaldehyde (MDA) content in spleen, liver and serum was decreased in Cu supplemented groups, which was the lowest in 13 mg Cu/kg diet group. The adequate dietary Cu supplementation in growing grouper was 13 mg/kg diet.

Keywords: Grouper; Copper; Growth; Digestive enzyme activity; Antioxidant ability

*E-mail address: wqkmail@yahoo.com.cn (Wang Qing-kui).*
Effect of dietary vitamin E level on growth, tissue lipid peroxidation, and tissue antioxidant capacity of juvenile turbot, *Scophthalmus maximus*

Yuting Wei*, Xiaojie Wang, Kangsen Mai, Qinghui Ai, Wei Xu
The Key Laboratory of Mariculture (Ministry of Education), Ocean University of China, Qingdao 266003, PR China

Abstract

This study was conducted to investigate the effect of dietary vitamin E concentration on growth performance, thiobarbituric acid reactive substances (TBARS), superoxide dimutase (SOD) activity and total antioxidant capacity (T-AOC) in liver and muscle tissue of juvenile turbot (*Scophthalmus maximus*). Fish were fed one of six isoenergetic and isonitrogenous experimental diets that contained either 26, 52, 66, 132, 191, 339 mg of vitamin E/kg. Following the 8-week feeding trial, no significant (*P* > 0.05) diet-related differences were detected in growth, whole body proximate composition. The vitamin E contents of liver and serum, however, were affected by the dietary treatment. The α-tocopherol concentrations of both tissues increased (*P* < 0.05) linearly (*r^2^* = 0.9553, in liver; *r^2^* = 0.9704, in serum) as vitamin E intake increased from 26 to 339 mg/kg diet. TBARS of liver and muscle tissue of fish fed elevated dietary vitamin E (≥52 mg vitamin E/kg diet) was significantly lower (*P* < 0.05) than that noted for fish fed the diet containing no supplemental vitamin E. Fish fed diets containing 66-132 mg of vitamin E/kg had significantly increased SOD activity than other treatments in liver. SOD activity in muscle and T-AOC in liver increased significantly according to the increasing vitamin E content of diet. The results indicated that changes in tissue lipid peroxidation measurements precede clinical signs of sub-optimal vitamin E intake and the vitamin E of the diet did promote the antioxidant capacity of juvenile turbot effectively.

**Keywords**: turbot, vitamin E; lipid peroxidation; antioxidant capacity

*Email: yutingwei0028@sina.com*
P-290

Studies on Dietary Phosphorus Requirement of Young Siberian Sturgeon *Acipenser baeri*

QiYou XU, QiuShan ZHENG, Hong XU, ChanAn WANG, Dajiang SUN
Heilongjiang River Fisheries Research Institute of Chinese Academy of Fishery Sciences, Harbin, 150070, China

Abstract

495 healthy young *Acipenser baeri* weighed average 14.50±1.00g were selected and randomly distributed into 33 tanks (eleven treatments with triplicates each) to study the phosphorus requirement for 56d. The growth performance of *Acipenser baeri* improved significantly with the level of phosphorus increasing, weight gain rate arrived at 431.94% for the maximum while the phosphorus level up to 0.60%. Body ash and phosphorus content increased with the level of phosphorus increasing and remained stable while dietary phosphorus was more than 0.99% (P>0.05). There were positive correlations between ash, calcium and phosphorus content in bone plates and dietary phosphorus. Plasma calcium, plasma phosphorus content and alkaline phosphatase activity were influenced significantly by dietary phosphorus levels (P<0.05). Based on WGR and phosphorus content in fish body presented in this report, we would suggest a value of 0.50-0.87% phosphorus be used in formulating sturgeon feed.

Keywords: phosphorus; requirement; *Acipenser baeri*; young

* E-mail address: xuqiyou@sina.com
Effects of dietary n-3 HUFA on growth, fatty acid metabolism and immunity of large yellow croaker (*Pseudosciaena crocea*)

Rantao Zuo, Qinghui Ai*, Kangsen Mai, Wei Xu, Wenbing Zhang, Jun Wang, Houguo Xu, Hiskia Asino

The key laboratory of Mariculture (Ministry Education of China), Ocean University of China, 5 Yushan Road, Qingdao, Shandong 266003, P.R. China

Abstract

The present study was conducted to investigate the n-3HUFA requirement for juvenile large yellow croaker and its effects on the fatty acid metabolism and immunity. Six isoproteic and isolipidic diets were made with six different levels of n-3 HUFA ranging from 0.08% to 2.08% of the dry diet and the DHA/EPA was approximately fixed at 1.8. Each diet was randomly allocated to triplicate groups of fish in floating sea cages, and each cage was stocked with 60 fish (initial average weight 9.79±0.6g). Fish were fed twice daily (05:00 and 17:00) to apparent satiation for 58 days. The results showed that low n-3 HUFA supplementation (0.88%) significantly enhanced growth compared with the control group (*P*<0.05) while the high n-3 HUFA supplementation (1.28%, 1.68 and 2.08%) had detrimental effects on the growth with no significance (*P*>0.05). The lipid content of fish fed the diet with 0.08% n-3 HUFA supplementation was the lowest, significantly lower than the other treatments except the 0.48% n-3 HUFA supplementation group. There were no significant differences in moisture and ash content among dietary treatments. No significant differences in phagocytosis percentage (PP) was observed among dietary treatments. Other immunological parameters including NBT positive test, lysozyme and SOD activity all showed a similar trend: ascending with the increase of n-3 HUFA level and then descending with the further increase of n-3 HUFA supplementation. The NBT positive test and SOD activity were significantly higher in fish fed the diet with 0.48% n-3 HUFA than those in fish fed with the control diet (*P*<0.05), but no significance was found neither between the control and the high supplementation groups nor between the low and the high supplementation groups. The cumulative mortality of fish after infected with *Ichthyophthirius marinus* was significantly lower in the 0.48% supplementation group than the other groups except the 0.88% supplementation group (*P*<0.05). The growth and immunity results of this experiment suggest that the optimal requirement for n-3 HUFA for juvenile large yellow croaker (initial average weight 9.79±0.6g) is 0.48%-0.88% of the dry diet.

**Keywords:** Large yellow croaker; *Pseudosciaena crocea*; N-3 HUFA; Immunity; Parasite challenge; Fatty acid metabolism

*E-mail address: qhai@ouc.edu.cn (Qinghui Ai).*
Dietary leucine requirement of juvenile Japanese seabass, *Lateolabrax japonicus* and large yellow croaker, *Pseudosciaena crocea* R

Yan Li, Qinghui Ai*, Kangsen Mai, Wei Xu, Zhenyan Cheng, Zhigang He, Xiaojie Wang
The key laboratory of Mariculture (Ministry Education of China), Ocean University of China, Qingdao 266003, P. R. China

**Abstract**

A 56 day feeding trials were conducted to examine dietary leucine requirement of Japanese seabass, *Lateolabrax japonicus* and large yellow croaker, *Pseudosciaena crocea* R. in seawater floating net cages (1.5 × 1.5 × 2.0 m or 1.0 × 1.0 × 1.5 m). The basal diet was supplemented with crystalline L-amino acid premix to simulate the whole body amino acid pattern of Japanese seabass (initial body weight 8.0 ± 0.20 g) and large yellow croaker (initial body weight 6.0 ± 0.10 g) except for leucine. Crystalline L-leucine was added to the basal diet at six graded levels to formulate six isonitrogenous (crude protein 44 %) and isoenergetic (18 kJ g⁻¹) practical diets replacing with equal Glu (0.90% to 3.88% dry diet in about 0.6% increment for Japanese seabass, 1.23% to 4.80% in about 0.7% increment for large yellow croaker). Each diet was randomly assigned to triplicate groups. Fish were fed twice daily (05:00 and 17:30) to apparent satiation. The water temperature fluctuated from 26 to 32 °C, salinity from 26‰ to 30‰ and dissolved oxygen was approximately 7 mg l⁻¹ during the experimental period. With increasing dietary leucine, specific growth ratio (SGR) significantly increased \((P<0.05)\). SGR were the highest in Japanese seabass fed the diet with 2.70% Leu and in large yellow croaker fed the diet with 3.30% Leu, and thereafter, declined. On the basis of SGR, the optimum dietary L-Leu requirements of juvenile Japanese seabass and large yellow croaker were separately estimated using second-order polynomial regression analysis to be 2.38% of diet (5.67% of dietary protein), 2.92% of diet (6.79% of dietary protein). The results indicate that for growth of Japanese seabass and large yellow croaker, L-Leu is essential and fish can be able to utilize crystalline forms of L-Leu, but there was a marked decline in growth response and feed utilization beyond the optimum requirement level of L-Leu.

**Key words:** Japanese seabass; large yellow croaker; leucine; requirement; feeding and nutrition

Email address: qhai@ouc.edu.cn (Q. Ai)
P-293

Comparison of nutritive component among four culters of Shinkai River

Chen Qing-Hua1 Liu Wei2 Tang Fu-Jiang2
1 College of Aqua-life Science and Technology, Shanghai Ocean University, Shanghai, 201306, China
2 Heilongjiang River Fishery Research Institute, Chinese Academy of Fishery Sciences, Harbin 150070, China

Abstract

This study briefly compares the muscle nutritive components and nutritional quality in four wild culters (Culter alburnus, Culter mongolicus, Culter dabryi, and Cultrichthys erythropterus) of Shinkai River by conventional biochemistry methods. The result showed that the content of crude protein and fat in muscles (wet weight) of these four culters were 17.92%–19.86% and 0.12%–0.68% respectively. The content of crude protein in the muscle of C.erythropterus was significant (P<0.05) higher than that of C.alburnus and C.dabryi, while closed to C.mongolicus (P>0.05); the content of crude fat was the lowest in these four culters. So it was conclude that there were some differences in nutritional components of culter in different species. The content of total amino acid (TAA) in muscles (dry weight) from high to low were: C.erythropterus (75.08%) > C.dabryi (73.05%) > C. alburnus (72.38%) > C.mongolicus (67.20%). The order of total essential amino acid (EAA) of expermental population was: C.erythropterus (33.04%) > C.dabryi (32.89%) > C. mongolicus (32.45%) > C.alburnus (31.89%), and for contents of delicious amino acid (DAA), it was C.alburnus (24.64%) > C.erythropterus (24.52%) > C.dabryi (23.07%) > C.mongolicus (17.34%). The essential amino acids index (EAAI) was from 66.59 to 69.14, and the constitutional rate of the essential amino acids (EAA) met the Food and Agriculture Organization of the United Nations (FAO) / World Health Organization (WHO) Standard. The result demonstrated that all of four culters were high quality nutritional value fishes involved in high protein content, low fat content and comprehensive amino acids.

Key Words: Shinkai River; Culters; Muscle; Nutritional component; Amino acids

* E-mail address: dongshideshui@sina.com.
Effect of dietary lipid on the content of ω-3 fatty acids EPA and DHA in the fillet of rainbow trout (Oncorhynchus mykiss)

Susana Muñoz¹, José Caquilpan¹, Lilia Masson², Cesar Martinez³ and Paulo Palacios³
¹ Department of Animal Production, University of Chile, Santa Rosa 11315, Santiago, Chile
² Department of Food Science and Chemistry, University of Chile, Vicuña Mackenna 20, Santiago, Chile
³ Salmones Antartica, Ruta W- 853 KM 3,7 Huicha Rural, Chonchi-Isla Chiloe, X Region, Chile

Abstract

Alternatives to replace fish oil in salmonids feeds should not affect deposition or synthesis of HUFA, particularly EPA and DHA, to maintain its functional food status.

All vegetable oils by nature have low content of HUFA, however, their medium-chain fatty acids act as precursors of these. That is why the best alternative would be the one that contains a greater amount of fatty acid precursors of the family ω-3 α-linolenic acid which in turn has a low amount of fatty acid precursors of the linoleic acid family ω-6; this is because the elongation and desaturation pathways are shared. A raise in ω-3 fatty acids in the preharvest diet should increase EPA and DHA in rainbow trout fillet.

The objective of this trial was to assess the effect of modifying the fatty acid profile of the diet on fillet DHA and EPA, carcass quality and productive performance. Soybean oil was partially replaced by linseed oil in rainbow trout diet. Fish were raised in 6 sea cages under commercial condition, average initial fish live weight was 1200g and feed was supplied for 12 weeks. Fish were randomly sampled every 4 weeks to measure total weight, fork length, HG carcass weight, viscera and liver weight, sex was identified. At the same time, 12 fillet samples were taken for each treatment group, and kept frozen at -20ºC until time of analysis. Proximal analysis and fatty acid profile were conducted on raw skinless fillets including belly fat.

Productive performance didn’t show statistical differences (P<0.05) between diets. Increased α-linolenic acid in the diet significantly increased (P<0.05) content of this fatty acid measured as methyl ester % in rainbow trout fillet (4.84 vs 3.77 control), however, no effect on EPA+ DHA content as methyl ester% was observed (8.09 vs 7.92 control). Decrease in linoleic acid in the diet significantly decreased (P<0.05) content of this fatty acid measured as methyl ester % in rainbow trout fillet (28.31 vs 30.31 for the control). It can be concluded that addition of linseed oil in the diet, as 20% total oils, significantly affected linoleic and α-linolenic acids in rainbow trout fillet, so ratio ω-3 / ω-6 ratio was significantly higher (P<0.05) in the test group compared to the control group, this primarily due to linoleic and α- alpha linolenic acid content.

Keywords: Rainbow trout; EPA; DHA; Linseed oil; Linolenic acid;

Project partially financed by domeyko project, universidad de Chile and salmones antartica, Chile

* E-mail address: smunoz@uchile.cl
Microphotographic observation of gut contents and ingestion rates of fed different microalgae included diets by Gilthead Sea Bream larvae (*Sparus aurata* L. 1758)

Eryalçın, K.M.¹, Roo, J.¹, Betancour, M.¹, Atalah, E.¹, Benítez, T.¹ and Izquierdo, M.S¹

¹Grupo de Investigación en Acuicultura, ICCM & ULPGC, Ciencias Básicas, Tafira Baja, 35017 Las Palmas de Gran Canaria Canary Islands, Spain

Abstract

The aim of this study was to evaluate the effect of four microdiets on ingestion and digestion of gilthead seabream larvae. Larval starvation and mortality are mostly attributed to poor feeding ingestion and digestion of starter microdiets in marine fish larvae culture. From this point of view, observation of larval feeding activity during the feeding regime is an important topic for further evaluation of microdiets. Four microdiets were formulated with alternative single cell oil sources instead of the fish oil. Two thousand and one hundred gilthead sea bream larvae 17 DAH (day after hatching) were settled into each larval tank. After started feeding experiment, ten larvae were collected from each tank at day 17, day 24 and day 31 for determine microdiet ingestion rate and larval growth. Evaluation of ingestion rate calculated with Adobe Photoshop 7.0. Microphotographic study revealed that used different PUFA sources from single cell microorganisms *Nannochloropsis gaditana* and *Cryptothecodinum cohnii* included microdiets accepted efficiently by gilthead sea bream larvae during 14 days experimental period. There was no disorder detected in digestive system between the groups. These microdiets were accepted without any rotifer addition between 21 days to 31 days of the experiment by the larvae. Control and Cryptothecodinum cohnii group showed better growth rates than other groups. In terms of the final survival rate, there was no significant differences detected between groups. Further studies should be focused on earlier feeding regime and observation of digestion of the gilthead seabream with that types of microdiets without adding rotifer.

Keywords: Microdiets; Larval development; Feed ingestion; Gilthead seabream larvae

*E-mail address: eryalcin@istanbul.edu.tr (K.Mert ERYALÇIN).*
Evaluation of dietary cadmium threshold level of toxicity in juvenile olive flounder (Paralichthys olivaceus)

Liu Yi, Jun-Ho Lee, Seunghyung Lee, Young Chul Kim, Okorie E. Okorie, Mahmoud Mohseni, Gun Hyun Park, Nam Yong Hwang, Sungchul C. Bai*
Dept. of Aquaculture / Feeds and Foods Nutritional Research Center (FFNRC)
Pukyong Nat’l University, 599-1, Deanyeon-3-dong, Nam-gu, Busan 608-737, Korea

Abstract

This feeding trial was conducted to examine the dietary cadmium threshold level of toxicity in juvenile olive flounder, Paralichthys olivaceus. Fish averaging 5.1 ± 0.1 (mean ± SD) were fed one of the seven semi-purified diets containing 0, 200, 225, 250, 275, 300, 350, 400 and 600 mg CdCl2 kg-1 diet (Cd0, Cd200, Cd225, Cd250, Cd275, Cd300, Cd350, Cd400 and Cd600) for 6 weeks. Growth performance, Oxidative stress in tissues, cadmium accumulation in each tissues and whole-body proximate composition will be discussed later.

* E-mail address: scbai@pknu.ac.kr
Effects of dietary ethoxyquin on growth performance, and body composition of juvenile Japanese seabass, *Lateolabrax japonicus*

Jun Wang, Kangsen Mai*, Qinghui Ai, Houguo Xu, Rantao Zuo, Hiskia Asino
The key laboratory of Mariculture (Ministry Education of China), Ocean University of China, Qingdao 266003, P. R. China

Abstract

A growth experiment was conducted to investigate the effects of ethoxyquin (EQ), a synthetic antioxidant, in the diet on survival, growth, feed utilization, and body composition of Japanese seabass, *Lateolabrax japonicus*. Five experimental diets were formulated to contain graded levels of EQ (0, 50, 150, 450 and 1350 mg kg⁻¹). Each diet was randomly fed to three sea cages (1.5×1.5×2.0 m), and each cage was stocked with 30 fish. Survival was more than 78.9%, regardless of dietary EQ concentrations. Specific growth rate (SGR) was significantly influenced by dietary EQ level. Fish fed diets with or more than 150 mg kg⁻¹ EQ had a significantly lower growth rate compared with those fed diets without EQ. There was no significant difference in SGR among the fish fed diets with or less than 50 mg kg⁻¹ EQ content. Feed intake (FI) and feed efficiency ratio (FER) were not significantly different among dietary treatments. No significant differences were found in body composition among dietary treatments. The lipid content of fish fed diet with EQ was higher than those fed without EQ. The viscerosomatic index (VSI) and condition factor (CF) were lower in fish fed diet with EQ supplementation compared to those fed diet without EQ. The HSI was significantly lower in fish fed a diet containing EQ than in those fed diet without EQ, except that fed with 450 mg kg⁻¹ EQ. Results of this study suggest that EQ level in diets of large yellow croaker should not exceed more than 150 mg kg⁻¹.

Keywords: Japanese seabass; *Lateolabrax japonicus*; Ethoxyquin; Aquaculture; Growth; Feeding and Nutrition
P-298

Effect of marine bacteria (*Paracoccus* sp.) as an astaxanthin source in practical red sea bream culture

Agus KURNIA1*, Shuichi SATOH2, Hiroaki KUDO2, Makoto NAKADA2, Hiroaki MATSUMURA3, Yutaka WATANABE3 and Syougo ADACHI4

1 Department of aquaculture, Faculty of fisheries and marine science, Haluoleo University Kendari, Southeast Sulawesi, Indonesia
2 Laboratory of Fish Nutrition, Department of Marine Bioscience, Tokyo University of Marine Science and Technology, Konan, Minato-ku, Tokyo 108-8477, Japan
3 Meiji Seika Kaisha, Ltd, Veterinary products & Feed additives Tokyo Office Agricultural & Veterinary Division, Kawasaki-shi, Kanagawa-ken 212-0013, Japan
4 Nishimaru Suisan corporation, Mishima prefecture - Japan

Abstract

Two feeding experiments were conducted with different sizes of red sea bream (*Pagrus major*) to observe the effects of marine bacteria (*Paracoccus* sp.) as an astaxanthin (Asx) source on skin coloration of the fish that reared in net cage culture. In experiment I, young fish (initial weight 250 g) were fed with a diet containing lower Asx (25 mg Asx/kg) from marine bacteria for six months, and the skin coloration of the fish was compared to that of the fish on commercial feed. Fish sampling was performed once in two months. In the experiment II, marine bacteria were supplemented to the experimental feed at a level of 75 mg Asx/kg and fed to 1200 g red sea bream for three months. Skin coloration of these red sea bream was then compared to that of the red sea bream fed with commercial feed. Fish sampling was conducted in once a month.

Results in experiment I showed that total carotenoids and Asx content in skin of fish fed the diet supplemented with marine bacteria diet were not significant different with those of the fish on commercial feed. Adult fish fed diet supplemented with marine bacteria (test diet) in experiment II was also similar with fish fed on commercial diet in total carotenoids and Asx content in their skin. It was observed that marine bacteria could effect to skin coloration in short term of rearing. Therefore, it might be suggested that Asx derived from marine bacteria could be considered as a good coloration source for red sea bream in fish farming.

Key words: Marine bacteria; *Paracoccus* sp.; Adult fish; Young fish; Red sea bream

*E-mail address: fatmi_70@yahoo.com (A.Kurnia).
Studies on effects of carotenoid sources and its levels on growth performance, body color and total carotenoids contents in the Slender walking catfish (*Clarias nieuhofii*)

Suphada Kiriratnikom¹*, Anut Kiriratnikom², Punthasit Choksawatdikorn¹ and Kritsana Ruengklay¹

¹Aquacultural Biotechnology Research Unit, Faculty of Science, Thaksin University, Phatthalung Campus, 93110 Thailand.
²Division of Environmental Science, Faculty of Science, Thaksin University, Phatthalung Campus, 93110 Thailand.

Abstract

This studies were undertaken to improve body color of the Slender walking catfish (*Clarias nieuhofii*) cultured in hatchery condition. In trial I, 5 test diets were supplemented with 100 ppm of carotenoids from synthetic astaxanthin, β-carotene, canthaxanthin and dried Spirulina compared to control diet without carotenoid supplementation. Fifteen slender walking catfish (1.10-1.15 g avg. body weight) were kept in each 100 l glass aquaria with water flow thought system (3 replications per treatment). The fish in each group were fed experimental diet at satiation twice times per day. Growth performance, feed conversion ratio and survival were not significantly different (P>0.05) during 8 week of feeding trial. Colorimetric analysis, (L* a* b* color system) shown the lowest a* value in fish fed control diet and significantly different from all carotenoids supplemented diet (P<0.05). Whereas, the highest b* value found in fish fed test diet supplemented with dried Spirulina. However, total carotenoids content in fish fed astaxanthin, β-carotene and dried Spirulina supplemented diets were not significantly different (P>0.05) and higher than control fish (P<0.05). Trial II was carried out to determine the effect of Spirulina levels on growth performance, body color and total carotenoids content in the Slender walking catfish (0.68-0.72 g avg. body weight). Six test diet supplemented with dried Spirulina in the amount of 0, 2.69, 5.38, 10.75, 21.50 and 32.25 % (equivalent to 0, 25, 50, 100, 200 and 300 ppm total carotenoids) were prepared. Feeding the fish in each treatment (3 replications per treatment, 15 fish per replication) at satiation rate twice times per day for 8 week periods. Growth performances were highest in the groups fed control and the diets supplemented with 2.69-10.75 % dried Spirulina, whereas the lowest growth performance was observed in fish fed 32.25 % dried Spirulina. L* value were negatively correlated to the increase in dried Spirulina in test diet. The highest b* value found in the group fed 2.69-10.75 % dried Spirulina. However, total carotenoid were not significantly different among the fish fed all levels of dried Spirulina (P>0.05) and higher than those fed control diet (P<0.05). This result indicated that the supplementation of dried Spirulina as dietary carotenoid can improve body color and increase total carotenoid content. The practical level of dried Spirulina for improving body color of Slender walking catfish was 2.69 % or equivalent to 25 ppm total carotenoid.

Keywords: Slender walking catfish; carotenoid; Spirulina; *Clarias nieuhofii*

*E-mail address: ksuphada@yahoo.com*
Influences of oxidized fish oil with vitamin C supplementation on growth performance and reduction of oxidative stress in Red Sea Bream (*Pagrus major*)

Jian Gao¹*, Saichiro Yokoyama¹,², Manabu Ishikawa¹,², Shunsuke Koshio¹,²

¹ Science of Marine Resources, The United Graduate School of Agricultural Science, Kagoshima University, 1-21-24 Korimoto, Kagoshima 890-0065, Japan
² Faculty of Fisheries, Kagoshima University, 4-50-20 Shimoarta, Kagoshima 890-0056, Japan

Abstract

Due to fish oil contains large amount of essential n-3 highly unsaturated fatty acids is used in the formulation of commercial aquafeeds. However, it is susceptible to lipid peroxidation, and lead to undesirable influence on fish metabolism in several ways. On the other hand, vitamin C, water-soluble antioxidant is the first and major line of antioxidative defense system against radicals to prevent lipid peroxidation in plasma, however, only limit study has been performed on vitamin C requirement of fish fed oxidized lipid. Therefore, the objective of this study was to examine the influence of dietary oxidized lipid with vitamin C supplementation on growth performance, oxidative stress in red sea bream. A 50-day feeding trial on juvenile red sea bream (average weight 3.4g) was conducted in triplicate groups with seven test diets containing two degrees of oxidized fish oil (peroxide values of 83.8 and 151 meq/kg) with different level of vitamin C (0, 500 and 1000ppm) supplementation, respectively. And fresh fish oil with 500ppm vitamin C was employed as control group. Growth performance such as body weight gain (BWG), specific growth rate (SGR) and feed conversion ratio (FCR), vitamin C contents in tissues (eyes, brain, liver, muscle, heart and plasma), thiobarbituric acid reactive substances (TBARS) and blood chemical composition were taken at the end of trial. No significant difference were observed on growth performance of fish fed diets containing two degrees of oxidized lipid with 500 and 1000ppm vitamin C supplementation, TBARS in tissue and blood chemical composition showed same values compared to those in control group. However, fish fed diets containing oxidized fish oil without vitamin C supplementation indicated significantly lower growth performance, vitamin C contents and increase of TBARS value in tissues. In conclusion, oxidized lipid effect negatively on growth but more than 500 ppm vitamin C supplementation improves dietary value for red sea bream.

