Regulation of miR-33a and cholesterol metabolism related gene expression in rainbow trout (Oncorhynchus mykiss): in vivo and in vitro approaches

Mr. Tengfei Zhu INRA Dr. Geneviève Corraze (1) Ms. Elisabeth Plagnes-Juan (1) Dr. Sandrine Skiba-Cassy INRA

Cholesterol is an essential component of cell membranes and the precursor of several bioactive compounds, including bile acids, steroid hormones and vitamin D. The regulation of cholesterol metabolism is mediated by both transcriptional and posttranscriptional factors including microRNAs. In mammals, miR-33a is reported to be involved in the regulation of cholesterol homeostasis. Utilization of plant ingredients in aquafeed leads to great reduction in dietary cholesterol supply that can affect fish physiology and metabolism.

The objective in this study was to investigate the impact of cholesterol the expression of miR33a and genes involved in cholesterol metabolism in rainbow trout both in vivo and in vitro. For the in vivo study, juvenile rainbow trout were fed a total plant-based diet (V) or a marine diet containing fishmeal and fish oil diet (M) for ten weeks. In the in vitro experiment, primary cell cultures of rainbow trout hepatocytes were stimulated by increasing concentrations of 25-hydroxycholesterol for 16h. The expression of genes involved in cholesterol and lipid metabolism was investigated in liver and hepatocytes. Expression of miR-33a was also assessed in liver and plasma as well as in hepatocytes.

Utilization of plant-based diet increased the expression of genes involved in cholesterol biosynthetic pathway. The level of miR-33a also enhanced when fish were fed the V diet. In vitro, increasing concentration of 25-hydroxycholesterol leads to the down regulation of genes involved in cholesterol synthesis and efflux and decreased miR-33a expression in hepatocytes.

In conclusion, both in vivo and in vitro studies highlighted the involvement of cholesterol in the regulation of expression of miR-33a which is co-transcribed with the transcription factor SREBP2 in rainbow trout. In addition, positive statistical correlations between miR-33a and genes involved in cholesterol synthesis were found in vivo and in vitro suggesting a potential role of miR33a in the control of this pathway. Further investigations based on miR-33a inhibition are now necessary to confirm this role.
The Effects of Soy Lecithin-Enriched Artemia on Growth and Survival of the Early Stages of Green Tiger Shrimp (Penaeus semisulcatus)

Dr. H. Asuman YILMAZ University of Cukurova, Faculty of Fisheries Ms. Ece EVLİYAOĞLU (1) Mr. M. Bedrettin DUMAN (1) Ms. Burcu AK Prof. Metin KUMLU (1) Prof. O. Tufan EROLDOĞAN (1) Ms. Merve SARIİPEK Department of Aquaculture, University of Sinop, Faculty of Fisheries,

Lecithin (LST) requirements have been investigated for juvenile and adults but not for the early life stages of marine crustaceans. Therefore, our present study was designed to determine optimal Artemia enrichment levels with soy lecithin, that sustain better growth and survival during the early stages [mysis 1 (M1) to postlarva 7 (PL7)] of the green tiger shrimp Penaeus semisulcatus. Artemia enrichment at five different levels of lecithin (0.0, 1.0, 2.0, 3.0 and 4.0%) was performed in 2-L glass flasks at a density of 300,000 nauplii/L with strong aeration. The enrichment dose was split into two and applied twice a day at 12 h intervals. The whole enrichment period lasted 24 h and the shrimp larvae/postlarva fed newly harvested/thoroughly rinsed Artemia twice a day at 5 nauplii/mL per feeding. The culture of shrimp was carried out in 2-L glass flasks at four replicates per treatment at 100% water exchange rate per day.

At the end of the experiment, it was found that survival and total length were clearly affected by the enrichment process. The larvae grown from M1 to PL1 or to PL7 displayed higher survivals (93.1-93.3% at PL1 or 83.9-84.1% at PL7) in 2.0-3.0% LST-groups compared to those feeding on 0.0% LST or 1.0% LST treatments (P<0.05). The largest larval total lengths (TL) at PL1 (5.03-6.04 mm TL) or PL7 (8.99-9.06 mm TL) were measured in those feeding on 2-4% LST enriched-Artemia (P<0.05). Daily growth rates of the PLs (0.50-0.51 mm/day TL) at 2-4% LST enriched-groups were also significantly greater than those of the 0.0% LST-group. In general, growth and survival of shrimp PLs fed Artemia enriched with 2.0, 3.0 or 4.0% LST did not differ significantly from each other (P>0.05), but these groups performed significantly better than those fed on 0% or 1.0% LST-groups. In conclusion, this study confirms that 24 h enriched-Artemia with 2-3% soy lecithin can effectively improve growth and survival of the early stages of P. semisulcatus.

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The study investigated the characterization, mRNA expression and regulation of 3-hydroxy-3-methylglutaryl (HMG) CoA reductase in giant grouper (Epinephelus lanceolatus) fed a high soybean meal diet. After full-length cloning for the fish, the full-length 1,918 bp HMG-CoA reductase cDNA encoding 375 amino acid polypeptides was obtained. The diet with soybean meal substituting 50% fish meal protein was supplemented with 1% cholesterol. Basal diet without cholesterol supplementation and all fish meal diet were also involved for comparison. The three experimental diets were each fed to triplicate groups of juvenile grouper (initial wt: 12.39 ± 0.36 g) in a recirculating rearing system for 8 weeks. Weight gain was higher (P<0.05) in fish fed the all fish meal diet than that in fish fed the other two experimental diets. Fish fed the diet with 1% cholesterol had the highest plasma cholesterol concentration, followed by fish fed the all fish meal diet, and lowest in fish fed the basal diet. Hepatic cholesterol concentration and HMG-CoA reductase gene expression were higher in fish fed the basal diet than fish fed the other two diets. The results indicated that grouper can synthesize cholesterol de novo to meet their cholesterol requirement when the fish fed a diet with high soybean meal. It is not necessary to supplement cholesterol in diet for giant grouper even the diet containing high level of soybean meal.
Lipophagy is essential for lipid metabolism in fish

Prof. Zhen-Yu Du Laboratory of Aquaculture Nutrition and Environmental Health (LANEH), School of Life Sciences, East China Normal University, 200241 Shanghai, China Ms. Jing Wang Laboratory of Aquaculture Nutrition and Environmental Health (LANEH), School of Life Sciences, East China Normal University, 200241 Shanghai, China(1) Ms. Si-Lan Han (1)

Lipophagy is the process in which lipid droplets (LDs) are degraded through the lysosomal degradative pathway of autophagy. It plays important roles in regulating lipid metabolism in mammals. However, the existence and roles of lipophagy in metabolism have not been studied in fish. In the present study, we observed the structures of LDs sequestered in autophagic vacuoles in the liver of fasting zebrafish and a zebrafish liver cell line (ZFL) using electronic microscopy. Moreover, mRNA expression of the microtubule-associated protein 1 light chain 3 (LC3) and conversion of the protein from LC3-I to LC3-II increased during fasting. Inhibiting autophagy with chloroquine and 3-methyladenine significantly increased LD content and decreased fatty acid β-oxidation and esterification activities in zebrafish liver and ZFL cells. Inhibiting lipophagy depressed the expression of most genes in lipid catabolism and lipogenesis, but upregulated the genes involved in glycolysis, gluconeogenesis, and proteolysis. Altogether, this is the first study to demonstrate the existence of lipophagy and its essential regulatory roles in lipid metabolism in fish. Moreover, lipophagy is very likely to be an important evolutionarily conserved cellular process for maintaining energy homeostasis in vertebrates.

Keywords: Fish; Lipophagy; LC3; Lipid metabolism; Energy homeostasis
A Comparative Approach Improving Efficiency of Finishing Period in Gilthead sea bream (Sparus aurata) and European sea bass (Dicentrarchus labrax)

Prof. ORHAN TUFAN EROLDogan  
Cukurova University, Faculty of Fisheries, Department of Aquaculture

Prof. Yılmaz Emre  
Akdeniz University, Faculty of Science, Department of Biology

Prof. Giovanni M. Turchini  
Deakin University, School of Life and Environmental Sciences

Dr. Mabrouk Elsabagh  
Kafrelsheikh University, Faculty of Veterinary Medicine, Department of Nutrition and Clinical Nutrition

Dr. Hatice Asuman Yılmaz (1)  
Ms. Ece Evliyaoglu (1)  
Prof. Marina Paolucci  
Department of Science and Technologies, University of Sannio, Via Port’Ars, 11,

This study aimed to evaluate the potential effects of a period of starvation on the efficiency of finishing period in gilthead sea bream (Sparus aurata, 26.6g) and European sea bass (Dicentrarchus labrax, 28.8g). Fish were kept separately and were randomly distributed into 6 replicates at 40 fish per tank. Two groups of both species were fed for an 8-week grow-out period on a fish oil-based diet (FO) or canola oil-based diet (CO). At the end of the grow-out period, CO fed fish was split into three sub groups with three replicates: the negative control (CO/FO-Neg) was shifted to the FO diet for 8 weeks, while the others were starved for one week (COFO1S) and 3 weeks (COFO3S) then fed to apparent satiation twice daily with the FO diet for remaining weeks. The positive control (FO-Pos) was represented by fish continuously fed FO-based diet. Fish for both species were sampled and analyzed for first, third and eighth weeks of the finishing period for fatty acid, proximate analysis and blood parameters (cholesterol, triglyceride and glucose). CO/FO-Neg (151.1 g and 158.2 g) and FO-Pos (136.1 g and 167.3 g) treatments in sea bass and sea bream showed increasing growth performance compare to other treatments, respectively. Dietary fatty acid is mirrored in both species whereas no significant dietary effects found in linolenic acid in sea bass. LC-PUFA content in COFOS1 and COFOS3 accurately increased during finishing period suggesting a positive effect of starvation before this period in both species. The major results of blood parameters will be presented and discussed towards providing information on diet formulation for both species starved and re-fed.
Six isonitrogenous and isocaloric diets were formulated to contain 0% (control), 0.4%, 0.8%, 1.2%, 1.6% or 2.4% dietary cholesterol, and fed to juvenile Nile tilapia Oreochromis niloticus (2.20 ± 0.12 g) twice daily to apparent satiation for 8 weeks in triplicate at salinity 16. Fish fed 0.4% cholesterol showed higher weight gain, specific growth rate and lower feed coefficient ratio than fish fed other diets. No difference was found in survival of Nile tilapia fed various levels of cholesterol. Cholesterol in the serum and liver, and low-density lipoprotein cholesterol in the serum increased with the increase of diet cholesterol content. Relative to the control, no significant difference was found in head kidney P450scc mRNA expressions between treatment groups. The mRNA expressions of head kidney 11β-HSD2 was the highest in the control group, and decreased significantly with the increasing levels of diet cholesterol. Fish fed 0.4% or 1.2% cholesterol had higher mRNA expressions of 20β-HSD2 than those fed other diets in the head kidney. Fish fed 0.8% cholesterol had higher mRNA expressions of GR1 and GR2B in the liver than in other groups. Fish fed 0.4% cholesterol had the highest activity of gill Na+/K+-ATPase, while the control fish showed the lowest activity. Fish fed 0.8% to 2.4% cholesterol had higher serum cortisol contents than the fish in the control and fed 0.4% cholesterol. This study suggests that dietary cholesterol is not essential for Nile tilapia survival in brackish water, but cholesterol supplementation at 0.4% in the diet contributes to improvement of hyperosmotic adaptation on higher activity of gill Na+/K+-ATPase and content of serum cortisol and fish growth through regulating the activity of key enzymes in by regulating the hypothalamic-pituitary-interrenal stress axis.
Effects of different dietary levels EPA + DHA on egg quality of greater amberjack (Seriola dumerili).