Keywords: Oxidized lipid; Red sea bream; Vitamin C; TBARS

* E-mail address: gaojian0106@hotmail.com (J. Gao)
P-301

Effects of dietary supplementation of lutein on growth performance, skin colour and tissue accumulation of the Chinese soft-shelled turtle, *Pelodiscus sinensis*

Haiyan Liu**, Peng Jia¹, Min Xue², Xiufeng Wu², Zhencai Yang¹, Huaxia Mu¹, Peipei Zhang¹

¹ The College of Life Science, Hebei Normal University, Shijiazhuang, P. R. China
² Feed Research Institute, the Chinese Academy of Agricultural Sciences, Beijing; P. R. China

Abstract

Aquatic animals are unable to synthesize carotenoids *de novo*, they must depend on the natural food full of the carotenoids. In intensive aquaculture, the aquatic animals often lose their natural skin colour in the production process. Chinese soft-shelled turtle (*Pelodiscus sinensis*) is an important aquaculture species, and its skin yellowness is the favourite quality for the consumer. In this study, the effects of dietary supplementation of lutein on the feed intake, growth performance, skin colour and lutein accumulation in the skin and muscle of the Chinese soft-shelled turtle were investigated. The concentrations of lutein in the diets were 1.16, 70.3, 132.0, 239.0 and 3410 mg kg⁻¹ respectively, each diet was fed to 24 turtles (initial body weight 4.81±0.04g) in a rearing system for 56d. Results from the 56d growth trial showed significant differences in growth, feed intake and feed conversion rate among treatments (*P*<0.05). The feed intake and feed conversion rate in 1.16 and 3410 mg kg⁻¹ treatments were significantly higher than other treatments, no significant difference was observed among other treatments. The specific growth rates (SGR) in the lowest lutein treatment (1.16 mg kg⁻¹) were significantly lower than 70.3, 132.0, 239.0 and 3410 mg kg⁻¹ treatments, SGR in the highest lutein treatment (3410 mg kg⁻¹) was significantly lower than 132.0 mg kg⁻¹ treatment. Skin yellowness (*b**) increased significantly with lutein supplementation (*P*<0.05), but no significant difference was observed among 132.0, 239.0 and 3410 mg kg⁻¹ treatments. Skin redness (*a**) also increased significantly with increasing lutein concentration. Whereas skin lightness (*L**) decreased with increasing lutein concentration. Lutein concentrations in skin and muscle also augmented significantly with increasing lutein level. In conclusion, appropriate lutein supplementation (70.3 mg kg⁻¹) in diets improved the growth performance and skin yellowness of the Chinese soft-shelled turtle.

Keywords: Chinese soft-shelled turtle; lutein; growth; skin colour

*E-mail address: liuhaiyan@hebtu.edu.cn.*
Influence of dietary protein and water temperature on growth and flesh quality of *Cyprinus carpio specularis*

Chang-an Wang*, Qi-you Xu, Hong Xu, Jia-sheng Yin, Tong Zhang, Yang Wang, Ling Tang
Heilongjiang River Fisheries Institute of Chinese Academy of Fisheries Sciences, 86-150070, China

Abstract

The study to investigate the influence of dietary protein levels and water temperatures on growth, body composition and flesh quality of carp (*Cyprinus carpio specularis*) which initial weight 165.24±5.08 g. Practical diets were formulated to contain five protein levels (30%, 33%, 36%, 39%, 42%), and each diet was randomly assigned triplicate groups of 14 fish at three temperatures (18℃, 22℃, 26℃) in a recycling system. Each aquarium volume of the system was approximately 220 L, were supplied with aerated water and the dissolved oxygen level was always higher than 6.0 mg L⁻¹. Fish were fed twice daily (8:00 and 16:00) to apparent satiation for 56 days. Results indicated that water temperature had the more profound effect on growth than dietary protein level. Fish had the better weight gain at temperature 22℃ and 26℃ than temperature 18℃, but there were no significant differences between temperature 22℃ and 26℃ (*P* > 0.05). The weight gain had no significant differences that fed different protein level diets at each temperature (*P* > 0.05). The body composition (protein, lipid, moisture and ash) had no significances among these treatments (*P* > 0.05). The muscle pH value were higher at temperature 18℃ than temperature 22℃ and 26℃ (*P* < 0.05), but no differences in different dietary protein level at each temperature were observed. No significant differences in flesh quality (firmness, drop loss, collagen, glycogen and lactate) were found among all the treatments (*P* > 0.05). These results suggest that if carp fed to satiation, the growth mainly depends upon the temperature, but body composition and flesh quality were found to be almost constant and unaffected by temperature fluctuations and dietary protein levels.

Keywords: Temperature; Protein; *Cyprinus carpio specularis*; Growth; Flesh quality

*E-mail address: gordoncase@126.com (C.A. Wang).*
Primary Study $\beta$-carotene on the Growth, Colouring and Deposition of Red-white koi carp ($Cyprinus carpio$ var. koi $L$)

Dongqing Bai, Shanshan Yan
Tianjin Key Lab of AQUA-Ecology and Aquaculture, Department of Fishery Science, Tianjin Agricultural University, Tianjin 300384, P.R.China.

Abstract

Koi carp value increases with intensity of body colour, which is an important quality criterion. Fish are unable to fully synthesize their own carotenoid colourings, therefore carotenoid only can be added in their diet. The study was conducted to investigate the colouring and deposition of 450 red-white koi carp ($Cyprinus carpio$ var. koi) by spectrophotometer method and this trial was undertaken to body colour enhancement in koi carp (red-white of koi carp), by feeding with different amount of the $\beta$-carotene (0, 0.5, 1, 1.5, 2 and 2.5 g kg$^{-1}$diet). The pigment components of koi carp ($Cyprinus carpio$ var. koi) was investigated by thin layer chromatography. In the trial, six homogeneous duplicate groups of 25 juvenile red-white koi carp, (initial mean body weight 19.7±5.12 g, initial mean body extent: 7.45±0.78 cm) were fed, for 8 weeks. At the end of the trials, samples of skin, head, scale and fin ray of red-white, red, white were withdrawn for subsequent analysis of total carotenoid content, and solvent evaporated to dryness, thin-layer chromatography. The results showed that spectrum of total pigment of koi carp ($Cyprinus carpio$ var. koi) had two absorption peaks in visible light (454 nm and 470 nm). Growth rate, weight gain and specific growth rate of red-white of koi carp fed 1 g·kg$^{-1}$ $\beta$-carotene tended to be obviously higher than control group ($P<0.05$), while feed conversion rate of groups fed 1-2.5 g·kg$^{-1}$ $\beta$-carotene were significantly lower than the control group ($P<0.05$). The colouring of red-white and red regional in koi carp had better at 1.5-2 g·kg$^{-1}$ $\beta$-carotene in diet. While $\beta$-carotene added in the diets did not have any effects on total carotenoid concentration of skin/scale of white regional. $\beta$-carotene mainly deposited in skin and fin ray. And it mainly transformed into astaxanthin in koi carp.

Keywords: $\beta$-carotene; red-white koi carp; total carotenoid; thin layer chromatography

*E-mail address: baidongqing@tjau.edu.cn (D.Q. Bai)*
Effect of dietary protein level on protein content and quality of Arctic charr

Jón Arnason¹*, Ólafur Ingi Sigurgeirsson² and Áðalheiður Ólafsdóttir¹
¹Máts ohf, Iceland
²University Collage at Hólar, Iceland

Abstract

Dose response trials were conducted with six weight classes of Arctic charr. Iso-energetic diets with protein content ranging from 34.7% to 49.2% were fed ad libitum to Arctic charr ranging from 0.07 grams to 1050 grams in six dose response trials.

The protein percentage in whole fish or fillet was analysed in all weight classes and revealed an effect of protein content in the diet on the protein content in the fish. The effect was particularly pronounced in the smaller fish but did not appear at all in the biggest size classes. Low protein content of feed appears to result in reduced protein content of small fish but this effect is not detectable in fish over 10 g. At the end of the trial with the biggest fish growing from 400 grams to 1050 grams a sensory evaluation was undertaken in order to see if different dietary protein content had any effect on the quality of the fish as food.

The results of analyzes from the project will be presented and discussed.

Keywords: Protein; Arctic charr; Chemical composition; Sensory evaluation

*E-mail address: jon.arnason@matís.is
Effect of dietary carbohydrate on the growth performance, fillet composition and flesh quality of *Pangasius bocourti*

Kanjana Payooha¹, Jitra Simawan¹, Chamnan Kawmanee¹ and Chutima Thongkaew²

¹Department of Fisheries, Faculty of Agriculture, Ubon Ratchathani University, Ubon Ratchathani Thailand 34190
²Department of Food Science, Faculty of Agriculture, Ubon Ratchathani University, Ubon Ratchathani Thailand 34190

Abstract

*Pangasius bocourti* is one of the most important indigenous species cultured in cage along the Mekong river in Northeast of Thailand. Feeding this species with commercial feed is not practical for the small scale farmer in Thailand so the objective of the present study was to evaluate the effect of carbohydrate on growth and flesh quality of *P. bocourti*. Cassava meal was using as the main carbohydrate source. The field experiment was conducted in cages (2*2*1.5 m³) installed in earthen pond. The juvenile fish with initial average weight of 242 g. were stocked in cages at rate of 40 fish/cage. Four practical diets were formulated with 20% protein and 4 levels of carbohydrate as followed 36, 46, 56, and 66% respectively. Fish were fed to satiation twice a day for 7 months, growth was measured monthly. The maximum final weight and daily gain of 832 g and 2.7 g./day were obtained from fish fed 46% dietary carbohydrate with 2.53 of FCR. The best FCR (2.11) was obtained from fish fed 66% dietary carbohydrate. PER increased with the increasing of dietary carbohydrate which were between 1.52-2.35. The highest percentage of fillet protein was found in fish fed 46% dietary carbohydrate, while the percentage of fillet lipid was increasing with the increasing of dietary carbohydrate. The lowest pH (6.5) of fillet was obtained from fish fed 66% dietary carbohydrate which is correlated to the hardness value of the fillet. The dietary carbohydrate was not much affecting the color of fish fillet. The value of redness and yellowness were not significantly different (P<0.05) among four treatments. The result suggest that the juvenile and adult *P. bocourti* can use the cassava meal as the carbohydrate source quite well which is suitable for small scale farmers in Northeast of Thailand where is abundance of cassava plant.

Keywords: Pangasius; Carbohydrate; Growth; Fillet composition; Flesh quality

*E-mail address: kanjanapayooha@yahoo.com*
Changes in nutritional and sensory properties induced by a plant based diet in two lines of rainbow trout selected for muscle fat content.

Geneviève. Corraze*, Sadasivam J. Kaushik, Georges Choubert, Françoise Médale
1 INRA, UMR 1067 NuAGe, Pôle d’Hydrobiologie, F-64310 Saint Pée-sur-Nivelle, France

Abstract

The aim of the study was to compare the effects of total substitution of fish meal and fish oil by plant ingredients in two lines of rainbow trout selected for high or low muscle fat content. Triplicate groups of each line (IBW: 72-88g) were fed for seven months with two diets either high in marine products (M: 30% fish meal and 70% fish oil) or totally devoid of marine products (V: 100% plant protein sources and vegetable oils). As observed previously, the lean muscle line (L) grew faster than the fat muscle line (F) irrespective of the diets. In both lines, feeding the totally plant-based diet V resulted in lower growth rate than the marine-based diet M with a lower feed efficiency. Whole body and muscle lipid contents were higher in the F line whereas viscerosomatic index was higher in the L line. The plant based diet induced an increase in fat level in the muscle and the whole body only in the F line. There were several differences between lines regarding muscle fatty acid composition with more saturated and monoenes and less C18:2 n-6, EPA, DHA in the F line compared to the L line. Consequently the n3/n6 ratio was lower in F line than in L line. As expected, the dietary treatments affected the fatty acid composition of muscle lipids: less saturated FA (C14:0, C16:0), EPA, DHA and more C18:1, C18:2 n-6, C18:3 n-3 with the diet V and thus a lower ratio n-3/n-6 compared to diet M. We observed an interaction between line and diet for several FA: 18:1, 18: 2 n-6, EPA and for n-3/n-6 ratio. Although both diets contained similar amounts of astaxanthin, the marine ingredients–based diet M resulted in significantly higher values for all the colour parameters, lightness, hue and chroma than the plant-based diet. The volatile profiles of the flesh determined by electronic nose analysis showed that the incorporation of plant ingredients affect the olfactory fingerprint only in fish with high muscle lipid content (fat line). These results highlight differences between lines in their response to the plant based diet opening new perspectives for the genetic selection of fish having a high capacity to utilise plant based diets.

Keywords: Vegetable ingredients; Genotype; Flesh quality

E-mail address: corraze@st-pée.inra.fr
Comparison of nutritional components and physical characteristics of muscle of *Fugu rubripes* from different zones

GAO Lu-jiao¹, Huang Yan-qing¹, Xia Lian-jun¹, Lu Jian-xue¹, Liu Sheng-cong²

¹ East China Sea Fisheries Research Institute, Chinese Academy of Fisheries Sciences, Key Laboratory of Marine and Estuarine Fisheries Resources and Ecology, Ministry of Agriculture, China, Shanghai 200090
² Dalian Tianzheng Industrial Company Ltd., China, Dalian 116011

Abstract

The differences of muscle in nutritional components, delicious materials and physical characteristics among three series *Fugu rubripes* from Dalian, Hebei and Dandong, were tested and analyzed in order to evaluate its nutritive value and quality. The results showed that the content of crude protein was 84.13 to 86.65% dry matter and the content of crude fat was less than 2% dry matter. But there were no significant difference among the contents of moisture, crude protein, crude lipid and ash in muscle of the three series fugu. 18 common amino acids were found in the flesh, including 8 amino acids that are essential for human body. The total content of glutamic acid and delicious amino acids were above 8.10% and 29.9~32% in dry samples, and the content of adenosine 5’-monophosphate, (IMP) was the most among the nucleotide and its metabolites, which were the reasons for fugu flesh was so delicious. On the other hand, the pattern of amino acids, the high content of essential amino acids (EAA) and poly unsaturated fatty acids (PUFA) were indicated that fugu flesh was much nutritional and healthy to human.

Keywords: *Fugu rubripes*; muscle; nutritional components; delicious materials; physical characteristics

*E-mail address: gaolujiaoyxk@126.com.*
Effect of oxidized fish oil blended with palm oil on antioxidant capacity and histology of Japanese sea bass (*Lateolabrax maculatus*) juvenile

Yuzhe Han 1, Zhiqiang Jiang 1, Tongjun Ren 1, Shunsuke Koshio 2, Jian Gao 2, Hongyue Shi 1

1 Key Laboratory of Mariculture, Ministry of Agriculture, Dalian Fisheries University, Dalian, 116023 China

2 Laboratory of Aquatic Animal Nutrition, Faculty of Fisheries, Kagoshima University, Kagoshima, 8900056 Japan

Abstract

A 60-days feeding trial was conducted to determine the effects of varies diets on Japanese sea bass (*Lateolabrax maculatus*) juvenile with initial weight of (1.73±0.01) g. Seven experimental diets were formulated with 10P, 10F, 6F4P, 4F6P, 10OF, 6OF4P and 4OF6P (P: palm oil, F: fresh fish oil, OF: oxidized fish oil). After the feeding trial, antioxidant enzymes (Catalase CAT, Superoxide dismutase SOD and Glutathione peroxidase GSH-Px) and total antioxidant capacity of vitality (T-AOC) of the liver was measured as well as histology of liver, intestine and muscle were observed to determine the effects on histology and antioxidant enzymes. The results showed that in the oxidized fish oil groups, replaced 40% fish oil with palm oil could significantly promote the activity of CAT and GSH-Px of Japanese sea bass liver (*P*<0.05). But a slight promotion was found in SOD (*P*<0.05). No significant difference was observed in T-AOC (*P*<0.05), also palm oil did not significantly effect the antioxidant enzymes activity and total antioxidant capacity (*P*<0.05) in fresh fish oil groups. Liver and muscle were healthy and without any damage in non-oxidized groups. Disservices of liver and muscle were found in 10OF, but after blended with palm oil, the symptoms were mitigated. The structure of intestines was integral and healthy in all groups. Therefore, it is available to replace fish oil partly with palm oil in Japanese sea bass diets. Mix partly of palm oil in the diet may decline the toxicity of oxidized fish oil.

Keywords: Oxidized fish oil; Palm oil; *Lateolabrax maculates*; antioxidant enzymes; histology

*E-mail address: hanyuzhe1234@hotmail.com (Dalian, China)*
Comparison of biochemical compositions of muscle of wild large yellow croaker (*Pseudosciaena crocea* R.) from different parts of China Sea

Wang Qiu-rong1*, Lin Li-min1, Wang Zhi-yong1, Xi Feng1
1 Fisheries College of Jimei University, Xiamen 361021, China

Abstract

This study was conducted to analyze biochemical compositions of muscle of four groups wild large yellow croaker (*Pseudosciaena crocea* R.) collected from different sea area of Zhejiang (Zhoushan group), Fujian (Pingtan group) and Guangdong (Naozhou group and Huilai group) Provinces, respectively. Crude protein (CP) and total amino acid (TAA) contents in muscle of large yellow croaker were significantly higher in Naozhou (CP: 81.6%; TAA: 70.9%) and Zhoushan (CP: 80.3%; TAA: 71.2%) groups than those in Huilai (CP: 77.8%; TAA: 66.5%) and Pingtan (CP: 78.4%; TAA: 67.9%) groups (\(P<0.05\)). The total free amino acid (FAA) content in muscle of four groups samples showed in the following order: Pingtan group (1702.9mg/100g)> Zhoushan group (1270.9mg/100g)>Huilai group (1082.4mg/100g)> Naozhou group (989.0mg/100g). Arginine, lysine, Phenylalanine and leucine were the most abundant essential FAA. Alanine, glycine and asparagine were the most abundant non-essential FAA. In contrast, Crude lipid (CL) and total n-3 high unsaturated fatty acid (\(\Sigma n\)-3HUFA) contents of the large yellow croaker muscle in Pingtan (CL: 10.8%; \(\Sigma n\)-3HUFA:19.2%) and Huilai (CL: 10%; \(\Sigma n\)-3HUFA: 16.9%) groups were significantly higher than those in Naozhou (CL: 7.6%; \(\Sigma n\)-3HUFA: 12.8%) and Zhoushan (CL: 6.5%; \(\Sigma n\)-3HUFA: 12.7%) groups( \(P<0.05\)). There were no significant difference in the contents of total saturated and monounsaturated fatty acids in the muscle among large yellow croaker of four groups(\(P>0.05\)). The predominant fatty acids in muscle were C16:0, C16:1, C18:1n-9, C20:6n-3 (DHA). The percentage of C18.2n-6 and C20:1 was relatively low, only accounts for 0.5%~ 0.9% and 0.5%~1.4% of fatty acid total, respectively. These suggested that inhabited environment, nature food quality and quantity may play a major role in affecting the chemical compositions of muscle of wild large yellow croaker. The results also showed that wild large yellow croakers are good food sources of protein and n-3HUFA.

Keywords: Large yellow croaker; Wild; Fatty acid; Amino acid

*E-mail address: Wqiurong@hotmail.com*
P-310

Effects of dietary arginine on survival, growth, nonspecific immune responses and disease resistance in juvenile darkbarbel catfish (*Pelteobagrus vachelli*)

Fuxian Feng¹, Hongming Ma¹*, Kangsen Mai¹, Qinghui Ai¹, Wenbing Zhang¹, Yi Hu², Qingfei Li¹, Yun Huang²

¹The key laboratory of Mariculture (Ministry Education of China), Ocean University of China, 5 Yushan Road, Qingdao, Shandong 266003, P.R. China
²College of Animal Science And Technology, Hunan Agriculture University, Changsha, Hunan 410128, P.R. China

Abstract

A 10-week feeding trial was conducted to estimate dietary arginine (Arg) requirement of juvenile darkbarbel catfish and its effects on nonspecific immune responses and disease resistance when challenged with *Aeromonas hydrophila*. Six diet were formulated with grade levels of Arg (1.24, 1.48, 1.72, 1.96, 2.2 and 2.44% of the dry diet) with 42% crude protein and 10% crude lipid. Each diet was fed to triplicate groups of darkbarbel catfish (initial body weight of 1.02±0.01 g) to apparent satiation for 10 weeks and each group was stocked with 50 fish. After the feeding trial, growth, biochemical and nonspecific immune parameters were monitored. Percent survival, weight gain (WG) and specific growth ratio (SGR) were significantly affected by dietary arginine concentrations (*P* < 0.05). The percent survival was significantly higher in fish fed the unsupplemented diet (1.24% Arg of diet) compared with other dietary groups (*P* < 0.05). The arginine requirement of juvenile darkbarbel catfish on broken-line analyse of SGR were 2.10% of diet. The respiratory burst activity and phagocytosis percentage (PP) of head kidney leukocytes increased significantly with the increase of dietary Arg. Serum lysozyme activity was significantly (*P* < 0.05) higher in fish fed with 2.2% diet than that in other groups. However, there were no significantly differences in Nitric Oxide Synthase (NOS) in liver and serum and the challenge experiment with *Aeromonas hydrophila* also showed no difference between groups (*P* > 0.05). From the present experiment, it may be concluded that the Arg requirement of juvenile darkbarbel catfish based on SGR is 2.10% Arg of dry diet and Arg supplementation may have benefits on enhancing immune responses.

Keywords: Darkbarbel catfish; Arginine; Dietary requirement; Nonspecific immune response

E-mail address: mahongm@ouc.edu.cn
Effects of Lysine and methionamine supplementation to different protein level practical diet for grass carp *Ctenopharyngodon idella*

Gan Lian¹,² *, Yong-Jian Liu ¹, Li-Xia Tian ¹, Hui-Jun Yang ¹, Yi-Rong Yue ¹, Yong-Jun Chen ¹, Jian-Jun Liang ¹, Gui-Yin Liang ¹
1 Life science college, Sun Yat-Sen University, Guangzhou, China
2 Animal science college, South China Agriculture University, Guangzhou, China

Abstract

A 62-day growth trial was undertaken to estimate effects of crystalline amino acid (CAA) supplementation of diets with different protein levels on growth performance and feed utilization in Grass Carp (*Ctenopharyngodon idella*). Six plant-based practical diets were prepared, and they were formulated to contain three graded protein levels (Crude protein: 32%, 30%, and 28%) with CAA supplementation. In the supplementary group, lysine and methionine were added to ensure the amino acid requirement of Grass Carp according to the whole body composition at 32% CP. In the groups without CAA supplementation, weight gain (WG) of the fish fed 32% CP diets was significantly higher than that of 30% CP and 28% CP diets, but no significant difference were found between 30% CP and 28% CP groups. In the groups with CAA supplementation, higher improvement of WG was observed at fish fed 32% CP and 30% CP diets, while the SGR was not significantly different between them. Feed conversion ratio (FCR) was significantly lower in groups of 32% CP and 30% CP with CAA supplementation, but no difference were found at 28% CP groups. Viscerosomatic index (VSI), hepatosomatic index (HSI), intraperitoneal fat ratio (IPF), the whole body and muscle fat was reduced after CAA supplementation at every protein level. The results indicated that CAA supplementation to the plant meal-based practical diets at different protein levels can improve the growth performance and feed utilization of grass carp.

Keywords: Grass carp; Lysine; Methionine; Amino acid supplementation;

* E-mail address: edls@mail.sysu.edu.cn (Liu Yong Jian).
Effect of ghrelin on the food intake and growth of grouper 
( *Epinephelus coioides*)

Yu-Jie Gao*, Yong-Jian Liu, Li-Xia Tian, Gui-Ying Liang, Yi-Rong Yue, Wen-Jia Luo, Shu-Yun Zhou
Nutrition Laboratory, Institute of Aquatic Economic Animals, School of Life Science, Sun Yat-sen University, Guangzhou 510275, P.R. China

Abstract

This experiment was conducted to investigate the effect of grouper ghrelin on food intake and growth of grouper, *Epinephelus coioides*. Two kinds of ghrelin were supplemented in the basal diet, acyl and des-acyl ghrelin, and each added at the dose of 0, 2, 4 mg/kg diet. Each diet was fed to fourfold groups of 15 fish (initial body weight 84.80±0.38 g) grown in seawater at 22.5-28.2 °C for 8 weeks. Fish were fed twice a day to visual satiety.

After 8 weeks trial, the effects of ghrelin on food intake, growth, feed utilization and body composition were determined. Average food intake, weight gain (WG, %), average final body weight (FBW, g) and specific growth rate (SGR) of the groups added acyl-ghrelin at the dose of 4 mg/kg diet were significantly higher compare to the control groups (P<0.05), the groups added either acyl ghrelin or des-acyl ghrelin at other dose were higher than those in the control groups, but no significant difference were found. Though there were no significant differences in average food intake and growth performance (WG, FBW, and SGR) among the groups that fed 2, 4 mg/kg diet acyl ghrelin and 2, 4 mg/kg diet des-acyl ghrelin, the groups fed acyl ghrelin were higher than the groups fed des-acyl ghrelin and was found rely on the dose of ghrelin that supplemented. Supplemented ghrelin in the diet had no signification effect on feed utilization and body composition of the grouper.