Ms. Samira Sarih Grupo de Investigación en Acuicultura (GIA), IU-ECOAQUA, Universidad de Las Palmas de Gran Canaria

Mr. Adnane Djellata Grupo de Investigación en Acuicultura (GIA), IU-ECOAQUA, Universidad de Las Palmas de Gran Canaria(1)

Dr. Ramon Fontanillas Skretting Aquaculture Research Centre AS

Dr. Grethe Rosenlund Skretting Aquaculture Research Centre AS

Prof. Marisol Izquierdo Grupo de Investigación en Acuicultura (GIA), IU-ECOAQUA, Universidad de Las Palmas de Gran Canaria(1)

Dr. Hipólito Fernández-Palacios Grupo de Investigación en Acuicultura (GIA), IU-ECOAQUA, Universidad de Las Palmas de Gran Canaria(1)

Dietary lipids and fatty acids composition play an important role in the reproductive processes, embryo development and larval survival in marine fish (Izquierdo et al., 2001). Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are especially important for egg and larval quality (Tocher, 2010; Migaud et al., 2013). The main objective of the present study was to examine the effects of different dietary levels of EPA + DHA on egg quality of greater amberjack. For that purpose, sixteen mature broodfish were used in this experiment, distributed in four 40m3 circular tanks at the facilities of the Grupo de Investigación en Acuicultura (GIA), located in the ECOAQUA Institute (Universidad de Las Palmas de Gran Canaria, ULPGC). The fish were fed with four different diets: diet 1 with 0.96% EPA + DHA, diet 2 with 1.57% EPA + DHA, diet 3 with 2.17% EPA + DHA and diet 4 with 2.8% EPA + DHA, at a DHA/EPA ratio of 1.1. Fish were intramuscularly injected with gonadotropin releasing hormone analogue at a dose of 20 µg.kg⁻¹ (Fernández-Palacios et al., 2015). Spawning quality was determined as: percentage of fertilization, viable eggs at 24 hours (%), hatching and larval survival at 1 day post-hatching (dph), using two replicates of 96-well microtiter plates. Eggs samples were taken for proximate and fatty acid analysis.

The number of eggs per spawn was significantly largest (P<0.05) in broodstock fed diet 2. Moreover, a higher fertilization rate (P<0.05) was obtained from broodstock fed diet 2 (91.76 ± 3.12 %) and diet 1 (86.32 ± 1.67 %) in comparison to those fed diet 3 (69.02 ± 7.38 %) and diet 4 (52.42 ± 10.64 %). The same trend was found in egg viability at 24h, hatching and larval survival rates, where the highest values (P<0.05) were found when broodstock were fed diet 2 and 1. Besides, the fatty acid composition of eggs showed marked differences, reflecting the influence of fatty acid profiles in the broodstock diets. The results denoted the importance of specific fatty acids, specifically the EPA and DHA levels to optimize greater amberjack spawning quality.
EFFECT OF DIETARY ARA/EPA/DHA RATIOS ON GILTHEAD SEA BREAM (Sparus aurata) GROWTH PERFORMANCE AND HEPATIC INTERMEDIARY METABOLISM

Mr. Rui Magalhães CIMAR/CIIMAR – Centro Interdisciplinar de Investigação Marinha e Ambiental/Departamento de Biologia, Faculdade de Ciências, Universidade do Porto Dr. Inês Guerreiro CIMAR/CIIMAR – Centro Interdisciplinar de Investigação Marinha e Ambiental Dr. Filipe Coutinho CIMAR/CIIMAR – Centro Interdisciplinar de Investigação Marinha e Ambiental Dr. Rolf Olsen Norwegian University of Science and Technology, Department of Biology Dr. Helena Peres CIMAR/CIIMAR – Centro Interdisciplinar de Investigação Marinha e Ambiental/Departamento de Biologia, Faculdade de Ciências, Universidade do Porto Prof. Aires Oliva-Teles CIMAR/CIIMAR – Centro Interdisciplinar de Investigação Marinha e Ambiental/Departamento de Biologia, Faculdade de Ciências, Universidade do Porto

Marine fish oil (FO) has been traditionally used as major lipid source in marine fish diets to provide essential fatty acids (EFA) and to enhance feed palatability. Aquaculture development and FO shortage supply have driven the use of alternative lipids sources such as vegetable oils (VO). However, VO are deficient in n3-Long Chain-Polyunsaturated Fatty Acids (LC-PUFA) and marine fish are unable to produce LC-PUFA due to their limited ability for elongating and desaturating their C18 fatty acids precursors. Thus, this study aimed to evaluate the effect of different dietary LC-PUFA ratios (ARA, EPA, and DHA) growth, plasma metabolites, and hepatic lipid and carbohydrates metabolism of gilthead seabream juveniles.

A feeding trial was conducted with gilthead seabream (IBW=15g), for 56 days at 23 °C. Four isoproteic (47%CP) and isolipidic (18%CL) plant-based diets (17.5% DM fish meal+60% DM plant feedstuffs) were formulated containing a VO blend (rapeseed:20%+linseed:50%+palm: 30%) as main lipid source. Diets were further supplemented with purified sources of EFA to include different (n3/n6)LC-PUFA ratios(%DM): Diet A(2.2%ARA:0%EPA:0%DHA); Diet B(1.2%ARA: 0.4%EPA:0.4%DHA); Diet C (0%ARA:0.6% EPA:0.7%DHA); Diet D (0%ARA:0.3%EPA:1.6%DHA). Each diet was fed to triplicate groups of fish.

Different dietary EFA ratios did not affected final body weight, liver composition, and plasma metabolites. Nevertheless, feed efficiency and protein efficiency ratio were higher in fish fed diet B than diets C or D. Liver lipids increased as dietary n-3PUFA increased. Activity of key-enzymes involved in hepatic glycolysis (glucokinase, hexokinase, pyruvate-kinase), gluconeogenesis (fructose bisphosphatase), and lipogenesis (glucose 6-phosphate dehydrogenase, malic enzyme) were not affected by experimental diets. However, liver fatty acid oxidation was higher in fish fed diets with higher levels of ARA (diet A and B) as indicate by higher hepatic activity of β-hydroxyacyl-CoA dehydrogenase (HOAD). Overall, results indicate that the diet with a similar ARA: n-3 PUFA (with 1:1 EPA:DHA ratio) promoted higher feed and protein utilization.

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Ballan wrasse (Labrus bergylta) is an effective counter-measure against sea lice used by Atlantic salmon farmers, proving to be more effective and economical than drugs or chemical treatments alone. There are currently efforts underway to establish a robust culture system for this species, however, essential fatty acid dietary requirements are not known for ballan wrasse. In the present study, we isolated and functionally characterised ballan wrasse fatty acid desaturase (Fads) and elongation of very long-chain fatty acids (Elovl) protein cDNAs to elucidate their long-chain polyunsaturated fatty acid (LC-PUFA) biosynthetic capability. Sequence and phylogenetic analysis demonstrated that the cloned genes were fads2 and elovl5 orthologues of other teleost species. Functional characterisations of fads2 and elovl5 were performed using the yeast (Saccharomyces cerevisiae) heterologous expression system. The Fads2 showed Δ6 desaturase activity towards 18:3n–3, 18:2n–6 and 24:5n–3, and Δ8 desaturase activity towards 20:3n–6 and 20:2n–6. The Elovl5 showed elongase activities towards various C18 and C20 fatty acids. Therefore, 20:4n–3 and 20:3n–6 can be synthesised from 18:3n–3 and 18:2n–6, respectively in ballan wrasse via two possible pathways, the Δ6 (Δ6 desaturation–elongation) and Δ8 (elongation–Δ8 desaturation) pathways. However, due to the absence of Δ5 desaturase activity and no other Fads2 in their genome, 20:5n–3 (eicosapentaenoic acid, EPA) and 20:4n–6 (arachidonic acid, ARA) cannot be synthesised from C18 PUFA precursors and they could consequently be regarded as dietary essential fatty acids for ballan wrasse. Since no Δ4 desaturase activity was detected in ballan wrasse Fads2, 22:6n–3 (docosahexaenoic acid, DHA) can only be synthesised from EPA via the Sprecher pathway comprising two sequential elongation steps to produce 24:5n–3 followed by Δ6 desaturation and chain shortening. Although ballan wrasse Elovl5 had no elongase activity towards C22, other elongases such as Elovl4 exist in the ballan wrasse genome that may be able to produce 24:5n–3. Therefore, as ballan wrasse Fads2 can desaturate 24:5n–3 to produce 24:6n-3, it can be assumed that ballan wrasse can synthesise DHA from EPA.
Practical dietary long-chain omega-3 polyunsaturated fatty acid requirements for large Yellowtail Kingfish (Seriola lalandi)

Prof. David Stone South Australian Research and Development Institute Aquatic Sciences Centre; Flinders University, College of Science and Engineering; The University of Adelaide, School of Animal and Veterinary Sciences; Marine Innovation Southern Australia Dr. Matthew Bansemer South Australian Research and Development Institute Aquatic Sciences Centre; Flinders University, College of Science and Engineering Mr. Paul Skordas South Australian Research and Development Institute Aquatic Sciences Centre Ms. Samantha Chown The University of Adelaide, School of Agriculture, Food and Wine, Waite Campus Dr. Nicole Ruff Skretting Australia

Currently, understanding the dietary long chain omega-3 polyunsaturated fatty acid (LC n-3 PUFA; Σeicosapentaenoic acid [20:5n-3, EPA], docosapentaenoic acid [22:5n-3, DPA] and docosahexaenoic acid [22:6n-3, DHA]) requirements of aquaculture species is vital to sustainably utilise limited supplies of fish oil. However, the dietary LC n-3 PUFA level for large Yellowtail Kingfish (Seriola lalandi) is unknown. In this 84 day study, the practical LC n-3 PUFA dietary requirements of Yellowtail Kingfish (2.67 kg) at warm water temperatures were determined based on growth performance, feed efficiency and nutrient utilisation. Fish were fed to apparent satiation once daily with one of eight experimental diets, formulated to contain graded levels of dietary LC n-3 PUFA (0.753 to 2.950 g 100g⁻¹). The LC n-3 PUFA were provided by graded levels of dietary fish oil, using poultry oil as the replacement. There was a moderate positive significant quadratic relationship between dietary LC n-3 PUFA and SGR (ymax = 2.12 g 100g⁻¹; R² = 0.5697; r = 0.7548; P < 0.001). There was no improvement in SGR by increasing LC n-3 PUFA levels above 2.39 g 100g⁻¹. With regard to the feed conversion ratio (FCR), there was a moderate negative significant quadratic relationship between dietary LC n-3 PUFA level and FCR (ymin = 2.26 g 100g⁻¹; R² = 0.5758; r = 0.7588; P < 0.001). There was also a moderate positive significant quadratic relationship between dietary LC n-3 PUFA and energy deposition, which supported SGR and FCR results. Based on these results, we estimate that the practical dietary LC n-3 PUFA requirement for Yellowtail Kingfish (2.67 kg) to be between 2.12 and 2.26 g 100g⁻¹. However, before implementing this dietary level of LC n-3 PUFA in commercial diets, we recommend further research to validate these levels under commercial conditions.
Mixes of plant oils for Nile tilapia at optimal and cold suboptimal temperature

Dr. Camila Fernandes Corrêa Polo Regional Vale do Ribeira, Agência Paulista de Tecnologia dos Agronegócios (APTA) Dr. Bruna Mattioni Aquaculture Department, Federal University of Santa Catarina(1) Ms. Renata Oselame Nobrega (1) Prof. Débora Machado Fracalossi Aquaculture Department, Federal University of Santa Catarina

Tilapia is the most widespread fish group in world aquaculture, but its production is concentrated in the subtropics, where high differences of temperature between summer and winter are registered. Fatty acids are key dietary nutrients to help fish coping with temperature differences. In the present study, juvenile Nile tilapia were fed three plant oil mixes and fish oil (FO), at optimal (28°C) or suboptimal (22°C) temperatures, for 9 or 12 weeks, respectively. In plant-oil mix diets, a base of coconut oil and olive oil was added to sunflower oil (MIX-S), sunflower and linseed oil (MIX-SL) or linseed oil (MIX-L). The main difference among plant oil mixes was their n-3/n-6 ratio. Nile tilapia exhibited similar growth when fed the three plant-oil diets at both rearing temperatures. However, when compared to FO diet, plant-oil diets promoted lower growth, except in fish fed the MIX-S diet, at 22°C. Feed efficiency and protein utilization were similar at 28°C, but at 22°C, FO diet led to better results for both indexes. Apparent net utilization of n-3 and n-6 PUFA was higher when these PUFA were in very low concentrations in the diets. However, total PUFA in body fatty acid profile was similar among tilapia fed different diets, within each temperature trial. Also, total LC-PUFA in the body was similar among tilapia fed plant-oil diets but inferior in tilapia fed FO diet. Polyunsaturated fatty-acid retention and conversion to higher chain length homologs was a strategy to lower temperature acclimation in Nile tilapia. We conclude that the variation in n-3/n-6 PUFA ratio among diets with mixed plant oils does not alter Nile tilapia growth or feed utilization at optimal or cold suboptimal temperatures. However, n-3 LC-PUFA in fish oil promotes higher growth performance of Nile tilapia, especially at cold suboptimal temperature.
Dietary DHA/EPA ratio affected tissue fatty acid profiles, antioxidant capacity, hematological characteristics and expression of lipid-related genes but not growth in juvenile black seabream (Acanthopagrus schlegelii)

Dr. Min Jin Laboratory of Fish Nutrition, School of Marine Sciences, Ningbo University Dr. Óscar Monroig Institute of Aquaculture, School of Natural Sciences, University of Stirling Mr. You Lu (1) Mr. Ye Yuan (1) Prof. Douglas R. Tocher Institute of Aquaculture, School of Natural Sciences, University of Stirling Prof. Qi-Cun Zhou (1)

An 8-week feeding trial was conducted to investigate the effects of dietary docosahexaenoic to eicosapentaenoic acid ratio (DHA/EPA) on growth performance, fatty acid profiles, antioxidant capacity, hematological characteristics and expression of some lipid metabolism related genes of juvenile black seabream (Acanthopagrus schlegelii) of initial weight 9.47 ± 0.03 g. Five isonitrogenous and isolipidic diets (45 % crude protein and 14 % crude lipid) were formulated to contain graded DHA/EPA ratios of 0.65, 1.16, 1.60, 2.03 and 2.67. There were no differences in growth performance and feed utilization among treatments. Fish fed higher DHA/EPA ratios had higher malondialdehyde (MDA) contents in serum than lower ratios. Serum triacylglycerol (TAG) content was significantly higher in fish fed the lowest DHA/EPA ratio. Tissue fatty acid profiles reflected the diets despite down-regulation of LC-PUFA biosynthesis genes, fatty acyl desaturase 2 (fads2) and elongase of very long-chain fatty acids (elovl5), by high DHA/EPA ratios. Expression of acetyl-CoA carboxylase alpha (acca) and carnitine palmitoyltransferase 1A (cpt1a) were up-regulated by high DHA/EPA ratio, whereas sterol regulatory element-binding protein-1 (srebp-1) and hormone-sensitive lipase (hsl) were down-regulated. Fatty acid synthase (fas), 6-phosphogluconate dehydrogenase (6pgd) and peroxisome proliferator-activated receptor alpha (ppara) showed highest expression in fish fed intermediate (1.16) DHA/EPA ratio. Overall, this study indicated that dietary DHA/EPA ratio affected fatty acid profiles and significantly influenced lipid metabolism including LC-PUFA biosynthesis and other anabolic and catabolic pathways, and also had impacts on antioxidant capacity and hematological characteristics.
Saturated fatty acids in diets are better utilized by juvenile tiger puffer Takifugu rubripes than n-6 fatty acids

Dr. Houguo Xu Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences Mr. Zhangbin Liao (1)
Mr. Qinggong Zhang (1) Dr. Yuliang Wei
(1) Prof. Mengqing Liang (1)

A 74-day feeding trial was conducted to investigate the efficacy of different types of fatty acids in diets for juvenile tiger puffer. Six experimental diets with gradually increasing levels of n-6 fatty acids (n-6 FA), which replaced dietary saturated fatty acids (SFA) correspondingly, were formulated, designated as diet 1, 2, 3, 4, 5, and 6 respectively. Each diet was fed to triplicate tanks of 30 fish. Increasing replacement of dietary SFA with n-6 FA resulted in decreased fish weight gain, feed efficiency ratio, crude protein content of whole fish, and serum glucose content, but increased viserasomatic and hepatosomatic indices, crude lipid content in whole fish and liver, serum triglyceride content, and activities of glutamic-pyruvic transaminase and glutamic oxalacetic transaminase in serum. Liver samples from groups 1 and 6, which were most significantly different in the parameters above, were subjected to transcriptomic assay to gain more insight into the different physiological status between the two groups. Compared to diet 1, diet 6 up-regulated transcription of 1948 genes while down-regulated that of 1691 genes. The differentially expressed genes were primarily enriched in KEGG pathways related to vitamin metabolism, xenobiotics metabolism, glycolysis/gluconeogenesis, phagosome, amino acids biosynthesis, and glycerolipid metabolism. Particularly, high levels of n-6 FA but low levels of SFA in diet inhibited transcription of ribosomal proteins, and tended to induce glycolysis and triacylglycerol biosynthesis. The contents of SFA and C18:2n-6 in liver reflected those in the diets, but the contents of them in muscle was relatively constant among groups. Arachidonic acid content in both liver and muscle significantly increased with its increasing content in diets. In conclusion, juvenile tiger puffer could utilize dietary SFA better compared to n-6 FA. High levels of n-6 fatty acids and low levels of SFA in diets resulted in lowered growth performance and increased lipid accumulation.
Elucidating the biosynthesis of long-chain polyunsaturated fatty acid in a freshwater fish species, *Clarias gariepinus*

**Ms. Angela Oboh** *University of Stirling*  
**Ms. Monica B. Betancor (1) Mr. Naoki Kabeya (1) Mr. Juan C. Navarro**  
**Instituto de Acuicultura Torre de la Sal (IATS-CSIC)**  
**Mr. Douglas R. Tocher (1) Mr. Óscar Monroig (1)**

Investigating the biosynthesis of long-chain (C20–24) polyunsaturated fatty acids (LC-PUFA), physiologically important compounds including arachidonic acid (ARA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in fish is crucial to identify dietary requirements for essential fatty acids (EFA). Moreover, knowledge of the LC-PUFA biosynthetic capability of farmed fish species enables us to understand their ability to utilise commonly used raw materials such as vegetable oils, which naturally lack LC-PUFA but include C18 PUFA that are metabolic precursors of LC-PUFA. Studies have shown that the potential of a species for LC-PUFA biosynthesis is associated with the complement and function of fatty acyl desaturase (fads) and elongase of very long chain fatty acid (elovl) genes existing in that species. Even within closely related species the complement of these genes and their functionalities can vary remarkably, underscoring the need for studies on each individual farmed fish species. The present study aimed to investigate these genes in the African catfish (*Clarias gariepinus*), the most commercially important farmed fish in sub-Saharan Africa. Two fads2 and three elovl cDNAs were cloned and functionally characterised by heterologous expression in yeast. Both Fads2 were bifunctional desaturase enzymes with ∆6∆5 activities, and thus catalysing all the desaturation reactions required for ARA and EPA biosynthesis from C18 precursor fatty acids. Moreover, the C. gariepinus Fads2 enzymes also desaturated 24:5n-3 to 24:6n-3, a ∆6 desaturation required for the biosynthesis of DHA through the so-called “Spächer pathway”. With regards to elongases, the C. gariepinus Elovl2 demonstrated the ability to predominantly elongate C20 and C22 PUFA and thus complements the Elovl5 with elongase capability towards mainly C18 and C20 PUFA. Interestingly, two further elongases, namely Elovl4a and Elovl4b, enable the biosynthesis of very long-chain (>C24) fatty acids, compounds with major roles in vision and fertility of vertebrates. The present study confirmed that *C. gariepinus* possess all the enzymatic capabilities required for the biosynthesis of ARA, EPA and DHA and, therefore, its EFA requirements can be satisfied with dietary provision of C18 PUFA.
Effects of dietary nucleotide on growth and fatty acid composition of juvenile rainbow trout Oncorhynchus mykiss