Keywords: Acyl ghrelin, Des-acyl ghrelin, Food intake, Growth

* E-mail address: g723_yj@163.com (Yu-Jie Gao)
Effect of partial replacement of calcium dihydrogen phosphate with neutral phytase on growth performance, body compositions and haemato-biochemical parameters of *Ctenopharyngodon idellus*

Li-wei Liu*, Yu-liang Luo¹, Hong-li Hou²
1 College of Fisheries, Huazhong Agricultural University, Wuhan, China
2 WUHAN SUNHY BIOLOGY CO., LTD, WuHan, China

Abstract

A 9-week feeding trial was conducted in flow through system to examine the effect of partial replacement of calcium dihydrogen phosphate with neutral phytase on growth, body compositions, haemato-biochemical status and intestinal digestive enzyme activities of gass carp, *Ctenopharyngodon idellus* (initial average weight: 31.45±4.68 g). The aim of this experiment was to determine how many calcium dihydrogen phosphate in gass carp diet could be reduced if neutral phytase (500 U/kg) was supplemented. Control diet supplemented with 2% of calcium dihydrogen phosphate without neutral phytase supplementation (designated as P0). Three experimental diets were formulated to replace calcium dihydrogen phosphate by neutral phytase (500 U/kg) at 25%, 50%, and 75% (designated as P25, P50, P75, respectively). After acclimation of fish for 2 weeks, 15 fish were randomly stocked into triplicate tanks (300-L) for each of four treatments. Average water temperature during the experiment period was 26-28°C and DO was 7.06-7.83 mg L⁻¹. Results indicated that compared with control group, fish in P25 and P50 groups fed with the phytase-supplemented diets had no significant effect on weight gain (WG), specific growth rate (SGR), protein efficiency rate (PER) and feeding rate (FR). Crude protein, ash, moisture and phosphorus contents of whole body and muscle showed no significant differences from control group; however, muscle lipid of fish fed with the phytase-supplemented diets were significantly lower than control group (*P*<0.05). For serum indices, alkaline phosphatase (Alkp), alanine transaminase (ALT), aspartate transaminase (AST) and albumin (ALB) in fish fed with the phytase-supplemented diets were significantly higher than control group (*P*<0.05); meanwhile, significant increases were observed in P50 and P75 groups on blood calcium, cholesterol (CHO), high density lipoprotein (HDL) and total protein (TP) (*P*<0.05); but serum inorganic phosphorus and triglyceride (TG) contents were not significantly increased. Dietary phytase inclusions significantly influenced several digestive enzyme activities (*P*<0.05), but had no effect on the hepatic Alkp, ALT, AST and lipase activities. The present study indicates that the optimum level of calcium dihydrogen phosphate in gass carp diet replaced by neutral phytase (500 U/kg) is 25%, and almost no effect on growth was observed while neutral phytase (500 U/kg) replace up to approximately 50% calcium dihydrogen phosphate.

Keywords: Neutral phytase; Calcium dihydrogen phosphate; Growth performance; Body compositions; Haemato-biochemical parameters; *Ctenopharyngodon idellus*

* E-mail address: liulw.870226@webmail.hzau.edu.cn (Li-wei Liu)
Effects of different acidifiers on the growth performance, metabolism and non-specific immunity of *Ctenopharyngodon idellus*

Wei-min Shang 1, Li Luo 1, Si-chao Jia 1, Chaoming Wang 1, Guizhong Zhang 1
1 College of Animal Science and Technology, Southwest University, Beibei, Chongqing 400716, China

Abstract

A 8-week feeding trial was conducted to investigate effects of different dietary acid supplements on growth performance, metabolism and non-specific immunity of *Ctenopharyngodon idellus* (average body weight was about (6.30±0.22)g). 5 test diets were applied for triplicate tanks and 30 fish was stocked randomly in each tank. No acid supplement was added in basal diet which was applied as control, 0.21% of phosphoric acid, citric acid, lactic acid and composite acidifier were added in basal diet, respectively. The results showed that the weight gain, specific growth rate were higher and the feed conversion rate was lower than those of fish fed basal diet. The differences of feed efficiency in fish fed diets with phosphoric acid, citric acid and lactic acid were not significant (P>0.05). However, significant higher feed efficiency was observed in fish fed diet with composite acidifier than that of fish fed basal diet (P<0.05). No significant differences were observed in the whole body moisture, protein, lipid and ash(P>0.05). However, the contents of Ca, Mg, Fe, Mn and Zn mineral elements were significant higher in fish fed diet with composite acidifier than that of fish fed basal diet (P<0.05). The activity of protease in intestine and hepatopancreas in fish fed diets with different acidifiers was significantly higher than that of fish fed basal diet (P<0.05). Significantly higher activities of hepatic amylase were observed in fish fed diet with citric acid, lactic acid and composite acidifier than that of fish fed basal diet (P<0.05), while no significant differences were observed in fish fed diet with phosphoric acid (P>0.05), and significantly higher activities of hepatic lipase were observed in fish fed diet with lactic acid and composite acidifier than that of fish fed basal diet (P<0.05). The activities of SOD and GSH-PX in fish fed diets with different acidifiers were significantly higher than that of fish fed basal diet (P<0.05), while the activity of MDA was significantly lower than that of fish fed basal diet (P<0.05). The content of lysozyme was in fish fed diets with different acidifiers was significantly higher than that of fish fed basal diet (P<0.05). Significantly higher activities of AKP and SOD were observed in fish fed diets with lactic acid and composite acidifier than that of fish fed basal diet(P<0.05). The activity of intestinal AKP in fish fed diets with different acidifiers was improved, but with no significant difference (P>0.05). The results indicated that different acidifiers in fish feed diets can significantly improve growth performance, metabolism and non-specific immunity. Accordingly, the feed efficiency in fish fed diets with composite acidifier was best.

Key words: acidifiers; *Ctenopharyngodon idellus*; growth; metabolism; non-specific immunity
P-315

Effects of dietary phytase on growth and feed utilization of jade perch, *Scortum bacoo*

Song li ping, Wang ai ying, Hu bin, Mao shu quan, Han bo
Freshwater Fisheries Research Institute of Shandong province, 3 Weili Zhuang, Jinan Shandong 250117, China

Abstract

A 60-day feeding study was conducted to investigate the effect of dietary phytase on the growth performance, feed utilization and digestive enzyme of jade perch juveniles. The basal diet designed with fish meal as protein source was used as control, the trial diets were designed with partially replacing fish meal with soybean meal as replacing fish meal group, replacing fish meal group with 0.02% phytase as phytase group. The results showed that jade perch fed the control diet showed higher final body weight, daily gain (DG), specific growth rate (SGR), feed efficiency ratio (FER), protein efficiency ratio (PER), protease and lipase activities than replacing fish meal group ($P<0.05$), and no significant differences compared with phytase group ($P>0.05$). Fish fed the replacing fish meal group diet showed lower final body weight, DG, SGR, FER, PER, protease and lipase activities than phytase group ($P<0.05$). Fish fed basal and phytase groups diet exhibited better apparent digestibility coefficient (ADC) of protein, lipid, dry matter, total phosphorous and calcium than replacing fish meal group ($P<0.05$). Retention of nitrogen in replacing fish meal group was significantly lower than the control group ($P<0.05$), and load of nitrogen in replacing fish meal group was significantly higher than phytase group. No differences between control and phytase groups were found for retention and load of nitrogen ($P>0.05$). Fish fed phytase group diet exhibited higher retention and lower load of phosphorous than other groups ($P<0.05$). Load of phosphorus in control group was significantly higher than the other groups ($P<0.05$). Supplementation with soybean meal and phytase had no influence on retention of calcium ($P>0.05$) and affected load of calcium with a decreasing tendency ($P<0.05$).
**P-316**

**Effect of acidifier on growth performance and feed nutrient digestibility of common carp (*Cyprinus carpio*)**

**WANG Ji-ting**¹ **XIAO Xin-wu**¹ **SUN Meng-ming**¹ **WAN Wen-ju**²

1. College of Animal Science and Technology, Shandong Agricultural University, Tai’an, Shandong 271000;
2. Department of Basic Medicine, Taishan Medical College, Tai’an, Shandong)

**Abstract:**

A feeding trial was conducted to study the effects of acidifier on growth performance and feed nutrient digestibility of common carp. All of 360 fishes were randomly allotted to three groups (four replicates for each group) and fed diets containing different levels (0, 0.2%, 0.3%) of compound acidifier. The results showed that: (1) The relative weight gain, special growth rate, feed conversion rate, feed protein efficiency, protein deposition rate were improved significantly \((P<0.05)\) by supplement of compound acidifier in common carp diet. (2) Supplementation of compound acidifier in common carp diet significantly improved the apparent digestibility of dry material, crude protein and phosphor in diets. (3) Supplementation of compound acidifier in common carp diet did not significantly affect the fish body protein, lipid, ash, moisture contents, coefficient of fatness and viscerosomatic index. Result above showed that adding composite acidifier could significantly enhance the growth performance, by improving crude protein and phosphor digestibility in diets.

**Key words:** composite acidifier; Common carp; growth; nutrient digestibility

*E-mail address:* wwenju2003@yahoo.com.cn
Evaluation of GroBiotic®-A in regard to growth, muscle composition, immune responses and resistance against Aeromonas hydrophila in tilapia (*Oreochromis niloticus*)

Zonglin. Zheng 1, 2, Delbert M. Gatlin III 3, Kaiyu Wang 1
1School of Veterinary Medicine of Sichuan Agriculture University, Sichuan 625014, P. R. China
2Fisheries Breeding and Healthy Cultivation Research Centre, South-west University, Chongqing 402460, P. R. China
3Department of Wildlife and Fisheries Sciences and Faculty of Nutrition, Texas A&M University System, College Station, Texas 77843-2258, USA

Abstract

It is becoming more readily apparent that prebiotics, non-digestible dietary ingredients that can beneficially affect the host by selectively stimulating the growth of and/or activating the metabolism of health-promoting bacteria in the intestinal tract, can confer numerous beneficial effects to fish in aquaculture. Responses such as increased weight gain, feed efficiency and nutrient digestibility as well as enhanced disease resistance have been observed in numerous fish species fed GroBiotic®-A, a prebiotic composed of partially autolyzed brewers yeast, dairy sugars and fermentation products. This study was conducted to evaluate the potential of graded levels of GroBiotic®-A to reduce the diet protein requirement of tilapia. Two control diets were formulated to contain 33% (positive control) or 29% crude protein (negative control) to which three incremental levels of GroBiotic®-A (0.4%, 0.8% and 1.2% of diet) were added in place of cellulose.

Enhanced weight gain and feed efficiency were generally observed in fish fed the diets supplemented with GroBiotic®-A compared to the negative control diet. No significant differences in these responses were observed between fish fed diets supplemented with GroBiotic®-A compared to those fed the positive control diet. Supplementation of 0.8% and 1.2% GroBiotic®-A induced significantly lower condition factor and hepatosomatic index compared to fish fed the negative control diet, but those values were similar to that of fish fed the positive control diet. GroBiotic®-A supplementation and protein reduction had no effect on the viscerosomatic index of fish or moisture, lipid and protein content of muscle samples. However, muscle ash increased significantly with protein reduction (negative control), but GroBiotic®-A supplementation (0.8% and 1.2%) reduced muscle ash content. Activities of catalase and superoxide dismutase were markedly reduced in fish fed GroBiotic®-A (0.8% and 1.2%) compared to those fed the control diet. GroBiotic®-A supplementation also induced significantly higher neutrophil oxidative radical production compared to fish fed the negative control diet, but no significant difference was observed in comparison with the positive control diet. After 8 weeks of feeding, exposure to *A. hydrophila* for 3 weeks resulted in 40% (0.4%, 0.8% GroBiotic®-A) and 27% (1.2% GroBiotic®-A) mortality and reduced signs of disease, while 47% mortality was observed in fish fed the negative diet.

Based on the result of this study, it is concluded that 0.8% and 1.2% GroBiotic®-A positively influenced growth performance and feed efficiency of tilapia fed diets containing 29% crude protein to levels comparable to fish fed the positive control diet containing 33% crude protein. GroBiotic®-A supplementation also significantly increased neutrophil oxidative radical production as well as resistance to *A. hydrophila* infection.

Keywords: Prebiotics; Tilapia (*Oreochromis niloticus*); GroBiotic®-A; Growth performance; Immune response; Bacterial resistance

*E-mail address: zhengzonglin@126.com*
Effects of Microcapsule Lysine and Methionine on the Growth Performance of Grass Crap (*Ctenopharyngodon idellus*)

Tan Fangfang¹, Ye Yuantu¹*, Xiao Shunying¹, Ma Hong¹, Li Bing¹
¹School of Basic Medical and Biological Science, Soochow University, Suzhou 215123, China

Abstract:

(Objective) This experiment was conducted to study the effect of microcapsule lysine and methionine on the growth performance of grass carp. (Method) The grass carps, weighing an average of 62.7 ± 0.89 g, were fed with the basal diets added with 0.2% microcapsule lysine, 0.3% microcapsule lysine, 0.4% microcapsule lysine, 0.15% microcapsule lysine + 0.15% microcapsule methionine, 0.2% microcapsule methionine respectively, for 97 days. (Result) The results showed that: the specific growth rates (SGR) of the microcapsule amino acids groups were higher than the control except for the 0.2% and 0.4% microcapsule lysine groups; the feed conversion ratios (FCR) of the microcapsule amino acids groups were lower than the control group except for the 0.4% microcapsule lysine group. The protein deposition rate of the microcapsule amino acids groups were increased than the control group, as well as the crude protein content of muscle and liver. The liver body weight ratio and the dirty body weight ratio of the microcapsule amino acids groups were less than the control group. (Conclusion) The results indicated that: ① The microcapsule amino acids could improve the growth performance of grass carp, decrease feed conversion ratio, increase the protein deposition rate, but adding excess microcapsule lysine slowed down the growth of grass carp, adding 0.3% microcapsule lysine was the most appropriate; ② Adding microcapsule amino acids to diet could decrease the liver body weight ratio and the dirty body weight ratio of the grass carp, improve the muscle and liver protein content.

Keywords: Microcapsule lysine; Microcapsule Methionine; Grass carp; Growth performance

*E-mail address: yeyuant@pub.sz.jsinfo.net
Does amino acids, glycosidase and phytase supplementation help to increase replacement level of fish meal by soybean meal in the diet of Pacific bluefin tuna juveniles?

Amal Biswas¹, Biswajit K. Biswas¹, Yang S. Kim¹, Norishige Yagi², Kenji Takii¹
¹Fisheries Laboratories, Kinki University, Uragami, Nachikatsuura, Wakayama 649-5145, Japan
²Chubu Feed Co. Ltd., Osaka, Japan

Abstract

Although we have been established successfully the nutritionally balanced formulated diet for juvenile Pacific bluefin tuna (PBT), *Thunnus orientalis* using enzyme treated fish meal (EFM) as protein source, it is necessary to search out alternative protein sources because of high price and less availability of fish meal. Previous study revealed that 10% soybean meal can be included in PBT juvenile’s diet without compromising growth. Soybean meal is known to have inferior amino acids balance and high carbohydrate contents. Therefore, we have investigated whether addition of amino acids and glycosidase in soybean meal diet can help to replace more EFM in PBT juvenile’s diet. Six diets were prepared: only EFM as protein source (EFM, control), 45.84% EFM + 20% soybean meal (SM), and SM was added with either amino acids (histidine, lysine, methionine and threonine; SM+AA), glycosidase (SM+Gly), phytase (SM+Phy) or combination of amino acids, glycosidase and phytase (SAGP). Twenty nine days old 200 juveniles of mean body weight ca. 0.45g were stocked into each of duplicate 15 t tank for each treatment. The PBT juveniles were fed six times daily until apparent satiation with experimental diets for 14 days. Almost all growth parameters showed significant differences among the treatments. Final mean body weight and specific growth rate showed similar pattern where there were no significant differences among EFM, SM and SM+Phy; however, EFM showed significantly higher values than SM+AA, SM+Gly and SAGP. Again, there was no significant difference in feed conversion efficiency among EFM, SM and SM+Phy; but EFM had significantly higher value than SM+AA and SM+Gly. Survival rate was significantly higher in SM diet followed by SM+Phy, EFM, SM+AA, SM+Gly and SAGP diets. The results suggested that 20% SBM can be included in the diet of PBT juveniles; however, addition of either amino acids or glycosidase could not able to stimulate the growth performance at least at the dietary formula used here.

Keywords: Pacific bluefin tuna; Fish meal replacement; Growth performance; Amino acids; Glycosidase; Phytase

*E-mail address: akbiswas04@yahoo.com or ns_akb@nara.kindai.ac.jp (A. Biswas)*
P-320

Effects of dietary levels of potassium diformate on growth, feed utilization and resistance to *Streptococcus iniae* of Nile tilapia, *Oreochromis niloticus*

Chhorn Lim1*, Phillip H. Klesius1 and Christian Lückstädt2

1Aquatic Animal Health Research Unit, USDA-ARS, 990 Wire Road, Auburn, Alabama 36832
2Addcon Nordic AS 3908 Porsgrunn, Norway

Abstract

The use of antibiotics as growth promoters in animal production, including aquaculture, is increasingly under public scrutiny and criticism. In the EU countries, the use of antibiotics as growth promoters in livestock production has been banned since January 2006. Short-chain organic acids or their salts have received much attention as alternatives. Among these, potassium diformate (KDF) has been shown to be an effective growth stimulator in diets of pigs, but its effects on aquaculture species are inconsistent. Thus, this study was conducted to evaluate the effects of dietary levels of KDF on growth, feed utilization, hematology, immune response and resistance of Nile tilapia (*Oreochromis niloticus*) to *Streptococcus iniae* challenge.

Mixed-sex juvenile Nile tilapia (7.05 ± 0.14 g) were stocked in 27 flow-through 57-L aquaria at a rate of 35 fish/aquarium. Practical basal diets (32% protein, 6% fat and 2,900 kcal of DE/kg) supplemented with 0 (control), 0.25, 0.50, 0.75, 1.00, 1.25 and 1.50 % KDF (D-1 to D-7, respectively) were each fed to fish in four replicate aquaria twice daily to apparent satiation for 12 weeks. Survival at the end of week 12 did not differ among fish fed different diets. Weight gain and feed efficiency ratio of fish fed diet D-5 (1.00 % KDF) were significantly higher than those fed higher levels of dietary KDF (D-6 and D-7), but were not different from those fed diets with lower supplemental levels of KDF (D-1 to D-4). Dry matter feed intake was highest and lowest for fish fed diets 4 and 7, respectively. These values were significantly different from those of fish fed other diets. There were no significant differences among hematological parameters (total, red and white blood cell counts, hematocrit, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration) of fish fed different diets. Likewise, innate immune responses (serum protein, immunoglobulin, lysozyme and alternative complement) did not differ among treatments. Cumulative mortality 14 days post-challenge with *S. iniae* and post-challenge antibody titer against the same bacterium were also not affected by dietary treatments.

E-mail: chhorn.lim@ars.usda.gov (Chhorn Lim)
Efficiency of microbial phytase sources in low phosphorus plant based diets for Tilapia (*Oreochromis niloticus*)

F. Liebert

1 Dept. of Animal Sciences, Division Animal Nutrition Physiology, Georg-August-University Goettingen, Kellnerweg 6, 37077 Goettingen, Germany

**Abstract**

Current strategies to maximize substitution of fishmeal or other animal protein sources in omnivorous fish diets need to be connected with verified concepts to improve the phosphorus (P) supply in fish diets. Plant based feed ingredients may contain much more than 60% of the total P bound to phytic acid. Similar to monogastric land animals, fish need supplementation of the feed enzyme phytase to achieve a partial degradation of phytates in the digestive tract. Prevention of significant phytate hydrolysis provides insufficient P-utilization from plant based diets in connection with increased P-output and additional inorganic P-sources are required in the feed to overcome P-deficiency. At present, studies with added microbial phytase enzymes of varying origin and in diets for different species yielded inconsistent efficiency, indicating that efficacy of commercial feed enzymes for use in fish diets may differ markedly. Two experiments were conducted in a tank culture (recirculating system with triplicates) to yield more information due to efficacy of commercial phytase sources in Tilapia feeds. Exp. I examined the efficiency of graded supply (500U vs. 750U/kg) of the phytase FINASE®P (AB Enzymes, Germany) as compared to a negative control diet (0.33% P) and a positive control diet (+0.3% inorganic P), respectively. Exp. II examined two different sources of microbial phytase at 750U/kg (FINASE®P; SMIZYME PT; SMIZYME CT (Beijing Smile Feed Sci. & Tech. Co., Ltd., China)). The plant based low P basal diets contained wheat (32.5%), corn (22.5%), soybean meal (24.5%) and wheat gluten (11.5%) as protein sources. Exp. I led to conclusion, that the examined microbial phytase FINASE®P improved growth and feed efficiency numerical. A beneficial effect of the higher level of added phytase activity (750U/kg) was observed. Exp. II yielded growth response according to Exp. I when FINASE®P was added. However, both of the SMIZYME sources (pelleted or coated pelleted) improved Tilapia growth much more pronounced when compared to FINASE®P. In conclusion, the observed responses due to different sources of microbial phytase in Tilapia diets are in general agreement with our earlier findings. Commercial enzymes achieve different adaptation to the physiological conditions in the fish gut. Consequently, comparative studies need more attention for reliable evaluation of efficacy of different phytase enzymes in fish nutrition. More investigations are needed to define the “working conditions” in different fish species and to adapt feed enzymes according to physiological needs.

**Keywords**: Phytase sources; Phosphorus; Tilapia; Growth; Nutrient utilization

*E-mail address: flieber@gwdg.de (Frank Liebert).*
Efficiency of different methionine sources in low methionine diets for Tilapia (*Oreochromis niloticus*)

F.Liebert¹, Wen Gao¹, C. Kobler² and A.Lemme²

¹ Dept. of Animal Sciences, Division Animal Nutrition Physiology, Georg-August-University Goettingen, Kellnerweg 6, 37077 Goettingen, Germany  
² Evonik Degussa GmbH, Health & Nutrition, Hanau, Germany

Abstract

Similar to land animals, optimization of protein and amino acid (AA) supply in fish diets needs robust AA-recommendations based on AA-requirement studies. Finally, the optimal dietary AA-ratio is needed for diet formulation, dependent on species and age. In addition, individual AA-availability has to be taken into account to meet the AA-requirements. When optimized combinations of different feed proteins do not yield this aimed dietary AA-supply, crystalline AAs are useful to overcome individual AA-deficiency. However, at present these ideal preconditions to optimize fish feeds are not achieved. Additionally, the efficiency of supplemented AAs is under discussion due to possible leaching effects in aquatic environment or other unknown physiological factors. Several experiments with all male Tilapia (*Oreochromis niloticus*) under conditions of tank culture (water re-circulating systems; water temperature 28-29°C; 12 hours light) were conducted to investigate the efficiency of protein-bound methionine (Met) in low-Met basal diets containing corn, wheat, soybean meal, soybean protein concentrate, legumes or gelatin as protein sources, but differing between the individual experiments. Diets were supplemented with two graded levels of DL-Met (Standard) and different new sources of Met (Met dimers, including either DD-, LL-, DL-, and LD-Met dimers (MET1), only DD- and LL-Met dimers (MET2), or a DL- and LD-Met mixture (MET3)). All diets were pelleted and fed by hand feeding up to apparent satiation. In addition to zoo-technical data, whole body analyses yielded body composition data to quantify protein deposition. Statistical evaluation of protein deposition data utilized a modelling procedure for assessing of protein quality parameters independent on variation of protein intake and model parameters of dietary Met-efficiency, as reported earlier (Liebert and Benkendorff, Aquacult. Nutr., 13, 2007, 397-406). In conclusion, supplementation of either Met source yielded dose dependent responses, suggesting general evidence for supplemental amino acids being utilized for growth and protein deposition in fish. Making use of the nitrogen utilization model, the observed efficacy of total Met in diets supplemented with different Met-sources was lower when compared with the non-supplemented basal diet. This observation provides some advice, that utilization of added Met was somewhat lower than utilization of the protein-bound AA. Reasons for this observation need more investigations in context with the specific features of protein metabolism in fish. Comparing DL-Met with the new Met-sources under study (MET1, MET2, MET3), an advantage was established for efficacy of the new Met-sources in our experiments.

Keywords: Methionine; Methionine efficiency; Methionine sources; Tilapia; Growth; Nutrient utilization

*E-mail address: flieber@gwdg.de (Frank Liebert).*
Evaluation of probiotics and prebiotics to improve the growth of juvenile leopard grouper *Mycteroperca rosacea*

MACÍAS-RODRÍGUEZ, M.E.*, REYES-BECERRIL, M.1, ROJAS, M.2, GRACIA-LÓPEZ, V.1, ASCENCIO, F.1
1Centro de Investigaciones Biológicas del Noroeste (CIBNOR), Mar Bermejo 195, Col. Playa Palo de Santa Rita, La Paz, B.C.S. 23090, México. 2Universidad Autónoma de Baja California Sur. Km 5.5, Carretera al Sur, La Paz, B.C.S. 23080, México.

Abstract

In last years, aquaculture has become a very fast growing sector in animal production. Both probiotics and prebiotics have been used to improve health and productivity parameters. Whereas probiotics when administered in adequate amounts confer a health benefit on the host, prebiotics stimulate the develop of certain groups of bacteria inhabiting the gut. It has been suggested synbiotic products are more effective than either probiotics or prebiotics alone. Thus the aim of this study was to evaluate by *in vitro* and *in vivo* trials the effectiveness of the combination of probiotics (Lactic Acid Bacteria (LAB) or *Debaryomyces*) and prebiotics (Inulin) in order to improve the growth of juvenile *Mycteroperca rosacea*. To select probiotic strains and prebiotic levels to be used *in vivo* trials, twelve probiotics strains which were isolated from intestinal mucus of fish and one yeast strain isolated from citrus fruit, were evaluated by their ability to use inulin as carbon source. Growth of each strain on the LDM semi-defined medium contained 0.5, 1, 2% inulin or 2% glucose as positive control was evaluated after 24, 48 and 72 h of incubation. An *in vivo* experiment was conducted with diets containing the probiotics *Lactobacillus sakei* strain 5-4 (2X10⁷ ufc/gr) and *Debaryomyces hansenii* strain L2 (1X10⁶ ufc/gr) alone or combined with inulin at level of inclusion in the diet of 1%. Control diet not contained probiotics or prebiotic. Two replicate groups (n=14) of juvenile *M. rosacea* (initial average weight 30.58±2.23 g) were fed at 2% of body weight for 6 weeks. A group of organisms were sampled at weeks 4 and 6 (n=4 by aquarium) and Weight Gain (WG) and Specific Growth Rate (SGR) were calculated. Results showed 5 of the tested strains were able to grow into LDM medium containing 0.5, 1 and 2% inulin. Compared with the control diet, higher WG and SGR values in diets containing *L. sakei* 5-4, *D. hansenii* L2 or *L. sakei* 5-4 plus inulin, were observed at week 4. Additionally, diets containing inulin or *D. hansenii* plus inulin showed higher WG and SGR values at week 6. In both times differences of WG and SGR between control and experimental diets at 4 or 6 weeks were not significat (*P*<0.05). We can concluded, inclusion of both probiotics and prebiotics into diets is an attractive option for fish culture. At present analysis on modulation of intestinal microbiota is carried out.