Mr. Asep Ridwanudin Tokyo University of Marine Science and Technology Dr. Yutaka Haga (1) Dr. Shuichi Satoh (1)

A feeding trial was conducted to evaluate the effect of nucleotide (NT) supplementation on growth and fatty acid composition of rainbow trout Oncorhynchus mykiss. Six isonitrogenous (420 g kg\textsuperscript{-1}) and isolipidic (180 g kg\textsuperscript{-1}) diets were formulated containing fish meal and plant ingredients as main protein sources. The control diet was a basal diet without supplementation of nucleotide (NT), and 1.5 g kg\textsuperscript{-1} purified nucleotides (NT) either inosine 5\textsuperscript{\prime}-monophosphate (IMP), adenosine 5\textsuperscript{\prime}-monophosphate (AMP), guanosine 5\textsuperscript{\prime}-monophosphate (GMP), uridine 5\textsuperscript{\prime}-monophosphate (UMP) or cytidine 5\textsuperscript{\prime}-monophosphate (CMP) were supplemented to the basal diet. Each diet was fed twice a day until satiation to duplicate tanks of rainbow trout with an initial average body weight of 9.8 g. After 15 weeks feeding period, growth performance and feed utilization of rainbow trout were not significantly different between control and nucleotide-supplemented diet groups. Dietary nucleotide had also no effect on VSI of rainbow trout. However, HSI of fish fed AMP, GMP and CMP diets were significantly lower compared to control diet. Lipid content in the muscle of fish fed GMP, UMP and CMP diets were higher than AMP diet. However, there was not significantly different among diet treatments for proximate composition of muscle. EPA (22:5n-3), DHA (22:6n-3) and total n-3 PUFA contents showed decrease when fish fed IMP, GMP and CMP diets. Moreover, dietary nucleotide also significantly decrease ratio of n-3:n-6 in the muscle. In contrast, total PUFA and total n-6 PUFA did not influenced by dietary nucleotides. The results of the present study indicated that dietary nucleotide altered muscle fatty acid composition of rainbow trout particularly for n-3 series.

Keywords: nucleotide, diet, fatty acid, rainbow trout.
The nutrient metabolic characteristics of a low-carnitine zebrafish model

Prof. Zhen-Yu Du Laboratory of Aquaculture Nutrition and Environmental Health (LANEH), School of Life Sciences, East China Normal University, 200241 Shanghai, China Mr. Jia-Min Li (1)

Mammalian models of mitochondrial dysfunction have been widely used for metabolism studies, but no such model has been established in fish. To establish a mildronate-induced low-carnitine zebrafish model and illustrate its characteristics of nutrient metabolism, mildronate (0.05% body weight/d) was fed to male zebrafish for 6 wk, then the fish were fasted for 1 wk. Carnitine and triglyceride (TG) concentrations, fatty acid (FA) β-oxidation capability, and other molecular and biochemical assays of lipid, glucose and protein metabolism were measured in the feeding and fasting states. The results indicated that mildronate markedly decreased hepatic carnitine concentrations in the feeding and fasting states but only decreased muscle carnitine concentrations in the fasting state. Liver TG concentrations increased by more than 50% in both nutritional states in mildronate-treated fish. Mildronate decreased the efficiency of liver mitochondrial β-oxidation, increased the hepatic mRNA expression of genes related to FA β-oxidation and lipolysis, and decreased the expression of lipogenesis genes. Mildronate decreased whole body glycogen content, increased glucose metabolism rate, and upregulated the expression of glucose uptake and glycolysis genes. Mildronate also increased whole body protein content and hepatic mRNA expression of mechanistic target of rapamycin (mtor), and decreased the expression of a protein catabolism-related gene in the feeding state. Liver, rather than muscle, was the primary organ targeted by mildronate. In short, mildronate-induced low-carnitine zebrafish showed decreased mitochondrial FA β-oxidation efficiency, greater lipid accumulation, and altered glucose and protein metabolism. This model could be used as a novel fish model for future nutrient metabolism studies.

Key words: low carnitine zebrafish; mildronate; FA β-oxidation; dyslipidemia; fatty liver; nutrient metabolism
RESPIRATORY METABOLISM OF JUVENILE SPINY LOBSTER (SAGMARIASUS VERREAUXI) UNDER DIFFERENT FEEDING CONDITIONS

Mr. Shuangyao Wang Institute for Marine and Antarctic Studies (IMAS), University of Tasmania Prof. Chris Carter (1) Dr. Quinn Fitzgibbon (1) Prof. Gregory Smith (1)

Energetic costs of protein synthesis account for a significant proportion of feeding respiratory metabolism, named specific dynamic action (SDA). SDA in aquatic animals can be evaluated by accumulated post-feeding oxygen consumption (ṀO2) multiplied by an oxycalorific coefficient, or based on stoichiometric relationships amongst ṀO2, dissolved inorganic carbon production (ṀCO2) and nitrogenous waste (ammonia-N plus urea-N) excretion (ṀN). Instantaneous ṀO2, ṀCO2 and ṀN measurements provide a stoichiometric approach to determine energy substrates oxidized over that time. Cycloheximide (CHX) as a protein synthesis inhibitor has been applied in SDA research in teleosts. Relatively little is known about SDA in crustaceans, we therefore aimed to investigate respiratory metabolism in Sagmariasus verreauxi juveniles under different feeding conditions using an intermittent-flow respirometer system. ṀO2 and ṀN were measured in five treatments: i) fasted lobsters sham injected with saline; ii) fasted and injected with CHX; iii) starved and injected with CHX; iv) fed; v) fed and injected with CHX. A comparatively reduced routine metabolic rate (RMR) in starved lobsters indicated decreased protein synthesis. Considerably higher SDA duration, magnitude and factorial aerobic scope in Fed Group compared with Fed and CHX Group suggested SDA was inhibited by CHX. Energetic costs of protein synthesis accounted for 15% of RMR in fasted lobsters and 66% of SDA in fed lobsters. Ammonia-N compromised 59-92% of nitrogen output in fed lobsters and confirmed ammonia is the major metabolic N waste product. From the ammonia quotient, 16, 26, 14, 67 and 58% of ingested protein were oxidized respectively in Fasted and Saline, Fasted and CHX, Starved and CHX, Fed, and Fed and CHX groups, suggesting a secondary role of protein catabolism during starvation. This work demonstrated an increase in oxygen consumption after feeding, confirmed a causative link between protein synthesis and SDA, and indicated a secondary role of protein catabolism during starvation in Sagmariasus verreauxi. Further studies on ṀCO2 will be included to assess respiratory quotients to develop a more thorough understanding of changes in energy substrate use by crustaceans. This will provide a more accurate approach to evaluate crustacean nutritional factors such as dietary protein quality in relation to SDA.
Modelling protein, amino acid and energy requirements of tiger grouper Epinephelus fuscoguttatus

Dr. Igor Pirozzi Port Stephens Fisheries Institute, NSW Department of Primary Industries | Centre for Sustainable Tropical Fisheries and Aquaculture & College of Science and Engineering, James Cook University

Tiger grouper are a high value marine fish species yet relatively little information is available describing their basic nutritional requirements for protein and energy. Nutritional modelling has been successfully applied to many aquaculture species to provide quantitative estimates of nutrient and energy requirements throughout growth production. While models have been developed for several species describing protein and energy requirements, few have considered the requirements for essential amino acids.

This study used a bioenergetic approach to quantify the digestible protein, amino acid and energy utilisation efficiencies, maintenance requirements, carcass composition and growth potential of tiger grouper. The daily digestible protein (DP) intake to achieve maximum predicted protein retention efficiency was 2.0 g DP kg⁻⁰.⁷ day⁻¹. The utilization efficiency of dietary protein for tiger grouper was 0.58 with the corresponding cost of DP per unit of protein gain was 1.71 g g⁻¹. The daily digestible energy (DE) intake (kJ DE kg⁻⁰.⁸ day⁻¹) to achieve a maximum predicted energy retention efficiency was 101 kJ DE kg⁻⁰.⁸ day⁻¹. The utilization efficiency of energy for tiger grouper was 0.63 and equates to an energetic cost of production of 1.59 kJ DE kJ⁻¹ energy deposition. Utilisation efficiencies for digestible MET, LYS and HIS were 0.51, 0.54 and 0.30 respectively (Fig. 1). The reciprocal cost of production for these essential amino acids per unit gain was 2.0, 1.8 and 3.4 g g⁻¹ respectively.