Keywords: *Lactobacillus; Debaryomyces; Inulin; Probiotics; Prebiotics*

* E-mail address: mmacias@cibnor.mx (María Macías-Rodríguez).
Selection of yeast isolated from citrus fruit and marine water with potential probiotics for leopard grouper *Mycteroperca rosacea*

REYES-BECERRIL, M. 12*, MACIAS, R. M. E. 1, ASCENCIO, F. 1, MESEGUER, J. 2, ESTEBAN ABAD, M. A. 2

1Centro de Investigaciones Biológicas del Noroeste (CIBNOR), Mar Bermejo 195, Col. Playa Palo de Santa Rita, La Paz, B.C.S. 23090, México. 2Fish Innate Immune System Group, Faculty of Biology, University of Murcia, 30100 Murcia, Spain.

Abstract

The selection of probiotics for aquaculture is usually based on their antagonism towards pathogens. However, other criteria such as growth, attachment to intestinal mucus and production of beneficial compounds should also be considered. *Debaryomyces hansenii* is yeast with great biotechnological potential because it is salt- and high-pH tolerant yeast rich in β-glucans and polyamines. The aim of this study was to select a *Debaryomyces hansenii* with probiotics abilities suitable for in vivo studies in aquaculture. The collection yeast was provided by Centro de Investigaciones Biológicas del Noroeste (CIBNOR, México). *D. hansenii* were cultured in yeast peptone-dextrose broth (YPD medium) at 25 ºC with constant aeration until the early stationary phase. The cell suspension was centrifuged and the pellet was recovered. The concentration of polyamines (putrescine, spermine and spermidine) in twelve candidate yeasts isolated from citrus fruit and marine water was determined by high performance liquid chromatography (HPLC). The ability of *D. hansenii* to grow at different pHs and concentrations of NaCl was evaluated in the selected yeast. The actual adhesion of *D. hansenii* to the digestive tract of leopard grouper was investigated through an in vitro experiment (DTAF labeled yeast) using the intestine of juveniles leopard grouper. Results showed that, putrescine and spermidine was observed at the considerably high concentrations in the *D. hansenii* strain CBS004 (isolated from marine water) followed by the yeast *D. hansenii* strain L2 (isolated from citrus fruit). A fairly large amount of putrescine was determined in the strain L2. Therefore, the selected yeast for the microbiological assays was *D. hansenii* strain L2. *D. hansenii* isolated from citrus fruit resisted high concentrations of NaCl showed an ability to grow at different concentration (256, 427, 769 and 1111 mM). When we compared the pH resistance of this strain at pH 2.5, 3.5 and 4.5, we found that *D. hansenii* strain L2 showed little or no decrease in viable cell numbers. Finally, the incubation of leopard grouper foregut with DTAF-labeled *Debaryomyces hansenii* strain L2 revealed the presence of labeled yeast on the surface of microvilli. It is concluded that *Debaryomyces hansenii* L2, non-indigenous yeast isolated from citrus fruit, can be considered a probiotic candidate. However, further extensive testing is recommended, including microbial analyze, such as colonization assays, and challenge experiments in fish to explore their probiotics effects.

Keywords: *Debaryomyces hansenii*; polyamines; HPLC; leopard grouper

*E-mail address: mreyes04@cibnor.mx* (M. Reyes-Becerril).
The Study of Effect of dietary Mannan oligosaccharides as prebiotic on growth performance and some blood metabolites in rainbow trout (*Oncorhynchus mykiss*)

M. Norouzi 1*, H. Meftah 2, S. Karimzadeh 3

1&2 Dept. of Marine Biology and Fishery, Islamic Azad University, Tonekabon Branch, Zip Code: 4681416167, Tonekabon, Iran
3 Dept. of Animal sciences, Roudaki higher education Institute, Tonekabon Branch, Iran

Abstract

Preliminary experiments were carried out to evaluate the effect of dietary mannan oligosaccharides (Active MOS) as prebiotic on growth performance of fingerlings of rainbow trout. The basal diet contained 40% crude protein, 11% crude fat and metabolic energy levels of 3000 kcal/kg. Three levels (0.1, 0.15 and 0.2 %) of Active MOS were added to the basal diet. Each diet was fed to three replicate groups of fish in 500-L tanks twice daily at rates approximately satiation for 8 weeks. Significantly higher mean final weight, specific growth rate, feed efficiency, protein efficiency was observed in fish fed the diets supplemented with prebiotic compared to the basal diet after 8 weeks of feeding while body chemical composition were not affected by various dietary treatments. Relative weight of Liver and gastrointestinal tract were increased in fish fed diets supplemented with prebiotic at 0.15 and 0.2%. Mean serum cortisol levels in fish subjected to stress by air exposure increased slightly but did not reveal significant difference between treatments. Based on results, it is concluded that mannan oligosaccharide can serve as a functional feedstuffs in the diets of fingerlings of rainbow trout by enhancing growth performance.

**Keywords:** Prebiotic, Mannan oligosaccharides, Rainbow trout, Growth performance, Cortisol.
Effect of extracted soy peptide replacement level on growth performance and specific digestive enzyme activity in juvenile Japanese flounder, *Paralichthys olivaceus*

R.E.P. Mamauag¹, S. Yokoyama², M. Ishikawa², S. Koshio²  
¹ Science of Marine Resources, The United Graduate School of Agricultural Sciences, Kagoshima University, 1-21-24 Korimoto, Kagoshima 890-0056, Japan  
² Laboratory of Aquatic Animal Nutrition, Faculty of Fisheries, Kagoshima University, 4-50-20 Shimoarata, Kagoshima 890-0056, Japan

Abstract

The incorporation in aqua feed of a hydrolysate processed from a high quality protein source and containing a high proportion of peptide may be beneficial. To determine whether the incorporation of extracted soy peptide can improve the growth performance of juvenile Japanese flounder, *Paralichthys olivaceus*, a growth experiment was conducted for 45 days. Six isonitrogenous and isolipidic diets in which extracted soy peptide was substituted with 0, 5, 10, 15, 20% of the fish meal protein and the sixth diet serving as the negative control (soybean meal) were formulated and fed to the flounders. Twenty five juvenile Japanese flounder (0.517±0.016 g, mean ± SD) were stocked in a 100-L tanks and were fed *ad libitum* twice a day. Result showed that fish fed the diet containing 0, 5, 10 and 15% of protein from soy peptide did not significantly differ (P>0.05) in terms of final body weight, weight gain and specific growth rate. However, diets with an inclusion level of 20% soy peptide showed a significantly (P<0.05) marked decrease in body weight gain and specific growth rate. Fish fed diets with soybean meal as the main source of protein has the lowest observed growth performance parameters among diets. Survival and total feed intake were not significantly different (P>0.05) among diets.

Trypsin and chymotrypsin activity from the pancreas of the fish fed the diets with 0, 5, 10 and 15% extracted soy peptide showed similar levels (P>0.05) compared to a lower trypsin and chymotrypsin activity to fish fed 20% inclusion level extracted soy peptide and diet containing soy bean meal as the main source of protein.

The study showed that processing soybean ingredient can improve growth performance parameters of the fish and can be used to replace (30% inclusion) fish meal as the main source of protein.

Keywords: Soybean hydrolysate; Japanese flounder; Trypsin; Chymotrypsin

* E-mail address: edwardmamauag@yahoo.com
P-327

Effects of dietary α-lipoic acid on growth, antioxidative responses of juvenile abalone Haliotis discus hannai Ino

Wenbing Zhang, Qiyong Chen, Kangsen Mai, Wei Xu, Xiaojie Wang, Zhiguo Liu

Key Laboratory of Mariculture (Ministry of Education), Ocean University of China, Qingdao 266003, P. R. China

Abstract

A feeding experiment was conducted to determine the effects of α-lipoic acid (LA) on growth and antioxidative responses of juvenile abalone Haliotis discus hannai Ino. Six purified diets supplemented with 0, 200, 400, 800, 1600 and 3200 mg kg⁻¹ of LA, respectively, were fed to abalone for 16 weeks in a flow-through water system. The results showed that the weight gain ratio (WGR) increased with dietary LA levels, and reached the highest value in the group with 800 mg kg⁻¹ dietary LA supplement. Glutathione peroxidase (GPX) activity in 800 mg kg⁻¹ dietary LA group was significantly higher than that in dietary LA deficient (0 mg kg⁻¹) group. Superoxide dismutase (SOD) activities in 200, 400, 800 mg kg⁻¹ groups were significantly increased. Supplements of 200, 400, 800 mg kg⁻¹ dietary LA significantly elevated the total antioxidative capacity (T-AOC). Glutathione (GSH) level in hepatopancreas significantly increased with dietary LA supplements in a dose-dependent manner (except for the 200 mg kg⁻¹ group). Catalase (CAT) and malondiadehyde (MDA) in hepatopancreas were not significantly affected by dietary LA. In conclusion, dietary LA promoted the growth and stimulated the antioxidative defense capacity of abalone. Based on data of WGR, the optimal dietary LA supplement for juvenile abalone was found to be 709 mg kg⁻¹ using piece-wise linear analysis.

Keywords: Abalone; Haliotis discus hannai; Growth; Antioxidation; Lipoic acid

*E-mail address: wzhang@ouc.edu.cn
Effects of Dietary Phytase (Cibenza Phos) on The Growth of Juvenile Tilapia (*Oreochromis niloticus*)

Yin Long 1*, Yongjian Liu 1, Hong Cao2, Huijun Yang1, Fu CunGuo2, Guiyin Liang1, Craig Browdy2, Lixia Tian1
1 Institute of Aquatic Economic Animals, School of Life Science, Sun Yat-sen University, 135 Xingang West Guangzhou 510275, PR China ;
2 Novus International Inc.,20 Research Park Drive,St Charles,MO 63304, USA

Abstract

Two experiments were conducted to determine the effect of dietary phytase (Cibenza Phos) supplementation on growth performance of juvenile tilapia (*Oreochromis niloticus*). In experiment I, two control diets were supplemented with 2% (positive control) or 1% (negative control) Ca(H2PO4)2: H2O but not with phytase. Another five experimental diets with 1% Ca(H2PO4)2: H2O and graded levels of phytase (0.01%, 0.015%, 0.02%, 0.025%, 0.03%) were prepared using plant meal as main protein sources. Juvenile tilapia with an initial weight of 18.35±0.20 g were fed with one of the seven diets for 8 weeks with five replicate tanks in each group. Fish fed with the negative control diet confirmed the modest P deficiency, showed reduced growth, poor feed efficiency, reduced ADCs of CP, dry matter and total-phosphorus, lower mineral deposition in scales, vertebrae and whole body, and an increase in body lipid content. Diets of phytase supplementation in 1% Ca(H2PO4)2: H2O significantly increased weight gain, ADCs of CP, dry matter and total-phosphorus, mineral concentration in whole body (Ca, P, Mg, Zn, Mn) and scales(Ca, P), and also decreased FCR and body lipid content significantly (P<0.05) compared with the negative control diet. We conclude that dietary phytase has potential to improve phosphorus availability and nutritive quality of plant meal for juvenile tilapia. The optimum phytase dosage is about 0.03% for juvenile tilapia.

In experiment II, we determined the optimum replacement of Ca(H2PO4)2: H2O by phytase in diets for juvenile tilapia. The control diet was supplemented with 2% Ca(H2PO4)2: H2O and with no phytase. Six low-P experimental diets with 0.03% phytase and graded levels of Ca(H2PO4)2: H2O (1%, 0.8%, 0.6%, 0.4%, 0.2%, 0%) were prepared using plant meal as main protein sources. Fish with an initial weight of 10.05±0.10 g were fed with one of the seven diets for 8 weeks with five replicate tanks in each group. Phytase supplementation in low-P diet significantly increased weight gain, whole body ash content, minerals concentration (Ca, P, Mn) in scales and whole body and ADC of total-phosphorus (P<0.05), and significantly decreased FCR (P<0.05) compared to the control diet. Results from this study showed that 1% Ca (H2PO4)2: H2O in the diet can be replaced by adding 0.03% phytase.

Keywords: Phytase; Phosphorus; Apparent digestibility coefficients; Body composition; Tilapia

*E-mail address: edls@mail.sysu.edu.cn
Effects of Emodin, vitamin C and their combination on growth, physiological and biochemical parameters, and HSP70s mRNA expression of Wuchang bream, *Megalobrama amblycephala*

Jian-Hua Ming\(^1,2\)*, Jun Xie\(^2\), Pao Xu\(^2\), Wen-Bin Liu\(^3\)

\(^1\) Wuxi Fisheries College, Nanjing Agricultural University, Wuxi 214081, China
\(^2\) Key Laboratory of Genetic Breeding and Aquaculture Biology of Freshwater Fishes, Ministry of Agriculture, Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences, Wuxi 214081, China
\(^3\) College of Animal Science and Technology, Nanjing Agricultural University, Nanjing 210095, China

Abstract

In order to investigate the effects of dietary emodin, high doses vitamin C (Vc) and their combination on growth, non-specific immunity and disease resistance ability of Wuchang bream (*Megalobrama amblycephala*), 1200 specimens with body weight (133.44±3.60) g were randomly divided into four groups. The control group was fed with basal diet (Vc content 50 mg/kg, supplied the L-ascorbyl-2-phosphate as Vc source), the other treated groups were fed with basal diets supplemented with 60 mg/kg emodin, 450 mg/kg Vc and 60 mg/kg emodin + 450 mg/kg Vc, separately. After feeding 60 days, the growth, biochemical parameters of serum and liver, and heat shock protein 70 (HSP70s) mRNA level were determined. The results showed that, compared with control, diets supplemented with 60 mg/kg emodin, 450 mg/kg Vc could significantly improve \((P<0.05)\) the weight gain and specific growth rates, the contents of total protein (TP), lysozyme (LSZ) and the alkaline phosphatase (ALP) in serum, the activities of superoxide dismutase (SOD) and catalase (CAT), the basal expression level of inducible HSP70 mRNA in liver, reduce feed coefficient, mortality, serum cortisol (COR) and liver maleic dialdehyde (MDA) content \((P<0.05)\). In the combination group, serum TP, LSZ contents, and liver HSP70 mRNA levels significantly increased \((P<0.05)\), and liver MDA content also significantly decreased \((P<0.05)\), but it didn’t display synergy action, and other indicators were not significantly differences compared with the control group \((P>0.05)\). In addition, there were not significantly differences \((P>0.05)\) among serum glucose (GLU), glutamic-pyruvic transaminase (GPT), glutamic-oxaloacetic transaminase (GOT), triglycerides (TG), cholesterol (CHO), liver constitutive HSC70 mRNA level, fish body indices and muscle composition. The challenge experiment with *Aeromonas hydrophila* showed that fish fed the diet supplemented with 60 mg/kg emodin or 450 mg/kg Vc could reduce mortality effectively, improve the immune protection, while the two combining, the effects instead dropped. Therefore, the basal diets supplemented with 60 mg/kg emodin or 450 mg/kg Vc could enhance the non-specific immunity and disease resistance of Wuchang bream, promote the fish growth. However, their combination could be ineffective.

Key words: *Megalobrama amblycephala*; emodin; vitamin C; growth; non-specific immunity; HSP70s mRNA expression

\(^*\) E-mail address: mingjianhua686@yahoo.com.cn (J.H. Ming).
Effect of Aquanin Plus™ on Growth and Feed Utilization of Nile Tilapia, Oreochromis niloticus

Nontawith Areechon¹, Qingsong Tan²*
¹ Department Aquaculture, Kasetsart Agriculture University, Bangkok, Thailand;
² Walcom Group Limited, Shanghai 201203, P.R. China

Abstract

This study was conducted to investigate the effect of Aquanin Plus™ (Containing 30% of cysteamine hydrochloride) as an inhibitor of Somatotropin Releasing Inhibitory Factors (SRIF) in all-male Nile tilapia (Oreochromis niloticus). The study focused on the additional of Aquanin Plus™ at three different concentrations in the feed. The effect on growth, flesh quality and stress response were monitored.

Feed component designed to obtain about 30% protein was added with Aquanin Plus™ in the feed mesh at 0, 500, 1,000 and 1,500 mg/kg feed, respectively, before pelletization with the diameter of feed at 4 mm. There were 4 experimental groups consisting of 3 replicates/group with 100 tilapia/rep with initial average weight of about 240 gms. The experiment was conducted in 3 x 3 x 1.5 meters (W x H x D) nylon cages with mesh size of 5.0 cm. All 12 cages were placed in a 20 x 40 sq.m earthen pond equipped with running water facilities over each cage. After 45 days of the trial, tilapia that was fed with feed containing 500 mg/kg Aquanin Plus™ had the best average weight gain, average daily growth and feed conversion rate (FCR) which were significantly different from the control \((P<0.05)\) but not from 1,000 and 1,500 mg/kg Aquanin Plus™ \((P>0.05)\). Edible portion (%) and survival rates were not significantly different amongst control and treatment groups \((P>0.05)\). Cortisol levels in serum were not significantly different. It is recommended that addition of Aquanin Plus™ at 500mg/kg feed would improve the growth and utilization of Nile tilapia.

Keywords: Aquanin Plus™, Nile tilapia, Growth, Feed conversion rate.

* E-mail address: tanqs2000@163.com (Qingsong Tan)
P-331

Dietary L-alanyl-L-glutamine supplementation improves growth performance and physiological function of hybrid sturgeon *A. schrenckii*♀ × *A. baerii*♂

Chang-an Wang*, Qi-you Xu, Hong Xu, Qing Zhu, Jun-ling Yang, Da-jiang Sun
Heilongjiang River Fisheries Institute of Chinese Academy of Fisheries Sciences, 86-150070, China

Abstract

Studies were conducted to demonstrate the effects of L-alanyl-L-glutamine (L-AG) on the growth performance and physiological function of hybrid sturgeon *Acipenser schrenckii*♀ × *Acipenser baerii*♂ (initial body weight 0.42 ± 0.05 g). Five experimental diets supplemented with 0.0, 0.25, 0.5, 0.75 and 1.0% L-AG were fed to triplicate groups of fish for 56 days. Hybrid sturgeons were obtained from the Harbin Aquafarm. 1500 larval fish were randomly stocked in each aquarium with triplicates per treatment and fish were acclimated to experimental conditions for 2 weeks before the start of the experiment. The aquaria were connected to a recycling system. They had a volume of approximately 220 L, were supplied with aerated water and kept at 23.0 ± 0.5°C. The dissolved oxygen level was always higher than 6.0 mgL⁻¹. The water exchanged rate was approximately 30% of the system capacity per day. A photoperiod of 24 h light was maintained during the first 30 days and fish were fed every 2 hours by hand to satiation. After that initial period, the photoperiod was changed to 12 h light/12 h dark and fish were fed 4 times a day by hand to satiation. When the culture experiment was terminated, weight gain rate, specific growth rate, survival rate and condition factor were determined and served as indices for assessing growth performance. Na⁺-K⁺ ATPase, glutamine, superoxide dismutase (SOD), malondialdehyde (MDA), protease, lipase and amylase of the whole fish or intestine were also determined. The results showed that the growth performance improved significantly with increasing dietary L-AG supplementation levels from 0.0 to 1.0%. Fish fed diets containing higher L-AG level had greater Na⁺-K⁺ ATPase, glutamine, SOD and lower MDA when the supplementation level increased. Compared to the control group, however, there were no significantly differences among groups for components such as protease, lipase and amylase of the intestine supplemented dietary L-AG trials. In conclusion, dietary L-AG supplementation could help improving growth performance, and physiological functioning of larva hybrid sturgeon.

Keywords: L-alanyl-L-glutamine; Hybrid sturgeon; Growth; Physiological function

*E-mail address: gordoncase@126.com (C.A. Wang).*
Effect of two kinds of Chinese herbs polysaccharide on respiratory burst and proliferation of head kidney macrophages in *Pelteobagrus fulvidraco*

Dongqing Bai  Xuan Wu*  
Tianjin Key Lab of AQUA-Ecology and Aquaculture, Department of Fishery Science, Tianjin Agricultural University, Tianjin 300384, P.R.China.

Abstract

Yellow catfish (*Pelteobagrus fulvidraco*) were fed with different concentration of Lycium bararum Polysaccharides (LBP), Ganodeima luaidum Polysaccharides (GLP)(300, 600, 900, 1200, 1500 mg/L). The head kidney macrophages of yellow catfish were separated by Percol continuous density gradient centrifugation. NBT reduction and Griess reagent coloration respectively, to evaluate the immunostimulant of the two polysaccharides and compare their immune activities with the control group and positive control group (PMA). The effects of the two polysaccharides on the proliferation of head kidney macrophages were investigated by using MTT. The results indicated that LBP and GLP could enhanced significantly the activity of superoxide anion in Macrophages (p<0.05), while between high concentration groups had not significant difference (p>0.05); LBP and GLP at high concentration had conspicuous effect on nitrogen burst activity of macrophage (p<0.05), LBP at 1500 mg/L could inhibited thenitrogen burst activity, while GLP had not significant difference (p>0.05) between higher concentration groups; LBP and GLP could improved the head kidney macrophages, but lower concentration of LBP had not significant difference (p>0.05) between, while higher concentration of GLP could significantly improved effects on head kidney macrophages. The results showed that both of LBP and GLP could enhanced non-specific immune of Yellow catfish.

Key Words: Lycium bararum Polysaccharides; Ganodeima luaidum Polysaccharides; respiratory burst activity; Proliferation macrophages

E-mail address: wuxuanzhangliping@126.com
Responses of grass carp (*Ctenopharyngodon idellus*) to supplemental methionine and lysine

Tianyi Liu¹, Hongqin Li¹*, Xuan Zhu²**, and Andreas Lemme³

¹ New hope group, 4th Floor, Waltz Plaza 7# Hangkong Road, Chengdu, Sichuan, P. R. China 610041
² Evonik Degussa (China) Co., Ltd. / Beijing
³ Evonik Degussa GmbH, Rodenbacher Chaussee 4, 63457 Hanau, Germany

Abstract

The objective of an experiment with grass carp (*Ctenopharyngodon idellus*) was to examine whether supplemental DL-Methionine and L-lysine HCl can be utilised for performance in protein reduced diets. A total of 960 grass carp with average body weights of 115g was equally distributed to 16 cages (2.0m×2.0m×1.5m, 60fish/cage). Water temperature ranged between 25°C and 28°C during the course of the 56d-experiment with dissolved oxygen concentrations of >4.0 mg/L. Fish were fed the diets twice a day until apparent satiation every time. Four cages each were assigned to one of four dietary treatments comprising treatment I) high protein diet (positive control) consisting mainly of soybean meal, rapeseed meal, cottonseed meal, DDGS, corn gluten feed and wheat middlings but no supplemental amino acids (analysis in dry matter (DM):33.94% CP, 1.55% Lys, 0.56% Met, 1.19% Met+Cys), treatment II) negative control with reduced dietary protein consisting of similar components as control diet except without soybean meal, and also no supplemental amino acid. (analysis in DM: 25.86% CP, 1.01% Lys, 0.47% Met, 1.02% Met+Cys,) and treatment III –IV) as treatment II but with supplementation of DL-Met and Lysine.HCl (analysis of diets III-IV in DM: Met 0.51%, 0.57%; Met+Cys 1.05%, 1.10%). Specific growth rate of grass carp of negative control (treatment II) was significantly lower than that of positive control (treatment I), 1.66 a vs.1.86 c, increasing levels of dietary methionine and lysine (treatment III, treatment IV) improved performance significantly but still lower than that of positive control (treatment I: 1.86 c, treatments III, IV: 1.55bc 1.50ab g/g). Regression analysis of lysine and methionine+cystine intake against weight gain revealed a strong linear relationships (r² = 0.98 (Lys); 0.94 (Met+Cys)) suggesting that with higher dietary Lys and/or Met level the same performance as in treatment I would have been achieved. Results of the experiment confirm that supplemental DL-Methionine and L-lysine HCl were utilised as well as protein bound amino acids by grass carp for growth, supplemental amino acids in low protein diets were very effective in improving performance.

* Corresponding author, email address: lihq@newhopegroup.com
Responses of juvenile Nile tilapia (Oreochromis niloticus) to supplemental methionine

Xuan Zhu2*, Wenhua1, Ming Jiang1 and Andreas Lemme3
1 Yangtze River Fisheries Research Institute, Chinese Academy of Fishery Sciences, No 41 # Jianghanbei Road, Jinzhou 43400, Hubei, China
2 Evonik Degussa (China) Co., Ltd. / Beijing
3 Evonik Degussa GmbH, Rodenbacher Chaussee 4, 63457 Hanau, Germany

Abstract

The objective of an experiment with juvenile tilapia (Oreochromis niloticus) was to examine whether supplemental DL-Methionine can be utilised for performance in protein reduced diets. A total of 540 tilapia with average body weights of 5g was equally distributed to 18 tanks (350L, 30 fish/tank). Water temperature ranged between 25°C and 32°C during the course of the 56d-experiment with dissolved oxygen concentrations of >5.0 mg/L. Fish were fed the diets three times a day according to feeding schedule with a rate of 5-3% of body weight (adjusted fortnightly). Three tanks each were assigned to one of six dietary treatments comprising I) high protein diet (positive control) consisting mainly of soybean meal (24%) , rapeseed meal, cottonseed meal, blood meal and wheat middlings but no supplemental amino acids (analysis in dry matter (DM): 36.17% CP, 1.79% Lys, 0.54% Met, 1.15% Met+Cys), II) negative control with reduced dietary protein consisting mainly of soybean meal, peanut meal, wheat middlings as well as L-Lys HCl (0.40%), L-Thr (0.35%), and Glutamic acid (0.40%) (analysis in DM: 31.38% CP, 1.63% Lys, 0.40% Met, 0.88% Met+Cys,) and III – VI) as II but with incremental supplementation of DL-Met at the expense of Glutamic acid (analysis of diets III-VI in DM: Met 0.53%, 0.65%, 0.79%, 0.92%; Met+Cys 1.01%, 1.13%, 1.27%, 1.41%). Initial fish as well as selected fish of each tank at termination were used for analysis and calculation of nitrogen and methionine retention. Specific growth rate of tilapia of negative control was significantly lower than that of positive control but increasing levels supplemental DL-Methionine improved performance to significantly higher levels than positive control and without suggesting an asymptote (treatments I to VI: 3.28b, 3.17a, 3.15a, 3.21ab, 3.29b, 3.42c %). Similar pattern applies for feed conversion ratio (1.57abc, 1.63cd, 1.67d, 1.62bcd, 1.54ab, 1.46a g/g). Protein (N x 6.25) retention results were as follows (30.3d, 33.5c, 35.5abc, 34.6c, 37.4a, 36.6ab % of intake). Results of the experiment confirm that protein content in diets for juvenile tilapia can be reduced while maintaining performance with beneficial effects of protein utilisation. Moreover, supplemental DL-Methionine could be utilised for growth and optimal Met supply seems to be higher than assumed. As both dietary levels of methionine in diets I and III and the efficiency of utilisation in these treatments (54.2 vs 56.5 % of intake) were similar, it is concluded that supplemental amino acids are utilised as well as protein bound amino acids.