Detailed understanding of nutrient and energy requirements of tiger grouper throughout production will provide a platform to improve feed management and feed formulation.
Effects of dietary protein and lipid levels on growth performance, feed utilization and body composition of tahuina larva (Cichlasoma trimaculatum, Amphiliophus trimaculatus)

Dr. Francisco Javier Toledo Solis Laboratorio de Acuicultura Tropical, División Académica de Ciencias Biológicas, Universidad Juárez Autónoma de Tabasco Dr. Miguel Sáenz de Rodrigañez2 Laboratorio de Nutrición y Producción Acuícola, Campus del Mar, Universidad de Ciencias y Artes de Chiapas Dr. Carlos Alfonso Álvarez González (1) Dr. Rafael Martínez García

(1) Dr. Mario Galaviz Facultad de Ciencias Marinas, Universidad Autónoma de Baja California Dr. Francisco Javier Moyano Lopez Departamento de Biología Aplicada, Escuela Politécnica Superior, Universidad de Almería

One of the first steps to be able to design an efficient feed, for any species with potential for aquaculture, is to determine the exact nutritional requirements. Energy sources including proteins and lipids are the major expenditure in formulated feeds for early life stages in culture. In this case, these factors may be limiting for growth and organisms survival. Therefore, proteins and lipids must be incorporated in the right amounts, due to this deficiency or excess may cause physiological damage, added to that, high protein content is usually associated with high cost feed. So the adjustment of the protein content can reduce cost of feed. Thus, this study was aimed to evaluate the effects of dietary protein and lipid levels on growth, feed utilization and body composition of tahuina larva. A 5x2 factorial design with three replicates was used. Ten experimental diets were formulated to contain five levels of digestible protein (35, 40, 45, 50 and 55%) and two levels of digestible lipid (L) (15% and 20%). Semi purified diets were formulated used fishmeal and casein as protein sources. Fish oil and soy lecithin were added to each diet, in order to provide the two levels of lipids to evaluate. The dietary ingredients, proximate compositions and fatty acid compositions are shown in Table 1. Fish ranged in size from 0.16 ± 0.02 g were randomly distributed (30 fish per tank) in thirty 180 L cylindrical fiberglass tanks connected to a recirculating system and were acclimated for one week prior to the experimental period. The feeding experiment lasted for eight weeks and fish were fed three times per day (08:30, 12:30 and 17:30 h) and the amount of feed consumed was determined by daily recovery of excess feed, which was then dried and weighed. Standard formula was used to assess feed utilization and growth parameters over the eight week feeding trial. We obtained a 90% survival at the end of feeding trial. The best growth results obtained were from treatments with greater level protein (45, 50 y 55%) and 21 % lipids. The results are discussed.
Effect of different dietary methionine and lysine supplementation on growth performance in practical diets for adult Common Carp (Cyprinus carpio)

Dr. Juyun He Evonik Degussa (China) Co., Ltd. Guangzhou Branch Dr. Karthik Masagounder Evonik Nutrition and Care GmbH

A growth trial was conducted to investigate the effects of different dietary methionine and lysine supplementation on growth performance in practical diets for common carp (Cyprinus carpio). Fifteen iso-nitrogenous (310 g/kg crude protein) practical diets, with three lysine (Lys) supplementation levels (0, 6.0 and 9.0 g/kg) and five methionine (Met) supplementation levels (0, 1.5, 3.0, 4.5 and 6.0 g/kg), were hand-fed three times daily to four replicates groups of adult common carp (initial body weight 212.4±4.5 g) for 56 days. Weight gain (WG) and specific growth rate (SGR) of fish fed 6.0 and 9.0 g/kg Lys supplementation were significantly high than that of fish fed no Lys supplementation (P<0.05), while no difference was found between 6.0 and 9.0 g/kg Lys supplementation when Met supplementation was higher than 1.5 g/kg. Only when fish fed 6.0 g/kg Lys supplementation, WG and SGR were increased significantly with the increasing dietary Met content (P<0.05). While no interaction was found between dietary Met and Lys supplementation. Based on quadratic regression analysis between dietary methionine and weight gain (6.0 g/kg Lys supplementation, i.e. 1.95% dietary Lys concentration), optimal methionine requirement for maximum growth, expressed as g Met required kg-1 diet, was 9.30 g/kg diet (with the appearance of 4.9 g/kg cysteine). Thus the optimal total sulphur amino acid to lysine ratio (TSAA/Lys) was calculated to be 72%. The results indicated that Lys content affects methionine utilization and requirement in adult common carp, therefore, the available dietary lysine density and the knowledge of balanced amino acids profile must be taken into consideration in formulating cost-effective feeds.
Japanese eel (Anguilla japonica) is a commercially important teleost species in East Asian countries, in which they are cultured from naturally captured glass eel stages. This practice inevitably leads to depletion of wild stocks and hence technology for developing full-cycle culture of Japanese eel from eggs is demanded to expand its production in captivity. Several studies demonstrated that linoleic acid (LA; 18:2n-6) and α-linolenic acid (ALA; 18:3n-3), fatty acids typically found in vegetable oils (VO), can satisfy the essential fatty acid requirements ensuring survival and normal growth of A. japonica and other eel species. This implied that eels possess a repertoire of fatty acid desaturases (Fads) and elongation of very long-chain fatty acid (Elovl) proteins enabling them to biosynthesise physiologically relevant fatty acids such as arachidonic acid (ARA; 20:4n-6), eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) from the C18 precursors LA and ALA. Previous studies confirmed that A. japonica had an Elovl5 elongase and a Fads2 desaturase, the latter having Δ6 and Δ8 desaturase activities. However, an enzyme with Δ5 desaturase activity required for the biosynthesis of ARA and EPA, had not yet been reported in A. japonica. In the present study, we identified a fads-like gene from Japanese eel and characterised its function by heterologous expression in yeast. Phylogenetic analyses confirmed that the herein studied A. japonica fads is an orthologue of FADS1, a desaturase believed to be absent in teleosts, the group of fish which the vast majority of farmed species belong to. Functional characterisation of the eel Fads showed this enzyme is a Δ5 desaturase able to convert 20:3n-6 and 20:4n-3 to ARA and EPA, respectively, in agreement with Fads1 from other vertebrates. In addition to this being the first demonstration of a Δ5 Fads1 among teleosts, the results confirmed that the Japanese eel possesses all desaturase activities required to biosynthesise essential LC-PUFA from C18 precursors and consequently, can efficiently utilise dietary VO to produce biologically active LC-PUFA.
Feeding of juvenile cobia Rachycentron canadum: evaluation of practical feeds, comparison of commercial fish meal replacers, and estimation of essential amino acids requirements

Mr. Thiago Raggi Coppens International B. V. Mr. Albert G. J. Tacon Aquaculture Laboratory (LAM) - University of São Paulo Mr. Daniel Lemos Aquaculture Laboratory (LAM) - University of São Paulo

Three studies were conducted with juvenile cobia Rachycentron canadum: (Study 1) a 10-week feeding trial within floating net cages to test the nutritional efficacy of different dietary feeding regimes (trash-fish control diet, a semi-moist diet, an in-house dry formulated diet, and a commercial cobia feed); (Study 2) a 10-week feeding trial within indoor water-recirculated tanks to test the nutritional efficiency of different potential dietary fish meal replacers (poultry by-product meal, soy protein concentrate, feather meal), a diet without taurine supplementation, and a commercial cobia feed; and (Study 3) an estimation of the essential amino acids (EAA) requirements based on the amounts of each amino acid retained in the carcass of the fish fed a high-quality protein diet (trash-fish), and compared with A/E ratio estimation method (the concentration of each essential amino acid as a percentage of the concentration of total essential amino acids, including cystine and tyrosine). Generally, fish performance was superior in the net-cage feeding trial compared with the indoor water-recirculated tank trial. Fish fed the trash-fish control diet displayed the best growth response and feed performance. Overall, the fish growth and performance of the experimental diets tested in the indoor trial were very similar, showing that the alternative ingredients could be included and replace part (50%) of the fishmeal component. Apparent crude protein digestibility coefficients of over 80% were obtained for all experimental diets from both trials. The quantitative EAA requirement values estimated by the protein accretion method was highly correlated to the average of each of the EAA requirement for carnivorous fish species reported in the literature, and could be recommended for formulation of commercial feed for cobia. The results from study 1 and 2 concluded that cobia requires practical diets with high levels of crude protein, and the inclusion of alternative plant-based and terrestrial animal protein sources was possible. Also, the results suggest the need for nutritional improvement of existing commercial diets currently employed for cobia farming in Brazil.
Metabolic response to dietary taurine levels in European sea bass (Dicentrarchus labrax, L) juveniles

Ms. Nicole M. Pires Interdisciplinary Centre of Marine and Environmental Research (CIIMAR-UP) Mr. Alexandre F. Diógenes (1) Mr. Rui Magalhães (1) Prof. Aires Oliva-Teles (1) Dr. Helena Peres (1)

Plant feedstuffs (PF) represent an economic and environmental sustainable alternative to fish meal. Nevertheless, PF contain residual amount of taurine (Tau), a neutral ß-amino acid that is naturally abundant in fish meal. For some species, including European sea bass, Tau have been reported to be essential to optimize growth, however its effects on liver metabolism is little known. Thus, this study aimed to evaluate the effect of increasing dietary Tau level on bile acid and intermediary metabolism, and liver oxidative status in European sea bass juveniles (ABW= 55g). Four isoproteic (45% crude protein) and isolipidic (18% crude lipid) diets were formulated, containing a mixture of plant feedstuffs and fish meal (corresponding to 80% and 20% of total dietary protein, respectively), and increasing levels of Tau (0.2, 0.5, 0.7, and 1.2%). Triplicate groups of European sea bass were fed these diets for 10 weeks at 24°C. Total hepatic bile acids content increased as dietary Tau levels increased, whereas the opposite was observed for total plasma bile acids. Plasma indirect bilirubin linearly increased with dietary Tau level. Plasma total, HDL, and LDL cholesterol decreased, though plasma triglycerides were not affected with the increase of dietary Tau level. The activity of hepatic key enzymes of lipogenesis, FAS and ME, decreased with the increase of dietary Tau up to 0.7%. Post-prandial plasma glycaemia and the activity of hepatic key enzymes of glycolysis and gluconeogenesis decreased with the increase of dietary Tau level. Liver peroxidation, glutathione peroxidase, and glutathione reductase activity linearly decreased as dietary Tau level increased, while catalase activity was not affected by dietary Tau level. Overall, present results indicate that Tau appear to be involved in different pathways of both lipid and glucose metabolism, has a hypocholesterolaemia and hypoglycaemia action and antioxidant proprieties in European sea bass.

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Supplementation of essential amino acids as a strategy to reduce dietary protein levels for Jian carp, Cyprinus carpio var. Jian

Dr. Mingchun Ren Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences, Dr. Karthik Masagounder Evonik Nutriton and Care GmbH Dr. Juyun He Evonik Degussa (China) Co., Ltd. Dr. Xianping Ge

It is important to know dietary protein reduction strategies in fish feed formulations. A ten-week feeding trial was conducted to investigate the effects of essential amino acids (EAA) supplementation on the success of protein reduction in practical diets of juvenile Jian carp (Cyprinus carpio var. Jian). Six diets were formulated including a positive control (PC, 40.3% dietary protein), a negative control (NC, 29.0% dietary protein level), and four test diets (37.8-29.9% dietary protein). The PC diet (Diet 1) contained high amount of fish meal (16% diet) and different plant protein sources, while the NC (Diet 6) diet contained low amount of fish meal (10% diet) and various plant protein sources, both without any supplemental amino acids. Other diets (Diets 2-5) contained decreasing levels of fish meal (14-10%) and plant protein sources with increasing levels of supplemental amino acids. Fish were fed three meals per day until visual satiety for 70 days. The results showed no significant decline in the growth performance and feed intake of fish fed decreased dietary protein with EAA supplementation, and these values were significantly higher than the negative control that contained no supplemental amino acids (Diet 6). Dietary protein reduction with EAA supplementation significantly increased apparent digestibility coefficient values of methionine, lysine and threonine. Dietary treatments did not affect whole body composition, but influenced EAAs retentions. Total ammonia nitrogen (TAN) excretion, plasma ammonia level showed a decreasing trend with the decreasing dietary protein levels, and the lowest values were found in fish fed Diet 5. In addition, plasma glucose concentration increased with the decreasing dietary protein reduction. Dietary EAA supplementation affected the metabolites related to taurine synthesis, TOR signaling and AMPK pathway by metabolomics analysis. These results indicated that low protein diets supplemented with EAA can maintain growth performance, and increase EAA digestibility and retention in Jian carp, when dietary protein reduced from 40.3% to 29.9%. Low dietary protein also has benefit in the reduction of TAN excretion. Results of our study demonstrate benefits to the industry in saving expensive protein sources and reducing dietary cost as well as increasing protein utilization and minimizing nitrogen excretion.
Dietary arginine levels affect the synthesis from glutamic acid to arginine in juvenile hybrid grouper (Epinephelus fuscoguttatus ♀ × Epinephelus lanceolatus ♂)

Mr. Dong Yu Hainan University Dr. Wu Xiaoyi Hainan University Dr. Gao Yujie (1) Mr. Li Xiaojun
(1) Mr. Yao Wei (1) Mr. Zhou Zhiyu (1) Mr. Jin Zibo Hainan University Ms. Wang Xiao Hainan University Mr. Mu Wei Hainan University Mr. Wang Cuijin
Hainan University Ms. Zhang Jienuo Hainan University

This study aimed to investigate the synthesis from glutamic acid to arginine (Arg) at different dietary Arg levels in juvenile hybrid grouper (Epinephelus fuscoguttatus ♀ × Epinephelus lanceolatus ♂). Three isoproteic (53.15% of dry matter) and isolipidic (7% of dry matter) experimental diets were formulated to contain 0.9%, 1.9% and 3.65% dietary Arg levels (dry matter basis). Dietary amino acid nitrogen contents were adjusted to be constant by replacing Arg with aspartic acid and glycine (1:1) mixture. Triplicate groups of 15 fish (initial body weight: 11.2 g) were fed to apparent satiation by hand twice daily for 10 weeks.

Results showed that fish fed 0.9% dietary Arg had lower weight gain% (WG%) than fish fed 1.9% and 3.65% dietary Arg, but the differences were not significant. Fish fed 3.66% dietary Arg had significantly higher protein productive value (PPV) than fish fed dietary 0.9% Arg. Hepatosomatic index (HSI) were declined with dietary Arg increasing. Fish fed 0.9% dietary Arg had significantly higher P5C synthetase (P5CS) specific activity in intestine than fish fed 1.9% and 3.66% dietary Arg. Intestinal ornithine aminotransferase (OAT) specific activity was reduced as dietary Arg increased. The specific activities of argininosuccinate synthase (ASS) and argininosuccinate lyase (ASL) in body kidney as well as ornithine carbamoyltransferase (OCT) in liver showed no obvious differences among different experimental treatments. The protein and gene expression levels of intestinal P5CS, OAT, OCT in liver as well as ASS, ASL in body kidney will be analyzed later, and free amino acids in serum will also be measured. Preliminary results from this study indicated that glutamate acid would be converted to arginine in hybrid grouper at a low dietary arginine level (0.9%), and at higher dietary Arg levels (1.9% and 3.65%), the synthesis from glutamic acid to arginine (Arg) was weak.
Effects of dietary lysine levels on growth, feed utilization and related gene expression of juvenile hybrid grouper (Epinephelus fuscoguttatus ♀ × Epinephelus lanceolatus ♂)

Mr. Li Xiaojun Hainan University Dr. Wu Xiaoyi Hainan University Dr. Gao Yujie Hainan University Ms. Dong Yu (1) Mr. Yao Wei (1) Ms. Jin Zibo (1)

An 8-week growth trial was undertaken to evaluate the effects of dietary lysine levels on growth, feed utilization and related gene expression of juvenile hybrid grouper (Epinephelus fuscoguttatus ♀ × Epinephelus lanceolatus ♂). Six isoproteic (53.5% of dry matter) and isolipidic (7% of dry matter) were formulated to contain 1.74%, 2.11%, 2.72%, 3.16%, 3.44% and 3.88% dietary lysine levels. Dietary amino acid nitrogen contents were adjusted to be constant by replacing lysine with aspartic acid and glycine (1:1) mixture. Triplicate groups of 15 fish (initial body weight: 11.44 g) were fed each experimental diet to apparent satiation by hand twice daily.

Results indicated that weight gain % (WG%) were significantly affected by different dietary lysine levels, and fish fed 1.74% and 2.11% dietary lysine had lower WG% than fish fed 2.72%, 3.16%, 3.44% and 3.88% dietary lysine. Daily feed intake (DFI) of fish fed 1.74% dietary lysine was higher than that of fish fed other levels of dietary lysine. Feed efficiency (FE), protein efficiency ratio (PER) as well as protein productive value (PPV) showed similar variation trends as WG. The relative mRNA expression levels of insulin like growth factor-I (IGF-1) and eIF4E-binding protein1 (4E-BP1) in liver of fish fed 1.74% lysine were significantly lower than those of fish fed 2.11%, 2.72%, 3.16%, 3.44% and 3.88% dietary lysine. Based on the quadratic broken-line analysis of weight gain against dietary lysine levels, the optimal dietary lysine requirement for hybrid grouper was estimated to be 2.34% of dry matter (corresponding to 6.36% of dietary protein on a dry weight basis). Hepatosomatic index (HSI) displayed a significantly declined trend as dietary lysine increased. The protein contents in whole body, muscle as well as liver of fish fed 1.74% lysine were significantly lower than those of fish fed 3.88% dietary lysine. Generally, the optimal dietary lysine requirement for best growth of hybrid grouper was estimated to be 2.34% of dry matter (corresponding to 6.36% of dietary protein on a dry weight basis).
Is dietary taurine supplementation beneficial for Senegalese sole juveniles?

Dr. Cláudia Aragão CCMAR Ms. Rita Colen (1) Dr. Nadège Richard (1) Ms. Rita Teodósio

(1) Prof. Ivar Rønnestad University of Bergen Dr. Jorge Dias SPAROS Lda.

Aquaculture has grown at a very fast rate during the last years and this growth has been supported by a significant research effort directed to increase the industry’s sustainability. Consequently, aquaculture has been able to reduce its dependence on fishmeal and fish oil as primary ingredients for fish diets. Dietary replacement of fishmeal by plant sources may result in an unbalanced supply of selected nutrients, among which taurine has been identified. Taurine is virtually absent in terrestrial plant ingredients, is involved in many important physiological processes (including a pivotal role in bile salt synthesis), and it is considered an essential nutrient for marine fish. Therefore, the objective of this work was to test the effect of taurine in diets with different levels of plant ingredients on growth, protein and lipid metabolism of Senegalese sole (Solea senegalensis) juveniles.

For the experiment two basal diets were formulated, one simulating a commercial diet (COM diet), in which 15% of plant ingredients are already incorporated, and another in which 85% of the ingredients from marine-origin in the COM diet were replaced by plant ingredients (PP85 diet). Taurine was supplemented to the basal diets, in order to obtain three additional diets: a PP85 diet with taurine concentrations similar to that found in COM diet; a COM and a PP85 based diets, with taurine concentrations similar to those found in polychaetes, the natural food of Senegalese sole in the wild. A six-week growth trial was conducted using Senegalese sole juveniles with an initial weight around 13g. Growth, feed conversion ratio, body composition, taurine concentration in different tissues, bile acid concentrations in bile, and expression of genes related with bile acid synthesis and transport, triglyceride and lipid metabolism were analysed.

Dietary taurine supplementation resulted in beneficial effects for Senegalese sole juveniles, especially when fed diets with high levels of plant ingredients. Dietary taurine supplementation to those diets seems more effective at levels that go beyond that found in commercial diets. These positive effects may eventually have a higher impact on growth performance and Senegalese sole condition in long term trials, up to commercial sizes.
RESPONSE OF NILE TILAPIA TO DECREASING LEVELS OF DIETARY PROTEIN BALANCED FOR ESSENTIAL AMINO ACIDS

Ms. Rita Teodósio CCMAR, Centro de Ciências do Mar, Universidade do Algarve Dr. Sofia Engrola CCMAR, Centro de Ciências do Mar, Universidade do Algarve Dr. Karthik Masagounder Evonik Nutrition & Care GmbH Dr. Cláudia Aragão CCMAR, Centro de Ciências do Mar, Universidade do Algarve

Protein is the most expensive qualitative nutrient in fish feed. Supplemental amino acids (AA) provide an opportunity to reduce excess dietary protein levels, while balancing the diets for AA needed to meet the requirements of animal. The objective of this study was to evaluate the performance of Nile tilapia to decreasing levels of dietary protein supplemented with increasing levels of AA. Juvenile Nile tilapia (5.91±1.66 g) were allotted into 15 tanks at 23 fish per tank. Triplicate tanks of fish were randomly assigned to one of the five dietary treatments (4550 kcal GE/kg) varying in protein levels (28, 30, 32, 34 and 36%). Diets were formulated to meet the minimum requirements of AA, on digestible basis, for Nile tilapia according to AMINOTilapia®. Fish were fed 3 times daily to their visual satiety for 8 weeks. After eight-week feeding period, no significant differences were detected among dietary treatments in final body weight, weight gain, daily growth index and feed intake. Fish reached a final body weight of 30.57±0.86 g (mean±SD). Feed conversion ratio (FCR) differed significantly only between the group fed 28%CP diet (1.30) and those fed the 36%CP diet (1.16). Dietary treatments affected final whole-body proximate composition. Fish fed low protein diets (28% or 30%) had lower moisture (74-75% vs. 76-77%) and higher body fat (~6% vs. ~5%) than those fed the high CP diets (34% or 36%). Fish fed 30%CP diet showed better body protein retention than those fed the 36%CP diet (41% vs. 36%), while others showed intermediate values. Similar trend was also observed on the retention of majority of the AA except for methionine which had better retention from 36%CP diet versus 28%CP diet. Overall, our study results demonstrated an opportunity to reduce dietary protein levels in juvenile Nile tilapia from 36% to 30% without any compromise in growth performance and FCR. In addition, diet with 30%CP supplemented with AA resulted in improved protein and AA utilization. Our study continues to evaluate metabolic flux of protein by feeding tilapia in metabolic chambers using the same dietary treatments labelled with a 14C-amino acid mixture (tracer).
SUPPLEMENTATION OF DL-METHIONYL-METHIONINE REDUCES THE DEPENDANCY ON FISHMEAL IN DIETS FOR JUVENILE Litopenaeus vannamei