* Email address: xuan.zhu@evonik.com
Effects of potential probiotics on growth performance and immune response of the juvenile sea cucumber *Apostichopus japonicus*

Huihui Zhou*, Hongming Ma, Wenbing Zhang, Wei Xu, Zhiguo Liu, Kangsen Mai  
The Key Laboratory of Mariculture (Education Ministry of China), Ocean University of China, Qingdao, 266003

Abstract  
The objective of this work was to select probiotic bacterial strains for juvenile sea cucumber *Apostichopus japonicus*. Three strains of potential probiotics isolated from the intestines of *A. japonicus* were identified by 16S rDNA sequence genotyping as *Bacillus GSC-1*, *Bacillus GSC-2* and *Enterococcus GSC-3*. The safety of these potential probiotics were evaluated by immersing juvenile *A. japonicus* in sea water with GSC-1, GSC-2, GSC-3 or Vibrio splendidus at 100, 103, 105 or 107 CFU/ml respectively for 7 days. Results showed that GSC-1 and GSC-3 treatments were safe under the designed concentrations based on the mortality rates. However, GSC-2 caused significantly higher mortality at 107 CFU/ml than the blank control (P<0.05). A 20-day feeding trial was conducted to investigate the effects of GSC-1 and GSC-3 on the growth performance and innate immune response of juvenile *A. japonicus*. Feeds were algae (*Sargassum thunbergii*) powder supplemented with GSC-1 or GSC-3 at 0 (control) and 109 CFU/g respectively. The specific growth rate and survival rate were significantly increased in treatment supplemented with GSC-1. Comparatively, survival rate and discoloration rate were remarkably improved in treatment of GSC-3 (P<0.05). Moreover, GSC-1 increased total bacteria counts and decreased *Vibrio* spp. counts significantly (P<0.05). Phenoloxidase and lysozyme activities in body tissue of the juvenile *A. japonicus* were significantly higher in the group treated with GSC-1 or GSC-3 than the control (P<0.05) while acid phosphatase and superoxide dismutase activities were remarkably higher than control in GSC-1 group only (P<0.05). Both GSC-1 and GSC-3 showed positive effects on disease resistance of juvenile *A. japonicus*, especially GSC-3 significantly improved disease resistance to *V. splendidus* exposure (P<0.05). In conclusion, both *Bacillus GSC-1* and *Enterococcus GSC-3* show probiotic effects, therefore they can be used as probiotics in the farming of juvenile sea cucumber *A. japonicus*.

Keywords: juvenile sea cucumber (*Apostichopus japonicus*), probiotics, growth, immune response

*E-mail address: huihuizhou2051@yahoo.cn*
Effects of Ala-Gln dietary supplementation on growth and some stress parameters of Jian Carp (Cyprinus carpio) juveniles, reared in different stocking density

Gui Qin-Wang*, Hong Mei-Lu, Xiao Tian-Niu, Zi Ping-Li
Department of Aquaculture, Faculty of Animal Science and Technology, Jilin Agricultural University, Changchun 130118, China

Abstract
High stocking density is an aquaculture-related situation under intensive culture, which causes crowding stress in fish and potentially affecting growth. The present study was conducted to evaluate the effects of N(2)-L-alanyl-L-glutamine (Ala-Gln) on growth and regulation of the stress response to Jian Carp reared in different stocking density. Diet containing three levels of Ala-Gln (0.0%, 0.3% and 0.8%) were randomly assigned to triplicate groups of Jian Carp (32.82±0.27 g initial mean weight) reared at low, middle and high stocking densities for 56 days. Some biological and stress parameters were studied at the end of the experimental period. Results indicate that: (1) stocking density significantly influenced growth, feed intake rate and coefficient of weight variation of carp \( P < 0.05 \). Specific growth rate of the high stocking density were significantly lower than those of the low stocking density carp \( P < 0.05 \), but plasma stress hormone and coefficient of weight variation contrast \( P < 0.05 \). (2) The dietary Ala-Gln significantly influenced specific growth rate and plasma stress hormone in high stocking density \( P < 0.05 \), but no significantly effect on coefficient of weight variation of carp in low stocking density \( P > 0.05 \). At same stocking density, the dietary Ala-Gln had no effect on production performance of the low stocking density \( P < 0.05 \) but significantly effect on that of the high stocking density \( P > 0.05 \). (3) Interaction of the dietary Ala-Gln levels and stocking density showed significantly effect on growth, feed intake rate and coefficient of weight variation of carp \( P < 0.05 \). It was concluded that adding Ala-Gln in diets could regulate growth, feed intake, decrease Individual differentiation and stress response with increased stocking density of Jian carp. These results suggest 0.8% Ala-Gln supplementation in diets of Jian Carp regulate growth and stress response in high stocking density.

Keywords: alanyl-L-glutamine; stocking density; growth; Stress; Cyprinus carpio

* E-mail address: wgqjlau@yahoo.com.cn
Effect of potassium diformate (KDF) on growth performance of male Nile Tilapia (Oreochromis niloticus)

Maria L. Cuvin-Aralar¹, K.-J. Kühlmann², K. Schroeder² and C. Lückstädt*³

¹ Southeast Asian Fisheries Development Center, AQD, Binangonan Freshwater Station Binangonan, 1940 Rizal, Philippines
² ADDCON Asia Ltd., 35/F Cnetral Plaza, 18 Harbour Rd., Wanchai, Hong Kong
³ ADDCON EUROPE GmbH, Kaiserstr. 1a, 53113 Bonn, Germany

Abstract

Growing awareness from consumers and producers of aquaculture species has resulted in a demand for responsible and sustainable aquaculture. The regulatory authorities in most exporting countries now focus on the misuse of antibiotic growth promoters (AGP) in aquaculture, while public attention has shifted towards sustainable production methods. As a result these methods had to be tested. Several feed additives, including acidifiers consisting of organic acids and their salts may promise to obtain high performance in aquaculture.

The current investigation aimed to examine effects of potassium diformate (0.3% KDF) in commercial tilapia feeds. Twenty-five male Nile tilapias with a mean weight of 7.84±0.90 g were stocked in eight 240 l polyethylene tanks in a static-renewal system in the Philippines (Binangonan Freshwater Station of SEAFDEC-AQD). Fish were reared for 74 days. Proximate composition of the commercial feed was 31.4% crude protein, 6.9% crude fat, 8.6% crude fibre, 52.3% NFE and 0.8% ash as well as a gross energy of 17.3 kJ g⁻¹. The fish in both the control and KDF treatment were given the appropriate feed with a daily ration equivalent to 5% of their body weight. Feed was dispensed thrice a day at 0800h, 1200h and 1600h. Water parameter as well as growth performance of fish were monitored regularly.

Diet supplemented with KDF yielded improved growth data, based on daily growth rate as well as specific growth rate (P<0.01). Tilapia in the control group reached a mean body weight of 45.5±1.1 g, while the fish fed with potassium diformate reached an average weight of 51.4±2.2 g. Likewise, feed conversion ratio was improved significantly (P<0.05).

The results show that addition of 0.3% KDF in the diets of Nile tilapia can help to improve its growth performance and thus, can achieve a more economic and sustainable tilapia production. Furthermore, the additive optimizes feed efficiency, which is in full agreement with previously reported improved digestibility parameters after the inclusion of KDF in fish feeds.

Keywords: Potassium diformate; Tilapia; Growth; Feed efficiency

* E-mail address: christian.lueckstaedt@addcon.net

Le Thanh Hung¹*, Dao Thi Thu Hang²*, Nguyen Huu Thinh¹
¹ The Faculty of Fisheries, Nong Lam University, Vietnam
² Provimi Vietnam

Abstract

Provimi Vietnam has developed a product called Nutribull. Nutribull contains a balanced blend of nucleotides, β-glucans, glucoronolactone and amino acids. Three groups of fish were difference by the addition of 0 % (control), 0.1 % and 0.2 % Nutribull in the feed. The feeds were fed for a period of 70 days to Pangasius fingerlings of initially 17g average body weight.

The growth rate of the fish responded linearly to the inclusion of Nutribull at 0.1% (89.09g) and 0.2% (93.73g) compare with control group (82.43g). The difference was statistically significant for the fish fed 0.2% Nutribull compared to the control diet. The FCR of the fish fed the 0.2% Nutribull (1.07) diet was significantly lower than the other groups (1.17 and 1.18). Nutribull shows a tendency to improve feed intake and reduces FCR significantly at the same time. Addition of Nutribull didn’t significantly affect the number of white blood cells but the Lysozyme activity increased significantly with increasing inclusion of Nutribull in the diet, were highest (26.9 units/ml) in the groups fed 0.2% Nutribull and reduced in 0.1% Nutribull groups to 19.4 units/ml and to 12.5 units/ml the control groups. In the bacterial challenge test with *Edwardsiella ictaluri* showed that 0.1% and 0.2% Nutribull supplementation improved the survival rate of fish (significant for 0.2% Nutribull) and improved the fish resistance to bacterial disease. In the environment challenge with ammonia (150mg/l), mortality rates of fish 24 hours post challenge were highest (61.7%) in the groups fed the control diets and reduced in the 0.1% Nutribull groups to 33.3% and to 16.67% in the 0.2% Nutribull fed groups. Similarly 24 hours post challenge the mortality rates of fish exposed to high salinity were reduced from 50% in the control treatment to 18.33% and 3.3% in the 0.1% and 0.2% Nutribull treatments respectively.

Nutribull combines three products each having its own function in fish metabolism, immune-stimulation and stress response. The present feeding trial and subsequent bacterial and environmental challenge tests clearly shows that Nutribull inclusion at 0.2% and to a lesser extend at 0.1% in Pangasius diet leads to major improvements in growth, feed conversion, a-specific and specific immune response as well as in the ability to cope with environmental stress.

Keywords: Pangasius catfish; Glucoronolactone;

*Email address*: hang.daothithu@nutriwayvn.com, lthungts@yahoo.com.vn
P-339

Effects of dietary garlic extracts on growth, feed utilization, whole body fatty acid profile, muscle free amino acids composition and blood plasma changes of Juvenile sterlet sturgeon, *Acipenser ruthenus*

Dong-Hoon Lee*, Jeong-Dae Kim

1Gyenoggi Province Freshwater Fisheries Research Institute, 235-1 Kwangtan-Ri, Yangpyeong-Gun 476-841, Korea
2Department of Animal Life System, College of Animal Life Sciences, Kangwon National University, Chuncheon 200-701, Korea.

Abstract

Three trials were carried out to investigate the supplemental effects of dietary garlic extracts on growth performance and whole body fatty acid composition of juvenile sterlet sturgeon (*Acipenser ruthenus*). The first experiment was designed to determine the optimum levels of garlic extracts as growth promoter during 10 weeks. Three groups (two replicates) of 240 fish with mean body weight of 85 g were fed the diets containing 0, 0.5 % and 1 % of garlic extracts. Mean value of final weight (g/fish), weight gain (%) and feed efficiency (%) of fish fed diet containing 0.5 % garlic extracts were highest (P < 0.05). The second trial was made to investigate the supplemental effects of dietary garlic extracts on growth, whole body fatty acid compositions and free amino acids compositions in the muscle of 6 month old sterlet sturgeon with average body weight of 59.6 g. Fish cultured in freshwater were randomly allotted to each of 10 tanks (two groups of five replicates, 20 fish/tank) and fed diets with (GE) or without (CD) garlic extracts respectively at the rate of 2% of fish body weight per day for 5 weeks. Weight gain (WG, 51.1 %), feed efficiency (FE, 76.9 %), specific growth rate (SGR, 1.18 %) and protein efficiency ratio (PER, 1.51) of fish fed diet GE were significantly higher (P < 0.05) than those of the other group. Fish fed GE also showed higher protein (PRE, 20.5 %) and lipid retention efficiencies (LRE, 78.4 %) than fish fed CD (P < 0.05). A difference was found in the whole body fatty acid profile between two groups. In particular, EPA (C22:6n3) and DHA (C22:5n3) of fish fed GE were significantly (P < 0.05) higher than those of fish fed CD. Third trial was carried out to investigate the supplemental effects of dietary garlic extracts on blood plasma changes of 6 month old sterlet sturgeon averaging 56.5 g. Fish were randomly allotted to each of 2 tanks (300 fish/tank) and hand-fed diet with (GE) or without (CD) garlic extracts, respectively at the rate of 5% of fish body weight per day for 23 days. Plasma glucose concentrations (mg/dl) between two groups were significantly different (P < 0.05) at 1 h (CD 65.4 vs. GE 40.4) and 24 h (CD 82.0 vs. GE 64.8) after a meal. Plasma insulin concentrations (µIU/ml) were also significantly different (P < 0.05) at 24 h (CD 2.96 vs. GE 32.56) and 1 h after meal (CD 5.06 vs. GE 10.56), respectively. The present results suggested that dietary garlic extracts could improve dietary glucose utilization through the insulin secretion, which result in better growth and feed utilization of juvenile sterlet sturgeons.

Keywords: Garlic extracts, *Acipenser ruthenus*, Growth, Fatty acid, Insulin, Glucose

*E-mail address: leedh27@gg.go.kr (D.H. Lee).*
Effects of diformates on growth and feed utilization of all male Nile Tilapia fingerlings (*Oreochromis niloticus*) reared in tank culture

F.Liebert¹, K. Mohamed² and C.Lückstädt³

¹ Dept. of Animal Sciences, Division Animal Nutrition Physiology, Georg-August-University Goettingen, Kellnerweg 6, 37077 Goettingen, Germany  
² Dept. of Animal and Fish Production, Faculty of Agriculture, Suez Canal University, 41522 Ismailia, Egypt  
³ ADDCON EUROPE GmbH, 53113 Bonn, Germany

Abstract

Similar to land animals, at present feeding strategies in aquaculture tend to increase application of feed additives which were successfully applied in other animal species and generally accepted from the viewpoint of feed and food safety. Global production of farmed Tilapia in at least 85 countries exceeded 2.5 million t in 2007 and requires high-quality fish feeds. Generally, diformates are able to stabilize feed quality by conservation effects, but only a few observations are available due to zootechnical effects of these feed additives when applied in Tilapia diets. The current investigation aimed to examine effects of potassium diformate (0.3% KDF) and sodium diformate (0.3% NaDF; 0.5% NaDF), when added separately to a basal diet (Soybean meal 30%; corn 25%; wheat gluten 15%; wheat 14%; fish meal 5%; L-Lys $\cdot$ HCl 0.18%; DL-Met 0.3%; premixes and wheat starch ad. 100%) without any further non-nutrient feed additive. Totally 32 fibreglass tanks (380 l/tank) of a semi-closed re-circulating system were utilized, each stocked randomly with 20 Tilapia fingerlings (Initial BW = 33.9±0.06g). During the whole experiment, no mortality was recorded. After finishing the growth study (56d), pooled samples of average fish were analyzed for whole body composition to evaluate the effects on deposition and utilization of protein. The results of the growth study demonstrated, that diets supplemented with KDF or NaDF yielded improved growth data, but statistical significance was not observed (p>0.05). In addition, feed efficiency was enhanced in the same manner, but significantly improved when 0.3% NaDF were added in the diet (p<0.05). Due to this dietary treatment (0.3% NaDF), also protein efficiency ratio and protein retention efficiency were significantly improved. This observed advantage of the diet with 0.3% NaDF was also supported by complex protein quality parameters yielded from application on an exponential N-utilization model, eliminating the influence of varying feed intake on response of protein deposition. In conclusion, the observed beneficial effect of added salts of formic acid on feed and nutrient efficiency of a low fishmeal Tilapia feed was significant with 0.3% of sodium diformate (NaDF) in the diet. These findings under experimental conditions with high hygienic standards led to the expectation of more pronounced beneficial effects under environmental conditions of applied Tilapia culture.

Keywords: Potassium diformate; Sodium diformate; Tilapia; Growth; Nutrient utilization

*E-mail address: flieber@gwdg.de (Frank Liebert).*
Effects of a probiotic feed additive (Enterococcus faecium) in diets for Tilapia (Oreochromis niloticus) on growth, feed efficiency and microbiological parameters of the gut

K. Mohamed and F. Liebert

1 Dept. of Animal Sciences, Division Animal Nutrition Physiology, Georg-August-University Goettingen, Kellnerweg 6, 37077 Goettingen, Germany
2 Dept. of Animal and Fish Production, Faculty of Agriculture, Suez Canal University, 41522 Ismailia, Egypt

Abstract

Currently, several feed additives are examined to improve feeding strategies in aquaculture. Probiotic feed additives are part of such investigations, but similar to studies in land animals up to now the observations are contradictory. As mentioned by Olvera-Novoa (2008, XIII ISFNF), microbes isolated from the gut may have a good potential as native probiotics. However, commercial probiotics contain microbes from different species and habitat. The current investigation was conducted as a preliminary study to examine the effect of a probiotic feed additive from Enterococcus faecium (Company DSM), when added (20g/kg) to a Tilapia diet containing no further non-nutrient feed additives (Soybean meal 30%; corn 25%; wheat gluten 15%; wheat 14%; fish meal 5%; L-Lys-HCl 0.18%; DL-Met 0.3%; premixes and wheat starch ad. 100%). The pelleting process of the final diets guaranteed in feed temperatures below 45°C to protect the feed additive. Totally 16 fibreglass tanks were utilized (380 l/tank; semi-closed re-circulating system; water temperature 28-29°C; 12 hours light per day), each randomly stocked with 20 all male Tilapia (Initial BW = 82±0.4g). During the growth experiment (56d), no mortality was observed. After finishing the growth trial, microbiological studies in pooled samples of the intestinal content from fish of each tank were conducted to examine dietary effects on microorganisms of the intestine. At the end of the growth study, no significant effect of the probiotic feed additive was established on feed intake (control 3.58 vs. 3.62 g/fish/d) and final BW (control 214.7 vs. 207.4 g/fish), respectively. The observed feed efficiency (control 1.52 vs. 1.62 g/g) tended to be lower due to the diet with the added probiotic feed additive, but not significantly. From observations in slaughtered fish, it can be speculated that the passage rate of the feed with added probiotics was more quickly, also supporting the observed decline in feed efficiency. This first advice about modulation of feed passage needs to be examined in further studies dependent on level of supplementation. Results of microbial investigations yielded no specific enrichment of the probiotic E. faecium in the intestine of Tilapia, but the observed variation between tanks was very high. In conclusion, the current study yielded no beneficial effects when the commercial probiotic under study was added to Tilapia diets. However, further investigations including dose-response-studies are needed for final conclusions.

Keywords: Probiotics; Tilapia; Growth; Nutrient utilization

* E-mail address: flieber@gwdg.de (Frank Liebert).
The effect of saponin supplementation to plant feeds on the growth performance, digestibility, and retention in Atlantic salmon.

Fredrik Venold¹, Elvis Chikwati¹, Michael Penn¹*, Junior Molatelo Madibana¹, Anne Marie Bakke¹, Jens Rohloff², Arne Guttvik³, Marie Hillestad³ and Åshild Krogdahl¹

¹ Aquaculture Protein Centre (a CoE), Department of Basic Sciences and Aquatic Medicine, Norwegian School of Veterinary Medicine, P.O. Box 8146 Dep, NO-0033 Oslo, Norway
² Plant Biocentre, Department of Biology, Norwegian University of Science and Technology, Trondheim
³ BioMar AS, Trondheim, Norway

Abstract

Plant feed material containing saponins is presently included as ingredients in fish feed for the aquaculture industry. Soysaponins are plant metabolites with a triterpenoid glycosidic structure. Both saponins and legumes have documented negative implications on intestinal function in Atlantic salmon. It is well established that full-fat or solvent extracted soybean meal causes a dose-dependent enteritis in the intestine of Atlantic salmon (Salmo salar L.). The causative agent(s) have not been conclusively identified although saponins are believed to be involved. The goal of the current study was to investigate the effects of supplementing soysaponins to diets containing legume or non-legume plant meals for Atlantic salmon.

An 80 day feeding trial with Atlantic salmon post smolts was carried out at Nofima Marine’s research station at Sunndalsøra. Five diet pairs containing the following 5 plant protein sources were made: corn gluten (CG), pea protein concentrate (PPC), sunflower (SFM), rapeseed (RSM), and horse bean (HB). Each pair consisted of one diet with (+S) and one without soysaponin supplementation. Saponins were supplemented based on the level present in soybean meal diets, 2g/kg, as for a diet with an inclusion level of 30%. In addition to these five diet pairs, a diet containing wheat gluten supplemented with soysaponin (WG+S) and a diet containing soybean meal (SBM) were also made. The twelve diets were formulated to have a DP/DE ratio (digestible protein (g/kg)/ digestible energy (MJ/kg)) of 20.0, dietary protein and energy varying according to the nutrient densities of the fish meal replacers.

During the first 19 days of the trial, feed intake was not affected by saponin supplementation. At day 30, body weights of the fish consuming diets containing saponins were slightly higher, except for the PPC+S diet. The feed conversion ratios (g g⁻¹) were lower for the diets containing saponins, except for SFM and HB+S, which was higher. Protein source had an effect on fecal dry matter (FDM), macronutrient digestibility (nitrogen, lipid, and starch), and apparent energy digestibility, while saponin inclusion had a minimal effect on FDM and lipid digestibility. Their combined effect was only significant for FDM, nitrogen and apparent energy digestibility. Saponin inclusion negatively affected apparent digestibility of minerals (ash, copper, and sodium).

Based on the results above, it can be concluded that soysaponins can have both positive and negative effects in fish. However, high levels of soysaponins combined with high levels of certain plant protein sources may have detrimental effects.

*E-mail address: fredrik.venold@nvh.no
Protease supplementation enhances the apparent digestibility of nutrients and energy in Nile tilapia fed plant-protein rich diets with variable crude protein content

Jorge Dias1*, Paulo Rema2, Viviane Verlhac3
1 SPAROS Lda. & CCMAR-CIMAR L.A., Centro de Ciências do Mar, University of Algarve – Campus Gambelas, 8005-139 Faro, Portugal.
2 CIIIMAR-CIMAR L.A., Centro Interdisciplinar de Investigação Marinha e Ambiental and Universidade de Trás-os-Montes e Alto Douro, Quinta dos Prados, P.O. Box 1013, 5001-801 Vila Real, Portugal.
3 DSM Nutritional Products France, Animal Nutrition & Health Research Centre , BP 170, 68305 Saint-Louis, France.

Abstract
A study was undertaken to evaluate the efficacy of a graded dietary supplementation of a protease (RONOZYME® ProAct) on the apparent digestibility of nutrients and energy of Nile tilapia fed low fishmeal diets. Two basal diets were formulated to contain soybean meal, cottonseed meal and rapeseed meal as major protein sources. One of these basal diets was formulated to contain a 34% crude protein level (CP34) while the other had a 31% crude protein level (CP31). Within each dietary crude protein level, diets were supplemented with 0, 200 and 400 mg/kg of the protease. Homogenous groups of 15 Nile tilapia (mean body weight: 85 g) were used to determine the apparent digestibility coefficients (ADC) of the dietary components, by the indirect method with diets containing 0.025% yttrium oxide as inert tracer. Fish were stocked in cylindro-conical tanks (volume: 60 L; water-flow rate: 5 L·min⁻¹) at a constant water temperature of 27 ± 1°C, and following adaptation, faeces were collected daily by means of the continuous outlet water filtration system (INRA system). A reduction on the dietary crude protein level (from 34 to 31% DM) significantly enhanced the apparent digestibility of dry matter, organic matter and energy, while it significantly reduced the apparent digestibility of protein. Within each dietary crude protein level, protease supplementation (200 and 400 mg/kg feed) significantly (P<0.05) enhanced the apparent digestibility of dry matter, protein and organic matter, in a dose response manner. Regarding energy digestibility, the improvement was only significant with the higher protease dose. ADC values of fat and phosphorus were significantly affected by the dietary crude protein level and protease dose, but also by the interaction of the two variables. Data from the current trial demonstrates the biological efficacy of the protease on improving the digestibility of protein derived from plant protein sources in Nile Tilapia.

Keywords: Nile tilapia; Protease; Vegetable proteins; Digestibility

* E-mail address: jorgedias@ualg.pt (J. Dias).
Evaluating CHD-FA Carbohydrate-derived Fulvic Acid for use in diets of Mozambique Tilapia Oreochromis mossambicus.