Dr. Karthik Masagounder Evonik Nutrition & Care GmbH Dr. Hassan Sabry-Neto LABOMAR – Instituto de Ciências do Mar Dr. Adhemar Rodrigues de Oliveira Neto Evonik Degussa do Brasil Ltda Dr. Claudia Figueiredo-Silva Evonik Nutrition & Care GmbH Dr. Alberto J.P. Nunes LABOMAR – Instituto de Ciências do Mar

Levels of dietary methionine (Met) are significantly impacted when fishmeal inclusion is restrained in aquafeeds. This work investigated the minimum amount dietary Met+Cys required for maximum growth of juvenile Litopenaeus vannamei fed diets with graded levels of fishmeal. Four sets of diets were prepared to contain 0, 6, 12 and 18% fishmeal. Each set was supplemented with DL-Methionyl-Methionine (AQUAVI® Met-Met) to result in a total dietary Met+Cys content of 1.05, 1.16 and 1.29% (% diet, as-is basis). A total of 6,120 juvenile shrimp (1.00±0.08g) were stocked in 60 outdoor, green-water tanks of 1m³ at 100 shrimp/m². Five tanks were randomly assigned to each dietary treatment. Shrimp were fed 4-8 times daily for 75 days. At harvest, shrimp final survival (92.7±4.7%, mean±SD), daily weight gain (0.17±0.01g), apparent feed intake (13.3±0.5g) and FCR (1.18±0.06) were unaffected by dietary fishmeal level or Met+Cys content. Final body weight (BW) was affected by both fishmeal and dietary Met+Cys content. Reducing fishmeal from 18% to 6% did not affect BW (13.3-13.7g), but 0% fishmeal diet resulted in reduced BW (12.7 g. No differences in BW were observed in shrimp fed diets containing 6% (13.27g) and 18% fishmeal. Comparatively, a significantly higher BW was observed when shrimp were fed diets containing 1.29% Met+Cys (13.44 g), followed by diets with 1.05% and 1.16% Met+Cys (13.25g and 13.11g, respectively). A significant interaction was detected between fishmeal level and dietary Met+Cys content. Under both 0% and 6% fishmeal, higher levels of dietary Met+Cys, 1.16% and 1.29%, respectively, were required to maximize shrimp BW. Differences in response of shrimp to Met+Cys content between high and low fishmeal diets could owe to the differences in digestibility. However, a complete withdrawn of fishmeal was detrimental to shrimp BW even at higher levels of dietary Met+Cys. In summary, our study results show opportunity to reduce dietary fishmeal level from 18% to 6% using supplemental DL-Methionyl-Methionine (0.34% diet) without any compromise in growth performance. In addition, our results suggest that a minimum Met+Cys level of 1.16% or 1.29% is required to maximize shrimp BW and yield especially in the low fishmeal diets, under green-water system.
Estimating Lysine and Phosphorus Requirements of Rainbow Trout and Nile Tilapia as a Function of Body Weight Using a Factorial Nutrient Requirement

Ms. Fatemeh (Neda) Nemati Shizari Department of Animal Biosciences, University of Guelph, Guelph. Prof. Dominique P Bureau (1)

Evidence suggest that essential nutrient requirements of fish changes as live weight of animal increases. Quantifying nutrient requirement of fish based on empirical dose-response trials is a difficult task since the weight of animal increases by several folds over the duration of trials. Nutrients requirement of fish can be predicted using factorial modeling approaches but there has been limited efforts to use such approaches to estimate essential nutrient requirements of different fish species. These models could be very valuable to help develop phase-feeding strategies and improve cost-effectiveness of feeds.

This study involved the development of a factorial approach to estimate the requirements of an organic (destructible) nutrient (lysine) and an inorganic (indestructible) nutrient (phosphorus) for a carnivorous (rainbow trout) and an omnivorous species (Nile tilapia).

Nutrient requirement was estimated as follows:

Nutrient (Lys or P) Requirements (g/fish/day) = Retained Nutrient + Maintenance Nutrient Requirement + Inevitable Catabolism (Lys) + Basal Non-Fecal Nutrient Losses (P)

Retained nutrient was estimated based on expected weight gain estimated using a growth model and whole body nutrient concentration. Maintenance requirements and inevitable catabolism of Lys was estimated from data from dose-response studies using a broken line model relating digestible nutrient intake to nutrient deposition. Lysine retention efficiency (LysRE) was estimated from data from dose-response studies that included estimation of lysine deposition as a function of digestible lysine intake and a linear regression of LysRE vs. live body weight. Inevitable catabolism of lysine was estimated as: 1 - LysRE. Basal non-fecal P losses were estimated from a review of studies that estimated urinary P excretion. Optimal dietary nutrient concentration was calculated from estimate nutrient requirement (g/fish/day) divided by predicted feed requirement (g/fish/day) which was estimated using a bioenergetics factorial model calibrated for each species.

Results from the model simulation suggested that the P requirements (expressed as dietary concentration) decreased in both species as fish grows. However, Lys requirement was predicted to remain relatively constant or even slightly increase as live weight of fish increases in both species. This investigation indicated a paucity of relevant and reliable data to calibrate factorial nutrient requirement models for commercially important species.
IMPORTANCE OF DIFFERENT Ca/P RATIOS IN PIKEPERCH (SANDER LUCIOPERCA) DURING THE EARLY LIFE STAGE

Ms. Najlae El Kertaoui Research Unit in Environmental and Evolutionary Biology (URBE), Institute of Life, Earth & Environment, University of Namur Dr. Syaghalirwa N.M. Mandiki (1) Dr. Ivar Lund Technical University of Denmark, DTU Aqua, Section for Aquaculture. Prof. Patrick Kestemont (1)

Pikeperch (Sander lucioperca) is considered as one of the most potential and promising new species in intensive freshwater fish farming, however, the high rate of mortality, cannibalism and incidence of skeletal deformities are among the major bottlenecks hindering the expansion of its culture. Nutritional requirements during the early life stage must be defined in order to develop an effective weaning diets for this species. At present, formulated feeds developed for marine fish larvae are used for weaning pikeperch, with Ca/P much higher than those observed in common freshwater fish diets. In fact, besides the importance to study the optimum dietary values for Ca and P in fish feeds, authors have suggested that Ca/P should be considered as well as individual dietary levels of minerals. In a previous study, low growth with an increase in skeletal deformities were observed in larvae fed high Ca/P, suggesting the importance of the dietary Ca/P effect in pikeperch larvae (El Kertaoui et al. 2017). In this respect, the present study was conducted to deeply investigate the effects of dietary Ca/P on pikeperch larval development and performance.

Six isonitrogenous and isoenergetic diets, containing different graded levels of Ca, P and Ca/P were tested. Triplicate groups of pikeperch larvae were fed the different experimental diets during three weeks. The results will report on final survival and growth rates calculated at the end of the experiment. Assays of digestive enzymes will be performed in order to better understand the role of Ca/P in the digestive physiology in early life stage of pikeperch. Biochemical analysis will be conducted focusing on the whole body mineral contents to evaluate the effects of Ca/ P on the absorption and retention of the other minerals. In addition, results will be discussed with respects to the role of Ca/P in the osteological development and the incidence of skeletal deformities. Therefore, the histological study of bone ossification as well as the molecular study of the expression of some relevant genes involved in skeletal system will be conducted. Thus, the results will increase the understanding of the nutritional requirements of Ca and P in percid fish.
Effect of inclusion of krill meal in on-growing diets on growth, survival, nutritional utilization and fry quality of seabream

Dr. REDA SALEH Aquaculture Research Group (GIA), Institute of Sustainable Aquaculture and Marine Ecosystems (ECOAQUA), Universidad de Las Palmas de Gran Canaria Dr. Lena Burri Aker BioMarine Antarctic AS, Dr. Tibiabin Benitez-Santana Aker BioMarine Antarctic AS, Dr. Line Johnsen Aker BioMarine Antarctic AS, Mr. Serhat Turkmen Aquaculture Research Group (Gia), Institute of Sustainable Aquaculture and Marine Ecosystems (ECOAQUA), Universidad de Las Palmas de Gran Canaria Mr. Pedro Castro Aquaculture Research Group (Gia), Institute of Sustainable Aquaculture and Marine Ecosystems (ECOAQUA), Universidad de Las Palmas de Gran Canaria Prof. Marisol Izquierdo Aquaculture Research Group (GIA), Institute of Sustainable Aquaculture and Marine Ecosystems (ECOAQUA), Universidad de Las Palmas de Gran Canaria

There is a need to find sustainable alternatives to fish meal (FM) and fish oil (FO) to support the continued growth of aquaculture. FM is mostly produced from mass-caught pelagic species, but the production has been relatively constant for several decades. The aim of the present study was to investigate the potential of dietary krill meal inclusion as a sustainable alternative to FM and FO. A feeding trial with Gilthead seabream juveniles was conducted to evaluate if 9% dietary krill meal inclusion improves growth performance when compared to a control diet. FM replacement by krill meal tended to increase fish total length, although no significant differences were obtained. However, at the end of study, fish in the 9% krill meal group showed significantly higher body weight compared to fish fed the control diet. Moreover, FM replacement by 9% krill meal indicated a reduction in the damage and accumulation of lipid droplets in the hepatocytes and around the pancreatic islets.