Lourens F de Wet¹, Willem Visagie¹
¹ Feed Technology Group, Division of Aquaculture, Stellenbosch University, Stellenbosch, South Africa

Abstract

An experiment was designed to evaluate the effect of Carbohydrate-derived Fulvic acid (CHD-FA®) as dietary source of Fulvic acid (16% concentration) on production performance promoter of Mozambique Tilapia. A number of 750 male tilapia with an average body length and weight of 10.121±0.501 cm and 18.543±2.390 g were allocated to thirty 100 liter tanks within a temperature-controlled experimental. Treatments consisted of a control diet with no Fulvic acid supplementation (F0), and four diets with increasing levels of CHD-FA: F0.5, F1, F5 and F10 containing 0.5, 1, 5 and 10 g kg⁻¹ CHD-FA®. These inclusion levels effectively delivered 0.02 (F0.5), 0.04 (F1), 0.2 (F5) and 0.4 (F10) g kg⁻¹ pure Fulvic acid. Each treatment was replicated 6 times. Effect of treatment on production performance results were compared for significant differences using one-way ANOVA with Tukey’s multiple comparison test. Results indicated that a maximum level of 1 g kg⁻¹ CHD-FA® that effectively delivered 0.04 g kg⁻¹ Fulvic acid increased bodyweight and length with 22 and 8 percent respectively without adversely affecting feed intake, feed conversion ratio or mortalities negatively. Failure to explain this improvement through antimicrobial effect is motivated due to lack of significant antimicrobial effect in a microbial plate-sensitivity test on tilapia gut microbes and human food borne pathogens.

Key words: AGP alternatives, organic acid, humic substances, fulvic acid, tilapia

*E-mail address: Lfdw@sun.ac.za (L.F. de Wet).
Effect of dietary different levels of probiotic on growth performance of rainbow trout *Oncorhynchus mykiss* in larvae stage

Alizadeh Morteza¹, A.farzanfar³, G.R. Lashtuaghai¹, M. Bayati⁴, R. Ghorbani⁵

¹ Inland Brackishwater Fishes Research Station, POBOX 89715-1123, Bafgh, Iran
², ³, ⁴ Cold water Fishes Research Center, POBOX 46815-467, Tonekabon, Iran
⁵ Department of Fisheries and Aquaculture, Gorgan University, Gorgan, Iran

Abstract

Current research conducted as a case study at Coldwater Fishes Research Center (CFRC) in Tonekabon, Iran. The main objectives of this study were evaluating some factors such as: growth performance, survival rates, FCR, PER, SGR, NPU and nutrition values of trout larvae which had fed from polluted pellets by a commercial probiotic "BioPlus 2B". This product contains two stains bacteria as *Bacillus subsitilis* and *B. licheniformis*. For starting this research, 12000 pieces of Rainbow trout, *Oncorhynchus mykiss* larvae in 400 mg were transferred from a well-known hatchery and after adaptation procedures they were kept through the 12 Californian troughs as 6 experimental treatments and 2 repetitions with density of 1000 pieces per trough. They were fed by polluted pellets with the probiotic supplement in various percentages (1.6×10⁹ CFUg⁻¹) of 0, 0/1, 0/2, 0/5, 0/75 and 1. The experiment had been continued for 36 days and biometry operation conducted in 6 day intervals.

After determination of research, all results were analyzed with accurate statistical methods. According to the results, there are harmonized relation ratio between increasing rate of fed probiotics and some parameters like WG, GR, SGR and NPU in all experimental larvae, except controls. After research duration, some specific factors of nutrition values, like protein and fatty component were determined. According to these results, increasing rate of fed probiotics is harmonized in protein values of all treatments, but inverse relationship in fatty values in most of the records. However, there are no any significant and harmonized variations in FCR and PER values.

The main reasons of the achieved results might be related to the ability of bacillus groups for producing B complex vitamins and gastric enzymes like protease.

Keywords: Probiotic; Bacilus subsitilis; B.licheniformis; Rainbow trout; *Oncorhynchus mykiss*; Feeding; Growth.

* E-mail address: m_alizadeh47@yahoo.com (M.alizadeh).
Dietary supplementation of glutamate and arginine to Atlantic salmon (Salmo salar L.) increases growth during the first autumn in sea cages

Maike Oehme1,2, Fabian Grammes2,3, Harald Takle3, José-Luis Zambonino-Infante4, Ståle Refstie1, Thomassen M.S2,3, Kjell-Arne Rørvik2,3 and Bendik Fyhn Terjesen1

1 The Norwegian Institute of Food, Fisheries and Aquaculture Research (Nofima), NO-6600 Sunndalsøra, Norway
2 Institute of Animal and Aquaculture Sciences, Norwegian University of Life Sciences, P.O. Box 5003, N-1432 Ås-UMB, Norway
3 The Norwegian Institute of Food, Fisheries and Aquaculture Research (Nofima), P.O. Box 5010, N-1432 Ås, Norway
4Ifremer, PFOM-ARN laboratory, BP 70, 29280 Plouzané, France

Abstract

In the sea water phase, the fish is exposed to shifting environmental conditions during the seasons. Especially temperature and photoperiod vary considerably affecting the fish's physiology and metabolism. Strategic dietary supplements may enable the fish to sustain a higher growth rate during these critical periods. In this experiment, a set of two amino acids (L-arginine and L-glutamate) were used as dietary supplements due to the increasing evidence that these amino acids may directly act as metabolic regulators, influence growth, reproduction and the immune response. In the case of arginine, the control diet was set at previously assumed requirement, while the test diet was supplemented further.

The effect of this supplementation on feed intake and growth parameters was studied, as well as selected molecular and hormonal indicators. During the first experimental period (May - June), no significant differences were found. However, in the second period of the experiment (June - September), higher specific feeding rate (SFR), thermal growth coefficient (TGC) and specific growth rate (SGR) (p<0.05) occurred in fish fed the supplemented diet together with a trend (p<0.1) for increased final body weight. The measured gene expression levels of IGF/GH genes did not correlate with the increased growth observed after the second period in fish fed the supplemented diet. No difference in total plasma IGF-I concentrations was found after the first period. After the second period fish fed the supplemented diet had in fact a significantly lower plasma IGF-1 level. Additionally, we found higher relative weight of gastrointestinal sections and elevated levels of plasma arginine, urea and ornithine after the second period in fish fed the supplemented diet.

We conclude that dietary supplementation of arginine and glutamine significantly increased feed intake and growth during the second feeding period in autumn. This response was observed during rapidly decreasing day lengths and at high feed intake.

Keywords: amino acid supplementation; growth; feed intake; season

*E-mail address: maike.oehme@nofima.no
Dietary attractants improve feed consumption and growth of soft-shelled turtles fed semi-purified diets

Wei-Hsiang Lin*, Chen-Huei Huang
Department of Aquatic Biosciences, National Chiayi University, Taiwan

Abstract
Soft-shelled turtle, *Pelodiscus sinensis*, is a high-valued aquaculture species in Asia. This animal is considered an excellent nutraceutical in traditional Chinese medicine. Nonetheless, the nutrient requirements of this reptile have not yet been fully investigated. It is a common practice to use semi-purified diets in conducting a nutrient requirement trial. However, palatability may be a problem for some animals when this type of diet is used. Therefore, it is important to develop a suitable attractant for these experiments. Soft-shelled turtles with initial mean body weight of 4.9 ± 0.2g, were fed diets either with or without attractants (betaine, L-arginine, glycine, cystine, L-isoleucine, L-phenylalanine, L-leucine, L-valine, L-methionine, L-histidine-HCl, tryptophan, L-tyrosine) for 8 weeks. Feed intake, weight gain and feed conversion ratio were significantly higher for turtles fed diet containing 6% attractant than control diets ($p < 0.05$). Hematological parameters (Hct, WBC, RBC, HGB, MCV, MCH, MCHC) did not affect by the dietary attractant supplementation. Supplementation of dietary attractants improves growth and feed consumption of soft-shelled turtles fed semi-purified experimental diets.

Key words: soft-shelled turtles, attractant, amino acid

*E-mail address: s0980537@mail.nctu.edu.tw*
Effects of dietary supplementation of compound Chinese herbal medicine on antioxidant ability of *Claris lareza*

Chen Cheng-xun*, Wang Qing-kui, Xu Da-wei, Ji Yan-bin, Zhao Jing-quan, Yang Shu-yuan
Tianjin Key Laboratory of Aquaeology and Aquaculture, Fisheries Science Department, Tianjin Agricultural University, Tianjin, 300384 China

Abstract

A preliminary study was conducted to determine the effect of dietary compound Chinese herbal medicine (CCHM)(composed of radix Astragalus, radix Paeoniae, rheum Rhaponticum, radix Glycyrrhizae, Dipsacus asper and fructus Gardeniae) on antioxidant ability of *Claris lareza*. The basal diet was composed of 83% fresh viscera of poultry and 17% dry artificial feed. CCHM was added in dry artificial feed at 2.5‰ (CCHM 2.5) or 5.0‰ (CCHM 5.0) in the test groups. Each diet was fed to triplicate groups of fish (initial body weight 532.7±41.6 g) for 22 d. Fish were fed twice daily to near satiation. Activity of superoxide dismutase (SOD) and catalase (CAT) and content of malondialdehyde (MDA) in liver, spleen and head kidney were assayed on day 11 and 22. Activity of SOD and CAT in test groups was increased during feeding and statistically significant on day 11 (group CCHM 5.0) and on day 22 (group CCHM 2.5 and CCHM 5.0). SOD and CAT in CCHM 5.0 were the highest than that in the other two groups. MDA content in test groups was decreased during feeding and statistically significant on day 11 (group CCHM 5.0) and on day 22 (group CCHM 2.5 and CCHM 5.0). MDA in CCHM 5.0 was the lowest during test. These results indicated that CCHM supplemented in diet enhanced the antioxidant ability of *Claris lareza*.

Keywords: *Claris lareza*; Compound Chinese herbal medicine; Antioxidant ability

*E-mail address: ccxny@163.com (Chen Cheng-xun).*
Effect of supplementation of microcapsule or crystalline methionine in diets with low fish meal on growth performance of juvenile cobia *Rachycentron canadum* (Linnaeus)

Shuyan Chi1, 2*, Beiping Tan1, Kangsen Mai2, Qinghui Ai2, Xiaohui Dong1, Qihui Yang1, Qicun Zhou1

1Laboratory of Aquatic Economic Animal Nutrition and Feed, College of Fisheries, Guangdong Ocean University, Zhanjiang, PR China
2 The key Laboratory of Mariculture (Education Ministry of China), Ocean University of China, Qingdao PR China

Abstract

This study was conducted to evaluate the effect of supplementation of crystalline methionine or microcapsule methionine in diets on growth performance and feed utilization of juvenile cobia (*Rachycentron canadum* Linnaeus). Seven iso-nitrogen and iso-energy diets, including positive control (fish meal), negative control (low fish meal with methionine deficiency) and five methionine supplemented with crystalline L-methionine diet (MET), hydroxyl-methionine calcium (MHA), cellulose-acetate-phthalate microcapsule L-methionine (CAP), acrylic resin microcapsule L-methionine (RES) and tripalmitin-polyvinyl alcohol microcapsule L-methionine (TPA) respectively, were prepared to investigate utilization of microcapsule and crystalline methionine on juvenile cobia. Each treatment was randomly assigned to triplicate aquaria stocked with 20 fish (initial body weight 5.40±0.07 g) each. Fish were maintained in flow-through aquaria for 60-day at water temperature ranged from 29 to 31°C. The results showed that weight gain and specific growth rate of fish fed RES were the highest in methionine supplementation groups and significantly higher 23.64% and 7.99% than those of negative group (P<0.05). Protein efficiency ratio of fish fed MET was significantly higher than that of negative control and the other methionine supplementation groups (P<0.05); Feed conversion ratio of fish fed MET was significantly lower than that of the others (P<0.05). Condition factor of fish fed methionine had no significant difference with positive control (P>0.05). Crude protein contents and rate of methionine to essential amino acid (A/E) in muscle of fish fed microcapsule and crystalline methionine groups had no significant difference with positive control (P>0.05). These findings indicate that supplementation of crystalline methionine in diet with low fish meal could promote growth performance of juvenile cobia.

Keywords: Juvenile cobia (*Rachycentron canadum* Linnaeus); Methionine; Microcapsule; Growth performance

*E-mail address: chishuyan@yahoo.com.cn*
Effects of dietary compound probiotics on growth, survival, activities of digestive enzymes and stress resistance of large yellow croaker (\textit{Pseudosciaenid crocea}) larvae

Jin Gao*, Kangsen Mai, Qinghui Ai, Hongming Ma, Xiaojie Wang, Wenbing Zhang, Wei Xu, Zhiguo Liufu
The Key Laboratory of Mariculture (Ministry of Education), Fishery College of Ocean University of China, 5 Yushan Road, Qingdao 266003, China

Abstract

A 30-day feeding experiment was conducted in tanks to estimate the effects of dietary compound probiotics (Xiale333, \textit{Bacillus Subtilis}, lactobacillus and \textit{Arthrobacter} $10^7$cfu/g as primary effective ingredients) of large yellow croaker larvae. A basal diet was supplemented with 0.00% (control), 0.20%, 0.50% and 1.00% compound probiotics to formulate four experimental diets. Each diet was randomly assigned to triplicate groups of larvae (15 days post hatch, initial body weight 2.75±0.31mg), 3000 larvae each tank. Larvae were fed eight times daily to satiation during rearing period. Results showed that the specific growth rate (SGR) had an increasing trend with increasing Xiale333, but no significant difference was observed among dietary treatments. Obviously improved survival were observed in larvae fed diets with supplementation of compound probiotics, survival in larvae fed the diets with 0.20%, 0.50% and 1.00% supplementation were significantly higher than that of control group. The challenge experiment showed an analogous trend of that of survival, the supplementation of compound probiotics significantly elongated the half-lethal time of larvae when challenged with high temperature, but there were no remarkable difference between 0.20%, 0.50% and 1.00% supplementation groups. There were no significant difference in activities of amylase and trypsin in intestinal or pancreatic segments among fish fed different diets. The activities of alkaline phosphatase in intestinal segments of larvae in 0.20% treated group were significantly higher than that of the control group, indicated that compound probiotics supplements promoted the mature of digestive gut of large yellow croaker larvae. Results of the present study indicted that compound probiotics Xiale333 could promote growth, significantly improve survival and stress resistance and speed the development of digestive tract of large yellow croaker larvae.

Keywords: large yellow croaker larvae; probiotics; growth performance; activities of digestive enzymes; stress resistance

* E-mail address: xiaoxie851227@163.com (Jin Gao)
In vitro effects of arachidonic acid on leucocytes immune functions of large yellow croaker (Pseudosciaena crocea) head-kidney

Qingfei Li1, Qinghui Ai1, Kangsen Mai1, Wenbing Zhang1, Wei Xu1, Hongming Ma1, Xiaojie Wang1,Yuefu Zheng2

1The Key Laboratory of Mariculture (Ministry Education of China), Ocean University of China. 5 Yushan Road, Qingdao, Shandong 266003, P.R. China.
2Xiangshan Oceanic and Fishery Bureau. Danhe Road, Ningbo, Zhejiang 315700, P.R. China.

Abstract
Arachidonic acid (ARA) has been proved to play an important role in modulation of fish immunity. However, it is far from clear that how arachidonic acid affect fish health. To examine the mechanism by which arachidonic acid regulate immune functions of leucocytes, a series of in vitro manipulations were conducted with cells isolated from the head-kidney of large yellow croaker (average weight approximately 400 g) fed low-value fish. Leucocytes derived from head-kidney were maintained in Leibovitz’s L-15 medium supplemented with 5% fetal bovine serum as long as at least 4 days without a significant reduction of cell viability. Leucocytes were firstly cultured in serum-free medium for 1 day and then exposed to different levels of arachidonic acid (0, 5, 25, 50, 100, 200 and 1000 μM) which was incorporated to bovine serum albumin supplemented in culture medium for 6h, 22h and 44h. Following culture and stimulation, cell toxicity, phagocytosis, production of superoxide anion (O2-) were determined. Production of nitric oxide and secreted phospholipase A2 (sPLA2) were also investigated after an incubation of 22 hours. Results showed that there were no significant effects on cell toxicity for cultured cells with different levels of arachidonic acids. Phagocytosis and superoxide anion (O2-) production, however, were significantly (P<0.05) affected. Compared to the higher concentration (200 and 1000 μM), lower concentration arachidonic acid (5, 25 and 100 μM) can significantly enhance the phagocytosis and superoxide anion production. Moreover, after having been incubated for 44h, marked increases in Phagocytosis and superoxide anion (O2-) production were observed in higher concentration treatments (200 and 1000μM) in comparison with groups of 6h incubation. Arachidonic acid of all concentrations can significantly enhance both the production of nitric oxide and secreted phospholipase A2 with maximal responses at 25μM. All the findings suggest that arachidonic acid modulate the innate immune responses by increasing the production of secreted phospholipase A2 in vitro. The increased sPLA2 may be, in part, due to the exogenous arachidonic acid which has an influence on the fluidity and stability of cell membrane, which may activate sPLA2 to release the endogenous arachidonic located at the sn-2 position of the membrane phospholipids. For the first time, characterization and in vitro culture of Pseudosciaena crocea leucocytes was studied.

Keywords: Arachidonic acid, Large yellow croaker, Superoxide anion (O2-), Phagocytosis, Nitric Oxide, secreted phospholipase A2, Immune response, In vitro

*E-mail address: qflii0310@gmail.com
Effects of different feed additives and combination on growth and non-specific immunity of European eel (*Anguilla anguilla*)

Lin jian-bin¹, Zhu ing-guo¹, Ling Ping¹, Chen Du-huan¹, Qin Zhi-qing¹, Ai Chun-xian²

¹Fujian Provincial Institute of Freshwater Fisheries, Fuzhou 350002, china; ²College of Ocean and environment, Xiamen University, Xiamen 361005, China

Abstract

An experiment was conducted to investigate the effects of adding Chinese herbal medicine, enzymes preparations, cysteamine on growing performance and non-specific immune function of *Anguilla anguilla*. *Anguilla anguilla* with body weight of Experiment I (10.62±2.30)g, Experiment II (18.10±1.73) g were fed with basal diet without or with adding Chinese herbal medicine, enzymes preparations, cysteamine and their combinations (the control,Diet 1~6) for 8 weeks. The results showed that Chinese herbal medicine, cysteamine had a significant positive effect on growth and the superoxide dismutase activities (SOD) and lysozyme activities of *Anguilla anguilla* (p<0.05); the combinations of three additives had a significant positive effect on growth *Anguilla anguilla* (p<0.05), enzymes preparations only increased the SOD and the superoxide dismutase activities (SOD) and lysozyme activities of *Anguilla anguilla* (p<0.05). For diet 5, the rate of weight gain increased 31.67%, feed conversion ratio reduced 13.12% than the control group. The growth rate of diet 6 was lower than that of diet 5. There was no significant difference in the feed conversion ratio, protein efficiency and immunity (lysozyme, superoxide dismutase activities) between diet 5 and diet 6. However alkaline phosphatase (AKP) activities were not affected by all tested groups (p>0.05). Considering the cost and quantity of the additives, diet 6 was superior to select.

Keywords: *Anguilla anguilla*; additives; immunity; growth rate; feed coefficient
Effects of Phosphorus and Phytase on Growth Performance and Body Composition in Juvenile Tilapia Fed Fishmeal-free Diet

Zi-Jiao He, Qing Pan*, Yan Chen, Bin-Chong Qiu, Ying-Zuo Bi, Jinghua Fu, Yuan Sun
Dept. of Aquaculture, School of Animal Science, South China Agricultural University, Guangzhou 510642, P. R. China

Abstract
The objective of this study was to investigate growth performance and body composition of juvenile tilapia Oreochromis niloticus × O. aureus (with initial body weight of 2.5 g) reared in estuarine water with fishmeal-free diets supplemented with monobasic calcium phosphate and phytase. Five test diets were formulated in which fishmeal diet (D0) was applied as control. Fishmeal-free diets were supplemented without phytase (D1), and with phytase (D2), with monobasic calcium phosphate alone (D3) and with monobasic calcium phosphate and phytase as well (D4). Each test diet was fed to three replicate tanks of fish. 30 fish were randomly stocked into each tank and reared in a flow-through system for 8 weeks. Feeding rate was 5%-6% of body weight to apparent satiation. The results showed that specific growth rate, feed efficiency and protein efficiency in fish fed diets with supplements of P and phytase (D2, D3 and D4) were significantly increased but still lower than that in fishmeal group (D0) (P>0.05). Significantly higher apparent digestibility of phosphorus and magnesium was observed in fish fed diets with P and phytase supplements (D4), and no significant differences were observed with fish in fishmeal group. Apparent digestibility of energy in fish with fishmeal was significantly higher than other groups. Body crude protein and fat contents in fish with fishmeal diet was markedly higher whereas the moisture content was markedly lower. The contents of body ash in fish fed diets with P or phytase increased markedly but lower than that in fish with fishmeal diet. The ratio of P to Ca in fish fed diets with monobasic calcium phosphate supplement had no significant difference with that in fishmeal group. The contents of body Mg, Zn and the contents of ash, Ca, P in lipid-extracted bone increased in fish fed the diet with P and phytase supplements. The serum P content and the activities of serum alkaline phosphatase were significantly higher and lower, respectively. The results indicated that monobasic calcium phosphate supplemented in the fishmeal-free diet could improve the growth, feed utilization and the ratio of body edible part, and enhance the deposition of P, Mg and Zn in body. Adding phytase simultaneity could further increase utilization of dietary P and Mg but did not reach the effect of fishmeal.

Keywords: Tilapia; Phosphorus; Phytase; Growth performance; Body composition

E-mail address: qpan@scau.edu.cn (Qing Pan).
Effects of natural plant additives garlic stem powder and oregano leaf powder on growth performance, digestive enzyme activity and serum biochemical indexes of the carp (Cyprinus carpio L. minor)

Tang Ling 1, 2, Xu Qi-You 1
1. Heilongjiang River Fisheries Research Institute of Chinese Academy of Fishery Sciences, Harbin 150070, China
2. Fisheries and Life Science College of Shanghai Ocean University, Shanghai 201306, China

Abstract
The overuse of antibiotics in feed animals caused the "three-induced effects" and environment pollution which would affect human health. The study of natural plant additives is a hotspot currently, the garlic and oregano have no drug residues and side-effects. This trial investigated the effects of garlic stem powder and oregano leaf powder on growth performance, digestive enzyme activity and serum biochemical indexes of the mirror carp (Cyprinus carpio L. minor). A total of seven trial treatment groups: G1 was control group, G2 added 10mg/kg Flavomycin, G3, G4 added 0.5% and 2.5% garlic stem powder respectively, G5, G6 added 0.1% and 0.5% oregano leaf powder respectively, G7 added 0.5% garlic stem powder and 0.5% garlic stem powder commonly. Each treatment had 3 replicates of 10 fish with initial body weight (201.45 ± 16.25) g. The feeding trial was conducted for 8 weeks. The results showed: 1) Compared with G1 and G2, G3 garlic group was significantly increased specific growth rate and weight gain rate (P < 0.05), significantly reduced feed conversion rates (P < 0.05), G3, G4 garlic group and G5, G6 oregano group significantly increased the efficiency of the protein (P < 0.05). 2) Compared with G1, G3 garlic group was significantly increased hepatopancreas protease and lipase, foregut and midgut protease activity (P < 0.05), G4 garlic group was significantly increased protease activity of hepatopancreas (P < 0.05), G5, G6, G7 intestinal amylase and lipase activity were significantly higher than G1, G2 (P < 0.05). 3) Compared with G1, all the experimental groups except G7 reduced TP and ALB significantly (P < 0.05), C3 and C4 of G3 and G5 group were significantly increased (P < 0.05), ALT and AST of G2 were significantly reduced (P < 0.05), ALP of each trial group was significantly reduced (P < 0.05). Conclusion: The garlic stem powder can effectively improve the growth performance, digestive enzyme activities of hepatopancreas and serum biochemical level of mirror carp, oregano leaf powder can increase the protein efficiency, improve digestive enzyme activity of foregut and serum complement activity.

Keywords: mirror carp; garlic stem powder; oregano leaf powder; growth performance; digestive enzyme activity; serum biochemical index

*E-mail address: tangling2008v@yahoo.cn
A study on the reducing-drug-residue additives for *Anguilla japonica*

Xi Feng, Wang Qiurong, Lin Limin
(Fisheries and Food Safety Science and Technology Key Laboratory of Colleges and Universities in Jimei University, Xiamen, China, 361021)
(Xiamen Feed Detection and Safety Evaluation key Laboratory, Xiamen, China, 361021)

Abstract

The control of drug residues in eel for the preservation of human food safety has a positive meaning. To this end, *Anguilla japonica* were fed for a 5-day continuous Oxytetracycline (200mg/kg BW) in 20 °C ~ 22 °C, three kinds of additives were fed for 7 days respectively, and muscle sample were taken in the 1, 2, 3, 5, 8, 11, 13, 16, 19, and 21 day. UV-Vis Spectrophotometer were used to measure the changes of Oxytetracycline in the samples of eel muscle, and compared with the elimination of the normal metabolism. The results showed that, Three kinds of additives are reflected in a strong effect of lowering drug residues, in which the oxytetracycline residues in muscle of the chitin group Longdanxiengan group were 0.472 mg / kg and 0.275 mg / kg in 21th day, respectively, close to the level of exports which is 0.1 mg / kg of the oxytetracycline residues in muscle, and below the 0.867 mg / kg in the control group in 34th day which is the end of the feeding experiment; the self-made traditional Chinese medicine additive is the best additive which can lower oxytetracycline residues from 4.221 mg / kg in 11th day to 1.261mg/kg in 13th day, and further reduce to 0.699 mg / kg in 16th day, and has failed to detect oxytetracycline residues in 19th day later. Thus, three kinds of additives can accelerate the elimination rate of the oxytetracycline in eel muscle, and shorten the time of eliminating oxytetracycline residues. In compare with the oxytetracycline residue level of the control group in 34th day, the chitin and Longdanxiengan group can reduced by at least 13 days (38.2%), and the traditional Chinese medicine group could shorten above 18 days (52.9%), which will favor the development of the eel industry.

Key words: Oxytetracycline; additives; ell (*Anguilla japonica*); residue

*E-mail address: xifeng@jmu.edu.cn*
Effects of dietary lactosucrose on growth performance and serum biochemical indices in *ctenopharyngodon idellus*

Fa Lan Zhao¹², Wu Ying Chu¹, Rui Xue Zhou¹², Tao Meng¹², Jia Cheng¹, Jian She Zhang¹*

¹ College of Biological Engineering and Environmental Sciences, Changsha University, Changsha, China
² College of Life Science, Guangxi Normal University, Guilin, China

Abstract

A 7-weeks feeding trial was conducted to determine the effects of lactosucrose on growth performances and biochemical indices in grass carp *ctenopharyngodon idellus*. The fishes (initial body weight 20±1.7g) were randomly allotted to 4 groups with 150 repeats per group. Group I, as the control group, was fed with the basal diet, while group II, III and IV as trial groups, were fed with the basal diet added with 1%, 1.5% and 2% lactosucrose respectively. Results showed that with the increasing of dietary lactosucrose levels, weight gain rate (WGR) increased in II, III, IV groups by 0.71%, 21.31%, 2.65%, and specific growth rate (SGR) by 1.25%, 24.29%, 9.40% in comparison with control group respectively, but no significant differences (p > 0.05) were observed. Total protein (TP), lactic dehydrogenase (LDH) and blood urea nitrogen (BUN) in trial groups were higher (p > 0.05) than that of the control group. No significant differences were observed in the activities of lysozyme (LZM), serum alkaline phosphatase (ALP) (p > 0.05) among the four groups. Total cholesterol diagnostic kit (TC) and glutamic-oxaloacetic transaminase (GOT) were lower in trial groups compared with control group, and group III was significantly lower (p < 0.05) than the control group. Based on these results, supplemented lactosucrose in diets could improve growth performance and play an important role in maintaining normal liver function of *ctenopharyngodon idellus*.

Keywords: Lactosucrose; *Ctenopharyngodon idellus*; Growth performance; Serum biochemical indices

*E-mail address: jzhang@ccsu.cn (J.S. Zhang).*
P-357

Effects of different dietary levels of bile acid product on growth performance and serum biochemical indexes of hybrid Snakehead, *Channa maculate♀ × C.argus♂*

Zhu Wangming¹, Zhou Meng¹,²
ⁱ Guangzhou Sintun Aqua-tech CO., LTD, Guangzhou 510640, People's Republic of China
² College of Life Science, South China Normal University, Guangzhou 510640, People's Republic of China

Abstract:

0, 200, 500 and 1000mg/kg bile acid product (commercial name Y Sintun, containing bile acid 15%) were supplemented into commercial feed of hybrid snakehead, *Channa maculate♀ × C.argus♂* (IBW, 158.94±1.63g) to feed the animal for 12 weeks, the results showed that:

1. At the end of the 4, 8 and 12th week, WGR of the fish fed 200, 500 and 1000 mg/kg Y Sintun were averagely increased for 1.98%, 5.39% and 5.81% compared with the control, and showed significant difference (P<0.05) at the 1000mg/kg group at and of the 12th week;

2. At the end of the 12th week, crude protein content of the dorsal muscle of the fish fed 200 and 1000ppm Y Sintun were significantly higher than that of control (P<0.05), and crude lipid tent to decrease (P>0.05);

3. At the end of the 12th week, HSI, VR of the fish fed Y Sintun tent to decrease compare with those of control, and VR of the fish fed 500mg/kg Y Sintun was significantly lower than that of control (P<0.05);

4. Serum ALT content of the fish fed 200 and 500mg/kg Y Sintun were significantly lower than that of control (P<0.05), and TG content of all the Y Sintun groups tent to decrease (P>0.05);

5. Serum SOD content of all the Y Sintun groups tent to increase, and were significantly higher than that of control at the 1000 mg/kg group (P<0.05), while MDA content tent to decrease at all Y Sintun groups (P>0.05);

The results of the reseach suggested that, supplementation of Y Sintun in the feed helped to hence growth performance and muscle nutrient composition of hybrid snakehead, benifit to dietary transportation and utilization of the animal, and increased its anti-oxidation ability. Here, 1000mg/kg Y Sintun were recommeded to add into the feed.

Keywords: Hybrid snakehead; Bile acid product; Growth; ALT; SOD; MDA

*E-mail address: mzhdg@sina.com*
P-358

Cloning and functional characterization of fatty acyl desaturase and elongase genes from southern bluefin tuna Thunnus maccoyii

Kathryn A. Schuller*, Melissa K. Gregory, Valene H.L. See and Robert A. Gibson

School of Biological Sciences, Flinders University, GPO Box 2100, Adelaide SA 5001

The synthesis of long chain polyunsaturated fatty acids (LCPUFA) such as eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) involves fatty acyl desaturase and elongase enzymes. The marine fish species southern bluefin tuna (SBT) can accumulate large quantities of omega-3 (n-3) LCPUFA from its diet in its flesh but its capacity to synthesize EPA and DHA from the essential fatty acid alpha-linolenic acid (ALA; 18:3n-3) is uncertain. Here we describe the cloning of a delta-6-desaturase (D6D) and a fatty acyl elongase (Elov15) involved in the synthesis of EPA and DHA from ALA. The cDNAs were functionally characterized by expressing them in yeast, Saccharomyces cerevisiae, and supplying the yeast with potential fatty acid substrates for the enzymes the cDNAs encoded. The deduced amino acid sequence and the fatty acid substrate preference of the fatty acyl elongase were found to most closely resemble fish and mammalian Elov15 with preference for C18:2n6 PUFA over C22 PUFA. The D6D deduced amino acid sequence was very similar to that of other fish species studied to date. It was successfully expressed in yeast but the activity of the encoded enzyme was very low. This suggests that SBT may have limited capacity to synthesized EPA and DHA from ALA. The results are discussed in relation to the potential for fish oil replacement with vegetable oils in the diets of farmed SBT.

Fig. 1. Yeast cells expressing the SBT Elov15 enzyme were supplied with the substrates listed above each graph. The % distribution of substrate and product fatty acid in total cellular lipid was determined.

*E-mail address: kathy.schuller@flinders.edu.au

Fig. 2. The LCPUFA synthesis pathway in vertebrates commencing with the essential fatty acids ALA (18:3n-3) and linoleic acid (LA; 18:2n-6) and highlighting the role of the fatty acyl elongase enzymes Elov12 and Elov15.

Conclusion: DHA is the major contributor to the human health value of seafood. Fish oil replacement with vegetable oils will reduced the proportion of DHA in the flesh of farmed SBT.
Cloning and functional characterization of a 2-Cys peroxiredoxin from southern bluefin tuna *Thunnus maccocyii*

Kathryn A. Schuller*, Drew L. Sutton, Grace H. Loo and R. Ian Menz

School of Biological Sciences, Flinders University, GPO Box 2100, Adelaide SA 5001

Tuna lipids contain high proportions of long chain polyunsaturated fatty acids (LCPUFA), especially docosahexaenoic acid (DHA; 22:6n-3). LCPUFA, especially DHA, are major contributors to the human health value of seafood. However, LCPUFA are also highly susceptible to oxidative deterioration due to lipid peroxidation. Lipid peroxidation is counteracted by dietary antioxidant vitamins and minerals (e.g. vitamin E and selenium) interacting with cellular antioxidant enzymes. The peroxiredoxin (Prx) family is a relatively newly discovered antioxidant enzyme family. Here we describe the cloning and functional characterization of a 2-Cys Prx cDNA from SBT liver.

The SBT Prx sequence was very closely related (76-92% identical) to Prx 1 and 2 sequences from other fish and mammals. Phylogenetic analyses showed that it was most likely a Prx 2. The deduced amino acid sequence contained the peroxidatic and resolving Cys residues characteristic of typical 2-Cys Prx proteins from all kingdoms of life. It also contained the GGLG motif associated with the sensitivity of eukaryotic typical 2-Cys Prx proteins to overoxidation and inactivation by H$_2$O$_2$.

When the SBT Prx 2 was expressed in *E. coli*, it showed typical thioredoxin (Trx)-dependent peroxidase activity with H$_2$O$_2$, cumene hydroperoxide (CuOOH) and t-butyl hydroperoxide. The SBT Prx displayed Michaelis-Menten kinetics with Trx but sigmoidal kinetics with H$_2$O$_2$ and CuOOH (see figure opposite). The $K_m$(Trx) was 12 μM and the $S_{0.5}$ values for H$_2$O$_2$ and CuOOH were 29 and 25 μM, respectively. The native SBT Prx enzyme existed as a mixture of dimers, tetramers and decamers with the decamer being the predominant form. Prx gene expression was highest in the liver and gills of SBT.

*E-mail address: kathy.schuller@flinders.edu.au*
## Author Index for Presentations

<table>
<thead>
<tr>
<th>Name</th>
<th>Country</th>
<th>Presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aghazadeh A. Mirza</td>
<td>Iran</td>
<td>P-001</td>
</tr>
<tr>
<td>Ahmadi B.</td>
<td>Iran</td>
<td>P-232</td>
</tr>
<tr>
<td>Ai Chunxiang</td>
<td>China</td>
<td>P-114, P115</td>
</tr>
<tr>
<td>Albrektsen Sissel</td>
<td>Norway</td>
<td>O-033</td>
</tr>
<tr>
<td>Alhazzaa Ramez</td>
<td>Australia</td>
<td>O-013, P-063</td>
</tr>
<tr>
<td>Alizadeh Morteza</td>
<td>Iran</td>
<td>P-047, P-244, P-345</td>
</tr>
<tr>
<td>Areechon Nontawith</td>
<td>Thailand</td>
<td>P-330</td>
</tr>
<tr>
<td>Ariyaratne M.H.S.</td>
<td>Sri Lanka</td>
<td>P-020</td>
</tr>
<tr>
<td>Arnason Jón</td>
<td>Iceland</td>
<td>P-304</td>
</tr>
<tr>
<td>Arslan Murat</td>
<td>Turkey</td>
<td>P-016</td>
</tr>
<tr>
<td>Askarian Fatemeh</td>
<td>Iran</td>
<td>O-102</td>
</tr>
<tr>
<td>Atalah E.</td>
<td>Spain</td>
<td>P-161</td>
</tr>
<tr>
<td>Bae Kimin</td>
<td>Korea</td>
<td>P-042</td>
</tr>
<tr>
<td>Bai Dongqing</td>
<td>China</td>
<td>P-303, P-332</td>
</tr>
<tr>
<td>Bai Lirong</td>
<td>China</td>
<td>P-209</td>
</tr>
<tr>
<td>Bai Nan</td>
<td>China</td>
<td>O-059, P-150</td>
</tr>
<tr>
<td>Bake Gabriel Gana</td>
<td>Japan</td>
<td>O-092</td>
</tr>
<tr>
<td>Bakke Anne Marie</td>
<td>Norway</td>
<td>O-035</td>
</tr>
<tr>
<td>Baric-Rafaj Renata</td>
<td>Croatia</td>
<td>P-280</td>
</tr>
<tr>
<td>Benítez-Santana Tibiábin</td>
<td>Spain</td>
<td>O-018</td>
</tr>
<tr>
<td>Betancor Mónica B.</td>
<td>Spain</td>
<td>O-082</td>
</tr>
<tr>
<td>Bharadwaj Anant S.</td>
<td>USA</td>
<td>O-100</td>
</tr>
<tr>
<td>Biswas Amal</td>
<td>Japan</td>
<td>P-319</td>
</tr>
<tr>
<td>Bodin Noélie</td>
<td>Belgium</td>
<td>P-106</td>
</tr>
<tr>
<td>Boglino Anaïs</td>
<td>España</td>
<td>P-250</td>
</tr>
<tr>
<td>Bonaldo Alessio</td>
<td>Italy</td>
<td>P-035</td>
</tr>
<tr>
<td>Bórquez Aliro</td>
<td>Chile</td>
<td>P-010</td>
</tr>
<tr>
<td>Brel Jonathan</td>
<td>Belgium</td>
<td>P-281</td>
</tr>
<tr>
<td>Buentello J. Alejandro</td>
<td>USA</td>
<td>O-098</td>
</tr>
<tr>
<td>Bundit J.</td>
<td>Thailand</td>
<td>O-004</td>
</tr>
<tr>
<td>Bureau D.P.</td>
<td>Canada</td>
<td>O-024</td>
</tr>
<tr>
<td>Cahu Chantal L.</td>
<td>France</td>
<td>O-037</td>
</tr>
<tr>
<td>Cai Chunfang</td>
<td>China</td>
<td>O-008</td>
</tr>
<tr>
<td>Cam Tu Do</td>
<td>Vietnam</td>
<td>P-189</td>
</tr>
</tbody>
</table>
Cao Junming China P-219
Cao Junming China P-119
Cao Yanan China P-181
Chang Jie China P-113, P-184
Chang Qing China P-210
Chen Bing China P-140
Chen Chengxun China P-141, P-282, P-348
Chen Jinghua China P-053
Chen Naisong China P-251
Chen Qinghua China P-293
Chen Xiaobing China P-168
Chen Xiaoying China P-117
Chen Yongjun China P-065
Chen Yuhang China P-182
Chen Yuke China O-032
Cheng Lu China P-026
Chi Shuyan China P-349
Chikwati Elvis M. Norway O-021
Codabaccus Basseer M. Australia O-061, O-077
Colombo Stefanie M. Canada P-050
Corraze Geneviève France P-306
Crampton Vivian O. Norway P-024
Cui Xianjun China P-246
Cuvin-Aralar Maria L. Philippines P-337
Dadgar sh. Iran P-012
Dai Xiaofang China P-008
Dalsgaard Johanne Denmark O-099
Dantagnan Patricio Chile P-205
Daprá Franco France P-100
Dars B. A. Pakistan P-231
Davis Allen USA O-051
de Wet Lourens F South Africa P-344
Deng Dongfang USA P-238
Deng Shixi China O-094
Denstadli Vegard Norway P-165
Dias Jorge Portugal P-343
Döll S. Germany O-074
Duan Cuming USA O-036
<table>
<thead>
<tr>
<th>Name</th>
<th>Country</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duan Yuanhui</td>
<td>China</td>
<td>P-073</td>
</tr>
<tr>
<td>Engin Kenan</td>
<td>Turkey</td>
<td>O-010</td>
</tr>
<tr>
<td>Eroldoğan O. Tufan</td>
<td>Turkey</td>
<td>P-249</td>
</tr>
<tr>
<td>Eryalçın K.M.</td>
<td>Spain</td>
<td>P-295</td>
</tr>
<tr>
<td>Feng Fuxian</td>
<td>China</td>
<td>P-310</td>
</tr>
<tr>
<td>Feng Guangpeng</td>
<td>China</td>
<td>P-074</td>
</tr>
<tr>
<td>Feng Lin</td>
<td>China</td>
<td>P-082, P-227</td>
</tr>
<tr>
<td>Fernández I.</td>
<td>Spain</td>
<td>P-101, P-102</td>
</tr>
<tr>
<td>Fernández Ignacio</td>
<td>Spain</td>
<td>O-044</td>
</tr>
<tr>
<td>Fjelldal Per Gunnar</td>
<td>Norway</td>
<td>O-073</td>
</tr>
<tr>
<td>Ganga R.</td>
<td>Spain</td>
<td>P-172</td>
</tr>
<tr>
<td>Gao Jian</td>
<td>China</td>
<td>P-300</td>
</tr>
<tr>
<td>Gao Jin</td>
<td>China</td>
<td>P-350</td>
</tr>
<tr>
<td>Gao Lujiao</td>
<td>China</td>
<td>P-307</td>
</tr>
<tr>
<td>Gao Rongbin</td>
<td>China</td>
<td>P-027</td>
</tr>
<tr>
<td>Gao Wen</td>
<td>China</td>
<td>O-084, P-253, P-254</td>
</tr>
<tr>
<td>Gao Y.</td>
<td>China</td>
<td>O-058</td>
</tr>
<tr>
<td>Gao Yujie</td>
<td>China</td>
<td>P-312</td>
</tr>
<tr>
<td>Gatlin III Delbert M.</td>
<td>USA</td>
<td>O-071, O-026</td>
</tr>
<tr>
<td>Geurden I.</td>
<td>France</td>
<td>P-276</td>
</tr>
<tr>
<td>Giannenas Ilias A.</td>
<td>Greece</td>
<td>P-187</td>
</tr>
<tr>
<td>Gibtan Ashagrie</td>
<td>Ethiopia</td>
<td>P-177</td>
</tr>
<tr>
<td>Gisbert E.</td>
<td>Spain</td>
<td>P-097</td>
</tr>
<tr>
<td>Glencross Brett</td>
<td>Australia</td>
<td>O-060, P-093</td>
</tr>
<tr>
<td>Gonzalez Vecino José L.</td>
<td>Chile</td>
<td>P-204</td>
</tr>
<tr>
<td>Gu J.</td>
<td>Norway</td>
<td>O-072</td>
</tr>
<tr>
<td>Gu Min</td>
<td>China</td>
<td>P-194, P-200</td>
</tr>
<tr>
<td>Gunderersen Vidar</td>
<td>Norway</td>
<td>P-023</td>
</tr>
<tr>
<td>Guo Ran</td>
<td>China</td>
<td>P-174</td>
</tr>
<tr>
<td>Guo Yunxue</td>
<td>China</td>
<td>P-054</td>
</tr>
<tr>
<td>Haga Yutaka</td>
<td>Japan</td>
<td>O-047</td>
</tr>
<tr>
<td>Halver John E</td>
<td>USA</td>
<td>O-025</td>
</tr>
<tr>
<td>Hamilton Phil</td>
<td>USA</td>
<td>P-173</td>
</tr>
<tr>
<td>Hamre Kristin</td>
<td>Norway</td>
<td>P-266</td>
</tr>
<tr>
<td>Han Chunyuan</td>
<td>China</td>
<td>P-060</td>
</tr>
<tr>
<td>Han Yuzhe</td>
<td>China</td>
<td>P-308</td>
</tr>
<tr>
<td>Harakawa Shogo</td>
<td>Japan</td>
<td>P-134</td>
</tr>
<tr>
<td>Hartviksen Mali Bjerkhaug</td>
<td>Norway</td>
<td>P-242</td>
</tr>
<tr>
<td>Name</td>
<td>Country</td>
<td>Page</td>
</tr>
<tr>
<td>-----------------------</td>
<td>-------------</td>
<td>------</td>
</tr>
<tr>
<td>Hatice A. Yılmaz</td>
<td>Turkey</td>
<td>P-022</td>
</tr>
<tr>
<td>He Shuxu</td>
<td>China</td>
<td>P-190</td>
</tr>
<tr>
<td>He Zhi jiao</td>
<td>China</td>
<td>P-138, P-139</td>
</tr>
<tr>
<td>He Zijiao</td>
<td>China</td>
<td>P-353</td>
</tr>
<tr>
<td>Heinsbroek Leon T.N.</td>
<td>Netherlands</td>
<td>O-081</td>
</tr>
<tr>
<td>Hemre Gro-Ingunn</td>
<td>Norway</td>
<td>P-274</td>
</tr>
<tr>
<td>Hernández Adrián</td>
<td>Chile</td>
<td>P-034</td>
</tr>
<tr>
<td>Hernández Crisantema</td>
<td>México</td>
<td>O-029</td>
</tr>
<tr>
<td>Hevroy Ernst M.</td>
<td>Norway</td>
<td>O-027</td>
</tr>
<tr>
<td>Ho Wai Yee</td>
<td>Canada</td>
<td>O-083</td>
</tr>
<tr>
<td>Holme M.H.</td>
<td>Norway</td>
<td>P-178</td>
</tr>
<tr>
<td>Zhao Hong-Xia</td>
<td>China</td>
<td>P-116</td>
</tr>
<tr>
<td>Hu Liang</td>
<td>China</td>
<td>O-030, P-031</td>
</tr>
<tr>
<td>Hu Yi</td>
<td>China</td>
<td>P-255</td>
</tr>
<tr>
<td>Hua Xuemeng</td>
<td>China</td>
<td>P-156</td>
</tr>
<tr>
<td>Hua Ying</td>
<td>China</td>
<td>P-216</td>
</tr>
<tr>
<td>Huai Mingyan</td>
<td>China</td>
<td>O-053, P-125</td>
</tr>
<tr>
<td>Huang Yanhua</td>
<td>China</td>
<td>O-056</td>
</tr>
<tr>
<td>Huang Yanqing</td>
<td>China</td>
<td>P-055</td>
</tr>
<tr>
<td>Huang Ying</td>
<td>China</td>
<td>P-166</td>
</tr>
<tr>
<td>Hung Le Thanh</td>
<td>Vietnam</td>
<td>P-338</td>
</tr>
<tr>
<td>Hwang Namyong</td>
<td>Korea</td>
<td>P-155</td>
</tr>
<tr>
<td>Ji Hong</td>
<td>China</td>
<td>P-066, P-075</td>
</tr>
<tr>
<td>Ji Li</td>
<td>Japan</td>
<td>O-050</td>
</tr>
<tr>
<td>Jiang Guangzhen</td>
<td>China</td>
<td>P-256</td>
</tr>
<tr>
<td>Jiang Hongbo</td>
<td>China</td>
<td>P-143, P-144</td>
</tr>
<tr>
<td>Jiang Jun</td>
<td>China</td>
<td>P-081</td>
</tr>
<tr>
<td>Jiang Xueqin</td>
<td>China</td>
<td>O-076</td>
</tr>
<tr>
<td>Jin Lina</td>
<td>China</td>
<td>O-041</td>
</tr>
<tr>
<td>Ju Z.Y.</td>
<td>USA</td>
<td>P-025</td>
</tr>
<tr>
<td>Kader Md. Abdul</td>
<td>Japan</td>
<td>P-243</td>
</tr>
<tr>
<td>Kaushik S.J.</td>
<td>France</td>
<td>O-012</td>
</tr>
<tr>
<td>Kazemi M.</td>
<td>Iran</td>
<td>P-241</td>
</tr>
<tr>
<td>Kiessling Anders K.</td>
<td>Sweden</td>
<td>O-068</td>
</tr>
<tr>
<td>Kilimnik Anna Yu.</td>
<td>New Zealand</td>
<td>P-160</td>
</tr>
<tr>
<td>Kiriratnikom Anut</td>
<td>Thailand</td>
<td>P-037</td>
</tr>
<tr>
<td>Kiriratnikom Suphada</td>
<td>Thailand</td>
<td>P-299</td>
</tr>
<tr>
<td>Kitagima Renato E.</td>
<td>Brazil</td>
<td>P-273</td>
</tr>
<tr>
<td>Name</td>
<td>Country</td>
<td>Code</td>
</tr>
<tr>
<td>----------------------</td>
<td>---------------</td>
<td>------------</td>
</tr>
<tr>
<td>Kortner Trond M.</td>
<td>Norway</td>
<td>O-048, P-091</td>
</tr>
<tr>
<td>Kou Hongyan</td>
<td>China</td>
<td>P-283</td>
</tr>
<tr>
<td>Kousoulaki Katerina</td>
<td>Norway</td>
<td>O-009, P-015</td>
</tr>
<tr>
<td>Krogdahl Åshild</td>
<td>Norway</td>
<td>O-015</td>
</tr>
<tr>
<td>Kurnia Agus</td>
<td>Indonesia</td>
<td>P-298</td>
</tr>
<tr>
<td>Lee Donghoon</td>
<td>Korea</td>
<td>P-339</td>
</tr>
<tr>
<td>Lee Junho</td>
<td>Korea</td>
<td>P-201</td>
</tr>
<tr>
<td>Lee Sang-Min</td>
<td>South Korea</td>
<td>P-049</td>
</tr>
<tr>
<td>Lemme Andreas</td>
<td>Germany</td>
<td>P-153</td>
</tr>
<tr>
<td>Lemos Daniel</td>
<td>Brazil</td>
<td>P-233</td>
</tr>
<tr>
<td>Leng Xiangjin</td>
<td>China</td>
<td>O-105</td>
</tr>
<tr>
<td>Li Erchao</td>
<td>China</td>
<td>P-124</td>
</tr>
<tr>
<td>Li Jing</td>
<td>China</td>
<td>P-175</td>
</tr>
<tr>
<td>Li Peng</td>
<td>China</td>
<td>O-034</td>
</tr>
<tr>
<td>Li Qingfei</td>
<td>China</td>
<td>P-351</td>
</tr>
<tr>
<td>Li Xiaoning</td>
<td>China</td>
<td>P-284</td>
</tr>
<tr>
<td>Li Yan</td>
<td>China</td>
<td>P-285, P-292</td>
</tr>
<tr>
<td>Li Yong</td>
<td>China</td>
<td>P-149, P-157</td>
</tr>
<tr>
<td>Li Yuanyou</td>
<td>China</td>
<td>O-019</td>
</tr>
<tr>
<td>Li Zhuo-Jia</td>
<td>China</td>
<td>P-126</td>
</tr>
<tr>
<td>Lian Gan</td>
<td>China</td>
<td>P-311</td>
</tr>
<tr>
<td>Liang J.J.</td>
<td>China</td>
<td>P-185</td>
</tr>
<tr>
<td>Liang Mengqing</td>
<td>China</td>
<td>O-007</td>
</tr>
<tr>
<td>Liang Xufang</td>
<td>China</td>
<td>P-080</td>
</tr>
<tr>
<td>Liao W.L.</td>
<td>Taiwan</td>
<td>P-051</td>
</tr>
<tr>
<td>Liebert F.</td>
<td>Germany</td>
<td>P-321, P-322, P-340</td>
</tr>
<tr>
<td>Lim Chhorn</td>
<td>Norway</td>
<td>P-320</td>
</tr>
<tr>
<td>Lin Heizhao</td>
<td>China</td>
<td>P-127</td>
</tr>
<tr>
<td>Lin Jianbin</td>
<td>China</td>
<td>P-352</td>
</tr>
<tr>
<td>Lin Weihsiang</td>
<td>Taiwan</td>
<td>P-347</td>
</tr>
<tr>
<td>Lin Yingjhih</td>
<td>Taiwan</td>
<td>P-208</td>
</tr>
<tr>
<td>Liou Chynghwa</td>
<td>Taiwan</td>
<td>P-062, P133</td>
</tr>
<tr>
<td>Liu Bo</td>
<td>China</td>
<td>P-083</td>
</tr>
<tr>
<td>Liu Haiyan</td>
<td>China</td>
<td>P-301</td>
</tr>
<tr>
<td>Liu Haokun</td>
<td>China</td>
<td>P-040</td>
</tr>
<tr>
<td>Liu Kang</td>
<td>China</td>
<td>P-257</td>
</tr>
<tr>
<td>Liu Liwei</td>
<td>China</td>
<td>P-313</td>
</tr>
<tr>
<td>Liu Suihua</td>
<td>China</td>
<td>P-118</td>
</tr>
<tr>
<td>Name</td>
<td>Country</td>
<td>ID</td>
</tr>
<tr>
<td>------------------------</td>
<td>-------------</td>
<td>-----</td>
</tr>
<tr>
<td>Liu Tianyi</td>
<td>China</td>
<td>P-333</td>
</tr>
<tr>
<td>Liu X.J.</td>
<td>China</td>
<td>P-171</td>
</tr>
<tr>
<td>Liu Xingwang</td>
<td>China</td>
<td>P-286</td>
</tr>
<tr>
<td>Liu Yang</td>
<td>China</td>
<td>P-067</td>
</tr>
<tr>
<td>Liu Yi</td>
<td>China</td>
<td>P-296</td>
</tr>
<tr>
<td>Lock Erik-Jan</td>
<td>Norway</td>
<td>O-079</td>
</tr>
<tr>
<td>Long Yin</td>
<td>China</td>
<td>P-328</td>
</tr>
<tr>
<td>López-Garcia Jesús</td>
<td>México</td>
<td>P-269</td>
</tr>
<tr>
<td>Lu Chih-Sheng</td>
<td>Taiwan</td>
<td>P-270</td>
</tr>
<tr>
<td>Lu Feng</td>
<td>Japan</td>
<td>P-002</td>
</tr>
<tr>
<td>Lund Ivar</td>
<td>Denmark</td>
<td>P-036</td>
</tr>
<tr>
<td>Luo Li</td>
<td>China</td>
<td>P-220</td>
</tr>
<tr>
<td>Luo Lin</td>
<td>China</td>
<td>P-259</td>
</tr>
<tr>
<td>Lupatsch Ingrid</td>
<td>United Kingdom</td>
<td>O-054</td>
</tr>
<tr>
<td>Mabroke Rania S</td>
<td>Egypt</td>
<td>O-070</td>
</tr>
<tr>
<td>Macias-Rodriguez M.E.</td>
<td>México</td>
<td>P-323</td>
</tr>
<tr>
<td>Madrones-Ladja Jocelyn A.</td>
<td>Philippines</td>
<td>O-046</td>
</tr>
<tr>
<td>Mahaut de Vareilles</td>
<td>Portugal</td>
<td>P-089</td>
</tr>
<tr>
<td>Mai Kangsen</td>
<td>China</td>
<td>O-001, P-287</td>
</tr>
<tr>
<td>Mamauag R.E.P.</td>
<td>Japan</td>
<td>P-326</td>
</tr>
<tr>
<td>Mansfield Graeme S.</td>
<td>Canada</td>
<td>O-063</td>
</tr>
<tr>
<td>Martinez Laura</td>
<td>Norway</td>
<td>P-088</td>
</tr>
<tr>
<td>Måsøval Kjell A.</td>
<td>Norway</td>
<td>P-043</td>
</tr>
<tr>
<td>Médale F.</td>
<td>France</td>
<td>O-022</td>
</tr>
<tr>
<td>Meng Fanyi</td>
<td>China</td>
<td>O-096</td>
</tr>
<tr>
<td>Miao Linghong</td>
<td>China</td>
<td>P-195</td>
</tr>
<tr>
<td>Miao Youqing</td>
<td>China</td>
<td>P-003</td>
</tr>
<tr>
<td>Ming Jianhua</td>
<td>China</td>
<td>P-329</td>
</tr>
<tr>
<td>Mohamed Ibrahim R. S.</td>
<td>Spain</td>
<td>P-105</td>
</tr>
<tr>
<td>Mohamed K.</td>
<td>Germany</td>
<td>P-341</td>
</tr>
<tr>
<td>Mohammed Aliyu-Paiko</td>
<td>Malaysia</td>
<td>O-011</td>
</tr>
<tr>
<td>Mohseni M.</td>
<td>USA</td>
<td>P-217</td>
</tr>
<tr>
<td>Monroig Óscar</td>
<td>UK</td>
<td>O-039, P-090</td>
</tr>
<tr>
<td>Montero D.</td>
<td>Spain</td>
<td>O-038</td>
</tr>
<tr>
<td>Morais Sofia</td>
<td>Scotland</td>
<td>O-017</td>
</tr>
<tr>
<td>Morken Thea</td>
<td>Norway</td>
<td>O-064, P-169</td>
</tr>
<tr>
<td>Muñoz Susana</td>
<td>Chile</td>
<td>P-294</td>
</tr>
<tr>
<td>Mydland Liv Torunn</td>
<td>Norway</td>
<td>P-085</td>
</tr>
<tr>
<td>Name</td>
<td>Country</td>
<td>Code</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>------------</td>
<td>-------</td>
</tr>
<tr>
<td>Nadège Richard</td>
<td>Portugal</td>
<td>P-103</td>
</tr>
<tr>
<td>Nates Sergio F.</td>
<td>USA</td>
<td>O-023</td>
</tr>
<tr>
<td>Ng Wing-Keong</td>
<td>Malaysia</td>
<td>O-049, P-052</td>
</tr>
<tr>
<td>Ngandzali Bergo Owari</td>
<td>China</td>
<td>P-011</td>
</tr>
<tr>
<td>Nguyen Binh Thanh</td>
<td>Japan</td>
<td>P-123</td>
</tr>
<tr>
<td>Nilson Stephanie A.</td>
<td>Canada</td>
<td>P-021</td>
</tr>
<tr>
<td>Niu Jin</td>
<td>China</td>
<td>P-128</td>
</tr>
<tr>
<td>Norouzi M.</td>
<td>Iran</td>
<td>P-325</td>
</tr>
<tr>
<td>Nouaga Rodrigue Yossa</td>
<td>Canada</td>
<td>O-088</td>
</tr>
<tr>
<td>Oehme Maike</td>
<td>Norway</td>
<td>P-346</td>
</tr>
<tr>
<td>Oku Hiromi</td>
<td>Japan</td>
<td>P-087</td>
</tr>
<tr>
<td>Olli Jan</td>
<td>Chile</td>
<td>O-093</td>
</tr>
<tr>
<td>Orapint Jintasataporn</td>
<td>Thailand</td>
<td>P-237</td>
</tr>
<tr>
<td>Øverland M</td>
<td>Norway</td>
<td>O-002</td>
</tr>
<tr>
<td>Pan Lei</td>
<td>China</td>
<td>P-068</td>
</tr>
<tr>
<td>Payooha Kanjana</td>
<td>Thailand</td>
<td>P-305</td>
</tr>
<tr>
<td>Peng Shiming</td>
<td>China</td>
<td>P-260</td>
</tr>
<tr>
<td>Penn Michael H.</td>
<td>Norway</td>
<td>O-103</td>
</tr>
<tr>
<td>Phromkunthong Wutiporn</td>
<td>Thailand</td>
<td>P-135</td>
</tr>
<tr>
<td>Pirarat Nopadon</td>
<td>Thailand</td>
<td>O-014</td>
</tr>
<tr>
<td>Pirozzi I.</td>
<td>Australia</td>
<td>O-016</td>
</tr>
<tr>
<td>Poveda Jenny M. Moreno</td>
<td>Colombia</td>
<td>P-268</td>
</tr>
<tr>
<td>Qi Ye</td>
<td>China</td>
<td>O-085</td>
</tr>
<tr>
<td>Qi Zhanhui</td>
<td>China</td>
<td>P-223</td>
</tr>
<tr>
<td>Qin Chuanjie</td>
<td>China</td>
<td>P-064</td>
</tr>
<tr>
<td>Qiu Binchong</td>
<td>China</td>
<td>P-028</td>
</tr>
<tr>
<td>Qiu Xiaojie</td>
<td>China</td>
<td>P-252</td>
</tr>
<tr>
<td>Qiu Yan</td>
<td>China</td>
<td>P-007</td>
</tr>
<tr>
<td>Ragaza Janice A.</td>
<td>Japan</td>
<td>O-097</td>
</tr>
<tr>
<td>Randall K.M.</td>
<td>Canada</td>
<td>O-087</td>
</tr>
<tr>
<td>Rema Paulo</td>
<td>Portugal</td>
<td>P-041</td>
</tr>
<tr>
<td>Remø S. C.</td>
<td>Norway</td>
<td>P-192</td>
</tr>
<tr>
<td>Ren Mingchun</td>
<td>China</td>
<td>P-224</td>
</tr>
<tr>
<td>Ren T.</td>
<td>China</td>
<td>P-261</td>
</tr>
<tr>
<td>Reveco Felipe E.</td>
<td>Canada</td>
<td>P-014</td>
</tr>
<tr>
<td>Reyes-Becerril M.</td>
<td>Spain</td>
<td>P-324</td>
</tr>
<tr>
<td>Richard Lenaïg</td>
<td>France</td>
<td>O-055</td>
</tr>
<tr>
<td>Rios-Durán M. Gisela</td>
<td>México</td>
<td>P-278</td>
</tr>
<tr>
<td>Name</td>
<td>Nationality</td>
<td>Code</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-------------</td>
<td>-------</td>
</tr>
<tr>
<td>Rodrigues Ana Paula O.</td>
<td>Brazil</td>
<td>P-013</td>
</tr>
<tr>
<td>Rollin Xavier</td>
<td>Belgium</td>
<td>P-094, P-095, P-107, O-005, P-215</td>
</tr>
<tr>
<td>Romarheim Odd H.</td>
<td>Norway</td>
<td>O-005</td>
</tr>
<tr>
<td>Rotllant G.</td>
<td>Spain</td>
<td>P-098, P-275</td>
</tr>
<tr>
<td>Ruohononen K.</td>
<td>Norway</td>
<td>O-086</td>
</tr>
<tr>
<td>Saedi M.</td>
<td>Iran</td>
<td>P-046, P-277</td>
</tr>
<tr>
<td>Sahlmann Christian</td>
<td>Norway</td>
<td>P-202</td>
</tr>
<tr>
<td>Sakurai Takuma</td>
<td>Japan</td>
<td>P-206</td>
</tr>
<tr>
<td>Sanchez A. Daniel</td>
<td>México</td>
<td>P-044</td>
</tr>
<tr>
<td>Sandra Yesell Torres O.</td>
<td>México</td>
<td>P-045</td>
</tr>
<tr>
<td>Sangsue Dhanapong</td>
<td>Singapore</td>
<td>P-236</td>
</tr>
<tr>
<td>Santos Juliana F.</td>
<td>Brazil</td>
<td>P-272</td>
</tr>
<tr>
<td>Sarker Md. Al-Amin</td>
<td>Japan</td>
<td>O-069</td>
</tr>
<tr>
<td>Satoh Shuichi</td>
<td>Japan</td>
<td>O-031</td>
</tr>
<tr>
<td>Scabini Valeria S.</td>
<td>Chile</td>
<td>P-104</td>
</tr>
<tr>
<td>Schrama J.W.</td>
<td>Netherlands</td>
<td>O-075, P-071, P-072</td>
</tr>
<tr>
<td>Schwarz Frieder J.</td>
<td>Germany</td>
<td>O-062</td>
</tr>
<tr>
<td>Schuller Kathryn A.</td>
<td>Australia</td>
<td>P358, P359</td>
</tr>
<tr>
<td>Senadheera Shyamalie D.</td>
<td>Australia</td>
<td>P-235</td>
</tr>
<tr>
<td>Serrano Edison</td>
<td>Norway</td>
<td>O-101</td>
</tr>
<tr>
<td>Shang Liping</td>
<td>China</td>
<td>P-129</td>
</tr>
<tr>
<td>Shang Weimin</td>
<td>China</td>
<td>P-314</td>
</tr>
<tr>
<td>Shao Xianping</td>
<td>China</td>
<td>O-089</td>
</tr>
<tr>
<td>Shapawi Rossita</td>
<td>Malaysia</td>
<td>P-218</td>
</tr>
<tr>
<td>Shiau Wen-Chung</td>
<td>Taiwan</td>
<td>P-137</td>
</tr>
<tr>
<td>Shuai Ke</td>
<td>China</td>
<td>P-158, P-159</td>
</tr>
<tr>
<td>SigurgeirssonÓlafur Ingi</td>
<td>Iceland</td>
<td>P-245</td>
</tr>
<tr>
<td>Skugor Stanko</td>
<td>Norway</td>
<td>O-040</td>
</tr>
<tr>
<td>Song Liping</td>
<td>China</td>
<td>P-315</td>
</tr>
<tr>
<td>Sprague M.</td>
<td>Scotland</td>
<td>O-003</td>
</tr>
<tr>
<td>Storebakken Trond</td>
<td>Norway</td>
<td>O-065</td>
</tr>
<tr>
<td>Stubhaug Ingunn</td>
<td>France</td>
<td>P-070</td>
</tr>
<tr>
<td>Sugiuira Shozo</td>
<td>Japan</td>
<td>P-248</td>
</tr>
<tr>
<td>Suhaman Indra</td>
<td>Japan</td>
<td>P-077</td>
</tr>
<tr>
<td>Suloma Ashraf</td>
<td>Egypt</td>
<td>P-154</td>
</tr>
<tr>
<td>Sun Shengming</td>
<td>China</td>
<td>P-029, P-142</td>
</tr>
<tr>
<td>Supamattaya Kidchakan</td>
<td>Thailand</td>
<td>P-203</td>
</tr>
<tr>
<td>Synnøve Aas T.</td>
<td>Norway</td>
<td>O-066</td>
</tr>
<tr>
<td>Name</td>
<td>Nationality</td>
<td>P-Numbers</td>
</tr>
<tr>
<td>----------------------</td>
<td>-------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Tan Fangfang</td>
<td>China</td>
<td>P-318</td>
</tr>
<tr>
<td>Tan X.Y.</td>
<td>China</td>
<td>P-225</td>
</tr>
<tr>
<td>Tang Ling</td>
<td>China</td>
<td>P-262, P354</td>
</tr>
<tr>
<td>Tantikitti Chutima</td>
<td>Thailand</td>
<td>P-271</td>
</tr>
<tr>
<td>Thanuthong Thanongsak</td>
<td>Australia</td>
<td>P-234</td>
</tr>
<tr>
<td>Thomassen Magny S.</td>
<td>Norway</td>
<td>O-091, P-240</td>
</tr>
<tr>
<td>Tibbetts Sean M.</td>
<td>Canada</td>
<td>P-164</td>
</tr>
<tr>
<td>TODORČEVIĆ M</td>
<td>Norway</td>
<td>P-188</td>
</tr>
<tr>
<td>Toledo-Cuevas E. Mayra</td>
<td>México</td>
<td>P-279</td>
</tr>
<tr>
<td>Torrecillas Silvia</td>
<td>Spain</td>
<td>P-191, P-207</td>
</tr>
<tr>
<td>Torrisen Ole</td>
<td>Norway</td>
<td>O-006</td>
</tr>
<tr>
<td>Torstensen Bente E.</td>
<td>Norway</td>
<td>P-061</td>
</tr>
<tr>
<td>Tran Thi Nang Thu</td>
<td>Belgium</td>
<td>P-109</td>
</tr>
<tr>
<td>Trichet Viviane Verlhac</td>
<td>France</td>
<td>O-095</td>
</tr>
<tr>
<td>Turchini Giovanni M.</td>
<td>Australia</td>
<td>O-045</td>
</tr>
<tr>
<td>Valen Elin C</td>
<td>Norway</td>
<td>P-086</td>
</tr>
<tr>
<td>Venold Fredrik</td>
<td>Norway</td>
<td>P-342</td>
</tr>
<tr>
<td>Viayeh Reza Malekzadeh</td>
<td>Iran</td>
<td>P-096, P-170</td>
</tr>
<tr>
<td>Viriyaponsutee Boonkob</td>
<td>Thailand</td>
<td>P-214</td>
</tr>
<tr>
<td>Waagbø Rune</td>
<td>Norway</td>
<td>O-090, P-247</td>
</tr>
<tr>
<td>Wade Nick</td>
<td>Australia</td>
<td>O-052</td>
</tr>
<tr>
<td>Wahbi O.M.</td>
<td>Egypt</td>
<td>P-039</td>
</tr>
<tr>
<td>Wang Aimin</td>
<td>China</td>
<td>P-226</td>
</tr>
<tr>
<td>Wang C.</td>
<td>China</td>
<td>P-186</td>
</tr>
<tr>
<td>Wang Changan</td>
<td>China</td>
<td>P-302, P-331</td>
</tr>
<tr>
<td>Wang Chaoming</td>
<td>China</td>
<td>P-222</td>
</tr>
<tr>
<td>Wang Fubao</td>
<td>China</td>
<td>P-258</td>
</tr>
<tr>
<td>Wang Guiqin</td>
<td>China</td>
<td>P-336</td>
</tr>
<tr>
<td>Wang Jia</td>
<td>China</td>
<td>P-092</td>
</tr>
<tr>
<td>Wang Jiting</td>
<td>China</td>
<td>P-316</td>
</tr>
<tr>
<td>Wang Jun</td>
<td>China</td>
<td>P-297</td>
</tr>
<tr>
<td>Wang Qingkui</td>
<td>China</td>
<td>P-196, P-197, P-288</td>
</tr>
<tr>
<td>Wang Qiurong</td>
<td>China</td>
<td>P-309</td>
</tr>
<tr>
<td>Wang S.</td>
<td>China</td>
<td>O-078</td>
</tr>
<tr>
<td>Wang Shou</td>
<td>Norway</td>
<td>P-110</td>
</tr>
<tr>
<td>Wang Tuo</td>
<td>China</td>
<td>P-019</td>
</tr>
<tr>
<td>Wang Weifang</td>
<td>China</td>
<td>P-069</td>
</tr>
<tr>
<td>Wang Xingqiang</td>
<td>China</td>
<td>P-146</td>
</tr>
<tr>
<td>Name</td>
<td>Country</td>
<td>Code</td>
</tr>
<tr>
<td>-------------------</td>
<td>--------------</td>
<td>------</td>
</tr>
<tr>
<td>Wang Xinxia</td>
<td>China</td>
<td>P-009</td>
</tr>
<tr>
<td>Wang Yage</td>
<td>China</td>
<td>P-221</td>
</tr>
<tr>
<td>Wang Yan</td>
<td>China</td>
<td>O-028</td>
</tr>
<tr>
<td>Wang Yue</td>
<td>China</td>
<td>P-145</td>
</tr>
<tr>
<td>Wei Yu</td>
<td>China</td>
<td>P-111</td>
</tr>
<tr>
<td>Wei Yuting</td>
<td>China</td>
<td>P-289</td>
</tr>
<tr>
<td>Wocher Hendrik</td>
<td>Germany</td>
<td>P-162</td>
</tr>
<tr>
<td>Wu Chenglong</td>
<td>China</td>
<td>O-042, P-084</td>
</tr>
<tr>
<td>Wu Lifang</td>
<td>China</td>
<td>P-056</td>
</tr>
<tr>
<td>Wu Xiaoyi</td>
<td>China</td>
<td>P-176</td>
</tr>
<tr>
<td>Wu Xugan</td>
<td>Australia</td>
<td>O-057</td>
</tr>
<tr>
<td>Xi Feng</td>
<td>China</td>
<td>P-355</td>
</tr>
<tr>
<td>Xian Jianan</td>
<td>China</td>
<td>P-179</td>
</tr>
<tr>
<td>Xiang Xiao</td>
<td>China</td>
<td>P-180</td>
</tr>
<tr>
<td>Xiao Jinxing</td>
<td>China</td>
<td>P-151</td>
</tr>
<tr>
<td>Xiao Tiaozi</td>
<td>China</td>
<td>P-030</td>
</tr>
<tr>
<td>Xiao Xucheng</td>
<td>China</td>
<td>P-136</td>
</tr>
<tr>
<td>Xie Fengjun</td>
<td>China</td>
<td>P-267</td>
</tr>
<tr>
<td>Xie Quansen</td>
<td>China</td>
<td>P-167</td>
</tr>
<tr>
<td>Xie S.</td>
<td>China</td>
<td>O-067</td>
</tr>
<tr>
<td>Xu Dandan</td>
<td>China</td>
<td>P-112</td>
</tr>
<tr>
<td>Xu Houguo</td>
<td>China</td>
<td>P-213</td>
</tr>
<tr>
<td>Xu Qiyou</td>
<td>China</td>
<td>P-099, P-263, P-290</td>
</tr>
<tr>
<td>Xu Shude</td>
<td>China</td>
<td>P-057</td>
</tr>
<tr>
<td>Xu Weina</td>
<td>China</td>
<td>P-211</td>
</tr>
<tr>
<td>Yang Jun</td>
<td>China</td>
<td>P-017, P-018</td>
</tr>
<tr>
<td>Yang Qihui</td>
<td>China</td>
<td>P-147</td>
</tr>
<tr>
<td>Yang Yang</td>
<td>China</td>
<td>P-130</td>
</tr>
<tr>
<td>Yang Yinghao</td>
<td>China</td>
<td>P-058</td>
</tr>
<tr>
<td>Yasumaru Fanny</td>
<td>Brazil</td>
<td>P-132</td>
</tr>
<tr>
<td>Ye Chaoxia</td>
<td>China</td>
<td>P-131</td>
</tr>
<tr>
<td>Ye Jinyun</td>
<td>China</td>
<td>P-005</td>
</tr>
<tr>
<td>Yildiz Mustafa</td>
<td>Turkey</td>
<td>P-048</td>
</tr>
<tr>
<td>Ying Xiaoming</td>
<td>China</td>
<td>P-230</td>
</tr>
<tr>
<td>Yokoyama Saichiro</td>
<td>Japan</td>
<td>O-104</td>
</tr>
<tr>
<td>Yu Peiqiang</td>
<td>Canada</td>
<td>P-078</td>
</tr>
<tr>
<td>Yue Yirong</td>
<td>China</td>
<td>P-120</td>
</tr>
<tr>
<td>Zhang C.</td>
<td>Canada</td>
<td>P-038</td>
</tr>
<tr>
<td>Name</td>
<td>Country</td>
<td>Page</td>
</tr>
<tr>
<td>---------------</td>
<td>---------</td>
<td>------</td>
</tr>
<tr>
<td>Zhang Chunxiao</td>
<td>China</td>
<td>P-264</td>
</tr>
<tr>
<td>Zhang Guobin</td>
<td>China</td>
<td>P-193</td>
</tr>
<tr>
<td>Zhang Jun</td>
<td>China</td>
<td>P-229</td>
</tr>
<tr>
<td>Zhang Keke</td>
<td>China</td>
<td>P-152</td>
</tr>
<tr>
<td>Zhang Lu</td>
<td>China</td>
<td>O-020</td>
</tr>
<tr>
<td>Zhang Qin</td>
<td>China</td>
<td>P-198, P-199</td>
</tr>
<tr>
<td>Zhang Shiliang</td>
<td>China</td>
<td>P-076</td>
</tr>
<tr>
<td>Zhang Wenbing</td>
<td>China</td>
<td>P-327</td>
</tr>
<tr>
<td>Zhang Yuexing</td>
<td>China</td>
<td>P-032</td>
</tr>
<tr>
<td>Zhao Falan</td>
<td>China</td>
<td>P-356</td>
</tr>
<tr>
<td>Zhao Yancui</td>
<td>China</td>
<td>P-212</td>
</tr>
<tr>
<td>Zheng Qingmei</td>
<td>China</td>
<td>P-004</td>
</tr>
<tr>
<td>Zheng Xiaozhong</td>
<td>Scotland</td>
<td>P-079</td>
</tr>
<tr>
<td>Zhong Guo-fang</td>
<td>China</td>
<td>P-033</td>
</tr>
<tr>
<td>Zhong Weijing</td>
<td>China</td>
<td>P-148</td>
</tr>
<tr>
<td>Zhou Chuanpeng</td>
<td>China</td>
<td>O-043</td>
</tr>
<tr>
<td>Zhou Fan</td>
<td>China</td>
<td>P-239</td>
</tr>
<tr>
<td>Zhou Hengyong</td>
<td>China</td>
<td>P-265</td>
</tr>
<tr>
<td>Zhou Huihui</td>
<td>China</td>
<td>P-335</td>
</tr>
<tr>
<td>Zhou Jishu</td>
<td>China</td>
<td>P-059</td>
</tr>
<tr>
<td>Zhou Xiaqiu</td>
<td>China</td>
<td>O-080</td>
</tr>
<tr>
<td>Zhou Yi</td>
<td>China</td>
<td>P-121</td>
</tr>
<tr>
<td>Zhou Zhigang</td>
<td>China</td>
<td>P-183</td>
</tr>
<tr>
<td>Zhu Wangming</td>
<td>China</td>
<td>P-357</td>
</tr>
<tr>
<td>Zhu Xuan</td>
<td>China</td>
<td>P-334</td>
</tr>
<tr>
<td>Zhu Xuezhi</td>
<td>China</td>
<td>P-122</td>
</tr>
<tr>
<td>Zimonja O.</td>
<td>Norway</td>
<td>P-163</td>
</tr>
<tr>
<td>Zou Yurong</td>
<td>China</td>
<td>P-228</td>
</tr>
<tr>
<td>Zuo Rantao</td>
<td>China</td>
<td>P-291</td>
</tr>
</tbody>
</table>
THE 14TH INTERNATIONAL SYMPOSIUM ON
FISH NUTRITION & FEEDING

Gold Sponsors:
1. Ocean University of China
2. Qingdao Municipal Government
4. The 7th project of China (2018) National Natural Science Foundation of China

Silver Sponsors:
1. NOVUS International, Inc.
2. DSM Nutritional Products (China) Ltd
3. Diamond Mills, Inc.
4. Grand Master Bio-Technology Co., Ltd
5. Guangdong Huadong Feed Group Co., Ltd

Co-Operation Sponsors:
1. West Haplo Co., Ltd
2. Qingdao Great Star Bio-Tech Co., Ltd
4. K.C. Young Education Foundation
5. Aquatic Living Resources

Media Supports:
1. Feifei Feed Co., Ltd
2. Wefei Feed Co., Ltd
3. Weifang Feed Co., Ltd
4. Aquatic Living Resources
5. Aquatic Living Resources