In summary, the present study suggests that FM and FO can be reduced in diets for seabream without negatively affecting growth performance. On the contrary, krill meal enhances gilthead sea bream growth and improves health parameters, which will open an interesting innovation line to completely replace FM by alternative terrestrial protein sources and the partial inclusion of krill meal.
COPPER LEVELS IN DIETS HIGH IN VEGETABLE INGREDIENTS FOR GILTHEAD SEABREAM (SPARUS AURATA) FINGERLINGS

Mr. David Dominguez IU-ECOAQUA, University of Las Palmas de Gran Canaria Ms. Paula Sarmiento IU-ECOAQUA, University of Las Palmas de Gran Canaria(1) Mr. Zakarya Sehnine IU-ECOAQUA, University of Las Palmas de Gran Canaria(1) Dr. Ramon Fontanillas Skretting Dr. Philip Antony Jesu Prabhu Fish Nutrition Program, Institute of Marine Research Prof. Marisol Izquierdo IU-ECOAQUA), University of Las Palmas de Gran Canaria(1)

Gilthead seabream (GSB) is the third most produced fish in EU. In commercial feeds for this species, marine ingredients (fish meal, FM and fish oil, FO) are increasingly substituted with ingredients of terrestrial origin. Copper (Cu) is an essential trace element and also a potential toxicant of concern at high intake levels. In general, ingredients of terrestrial origin have higher Cu levels than marine ingredients. With the changing ingredient profile of commercial GSB feeds, it is necessary to define optimal dietary supply of Cu to ensure better performance, and to avoid excess supplementation. Therefore, the aim of this study was to evaluate optimal dietary inclusion level of Cu in low FM-FO diets for GSB fingerlings.

A basal diet closely mirroring practical GSB feeds was formulated with low inclusion of FM (10%) and FO (6%). Five different experimental diets were then produced by supplementing CuSO4, at 0, 1, 3, 7 and 29 mg Cu kg-1 diet. GSB fingerlings, 12.6 ± 1.4 g (mean ± SD) were distributed in 15 tanks with 30 fish per tank and randomly assigned one of the dietary treatments, in triplicates. The fish were fed three times a day until apparent visual satiation for 42 days. Water temperature and oxygen were monitored daily, while pH was registered weekly. Growth was recorded and tissue samples were taken for biochemical, mineral, histology, X-ray and hepatic gene expression analyses at the end of the trial.

Irrespective of the dietary treatments GSB fingerlings almost tripled their body weight, which was not significantly affected by dietary Cu levels. Moreover, there were no differences for productive parameters such as FCR, SGR or TGC. In the present study, as the basal diet in itself had 6 mg Cu kg-1, dietary Cu levels required for growth appear to be covered in GSB fingerlings by these levels. However, it is still an open question on whether growth is the most pertinent criteria to evaluate micro-nutrient requirements. The results of further analyses being conducted will provide more information towards optimizing Cu levels in practical diets of GSB fingerlings.
Substitution of fishmeal (FM) and fish oil (FO) with ingredients of terrestrial origin is of paramount importance for the sustainable production of gilthead seabream (GSB). Nevertheless, the mineral profiles of plant ingredients vary considerably from that of marine ingredients. Manganese (Mn) is a cofactor for essential metalloenzymes involved in the development of bone and preventing oxidative damage that tends to be in higher concentrations in ingredients of terrestrial origin. Mn requirements have been established for several finfish but not for GSB. Thus, the present study aims to establish the optimal dietary supplementation level of Mn in GSB fingerlings fed vegetable based diets.

GSB fingerlings (weight 12.6 ± 1.5 g (mean ± S.D.)) were fed five practical diets high in vegetable ingredients (FM: 10%, FO: 6%). The diets were supplemented with 0, 10, 16, 23 and 56 mg Mn kg⁻¹ as MnSO₄. GSB fingerlings of 450 were randomly distributed in 15 tanks and fed one of the five diets until apparent satiation three times per day for 42 days. At the end of the trial, samples were taken for biochemical, mineral, histological, gene expression and X-ray analyses. Performance parameters including feed intake, thermal growth coefficient and feed conversion ratio were calculated.

At the end of the trial fish almost tripled their weight, however dietary Mn did not affect growth, productive parameters or survival. The high substitution levels of FM led to high Mn levels in the basal diet, 20 mg Mn kg⁻¹ diet. This level was similar to those described as optimal for other finfish such as grouper or cobia. Body lipid composition was reduced without Mn supplementation compared to diets supplied with over 23 mg Mn kg⁻¹, while protein and ash were not affected by the dietary Mn. Supplementation up to 16 mg Mn kg⁻¹ increased the expression of MnSOD whilst reduced the expression of catalase. Their expression levels followed a significant inverse regression with the levels of dietary Mn. Further analyses are conducted to elucidate the effects of dietary Mn on whole body and vertebral mineral composition and histology in GSB fingerlings fed practical diets high in vegetable ingredients.
Substitution of marine ingredients by plant ones can alter Se levels in feeds. Se plays an important role in the anti-oxidative defense by forming part of selenoproteins and also improves growth. Moreover, Se is usually present at higher levels in marine ingredients than those of terrestrial origin. Despite being one of the most important marine finfish species of Mediterranean aquaculture, the Se requirements of gilthead seabream (GSB) fed low FM-FO diets has not been yet determined. Therefore, this study was conducted to establish recommendation for Se supplementation in low FM-FO practical diets for GSB fingerlings.

A plant-based diet (FM 10% and FO 6%) was formulated to contain 0.1 mg Se kg⁻¹ diet. Four other diets were supplemented with 0.2, 0.4, 0.7 or 1.1 mg Se kg⁻¹ diet, as sodium selenite. GSB fingerlings, 12.6 ± 1.4 g (mean ± S.D.) were distributed in 15 tanks in triplicates and fed until apparent satiation three times per day for 42 days. Growth and productive parameters were monitored, and samples for biochemical, mineral, histological, gene expression and X-ray analyses were taken at the end of the trial.

Se supplementation significantly improved growth up to 0.7 mg Se kg⁻¹. Inclusion of Se beyond 0.7 mg kg⁻¹ diet reduced growth, despite there were no differences on productive parameters such as FCR, SGR and TGC. Body proximal composition reflected an increased lipid deposition in fish fed 0.4 mg Se kg⁻¹, while protein and ash were not significantly affected by dietary Se. Supplementation up to 0.4 mg Se kg⁻¹ tended to reduce the expression of GPX, while further addition increased it. GR was significantly higher expressed on fish supplemented with 1.1 mg Se kg⁻¹. Selenosis in mammals produces signs of oxidative injury, thus the increase of GPX and GR at the highest supplementation levels may suggest a potential pro-oxidant effect of Se at these doses.

Even though high dietary Se had negative effects on several biomarkers, other response criteria are being analysed in order to assess optimum Se levels in diets with high inclusion of vegetable ingredients for GSB.
Phosphorus: problems and solutions

Dr. Shozo Sugiura The University of Shiga Prefecture, School of Environmental Sciences

Phosphorus (P) has been increasingly recognized as a key nutrient in aquafeeds and the aquatic environment. In this review, five major issues concerning P are highlighted and their solutions are given. (1) Diagnostic P indicators: Among numerous P indicators studied in fish and animal species, no sensitive and stable indicator has been identified. For the diagnosis of fish P status, gauge indicators are most reliable and practical. (2) Fish growth and N:P stoichiometry: Body N and P ratio is constant irrespective of the body size, except early ontogeny and under malnutrition. The P requirement, therefore, can be accurately expressed as per body N accretion. In practice, the dietary requirements of P can be and should be expressed accurately by normalizing the measured P requirement based on its feed efficiency; i.e., standard requirements. (3) Prevention of bone deformity: Larval-early juvenile fish are most vulnerable to bone deformity because their collagen matrix is only partially calcified and stabilized. Collagens have species-specific low melting temperatures. Thus, only a slight increase in temperature results in bone malformation, especially upon under-mineralized collagen. Hence, high temperature, low-P, and low-vitamin C cause bone deformity. Since small fish contribute little to effluent P, reducing dietary P during this vulnerable period should be avoided. (4) Digestibility and absorption: Our interest in digestibility has been shifting from fish meal to plant ingredients. Phytase supplementation is less effective at fish’s “body temperature” compared to livestock animals and birds. In vitro acid-dough incubation of plant materials (with phytase) can achieve near 100% digestion of plant P. Plant materials also contain substantial amounts of lignocelluloses. Developing technology to digest such fibrous components is becoming increasingly important to reduce the excretion of organic waste. (5) Making low-pollution feeds is simple and easy; as, for example, all aquarium feeds are made to be low-polluting. However, low-pollution feeds, if they are used for aquaculture, must meet various other standards, including the feed cost, fish growth, and product quality. Obviously, the goal is still far off. Sustainable development of aquaculture depends largely on problem-solving research rather than some peripheral efforts.
Vitamin K nutritional requirements, functions and underlying mechanisms in fish: new insights from a 6 year integrative research effort.

Dr. Ignacio Fernandez Centro de Ciências do Mar Prof. Jorge Manuel de Oliveira Fernandes Nord University Dr. Nadge Richard Phileo Lesaffre Animal Care Dr. Jorge Dias Sparos Ltd Dr. Elsa Cabrita (1) Dr. Paulo Gavaia (1)

Vitamin K (VK) is a liposoluble vitamin known to be essential for blood coagulation and bone metabolism by two different pathways: (i) the gamma-carboxylation of the known VK dependent proteins (VKDPs; Oldenburg et al., 2008) and (ii) the gene transcription through the pregnane X receptor (Tabb et al., 2003). Recently, new biological functions of VK have been suggested in mammalian systems. In this regard, while undercarboxylated BGP (a known VKDP) stimulates testosterone synthesis in testis, promoting male fertility (Karsenty and Ferron 2012), the expression of genes involved in the biosynthesis of cholesterol and steroid hormones and the concentrations of testosterone in plasma and testis was found to be decreased in rats fed with VK deficient diets (Shirakawa et al., 2006). Similar results regarding the role of MK-4 in testis and testosterone were also reported by Ito et al. (2011). Previous report stated that VK deficiency in the parental fish did not affect the hatching rate of the eggs or the mortality of the larvae (Udagawa and Hirose 1998).

We first determined the requirement of VK during fish larval development, unveiling new roles where VK might be involved by a proteomic approach (Richard et al., 2014). After, we characterized the effects of VK deficiency, and expanded the biological roles where it might be involved (Fernandez et al., 2014, 2015; Marques et al., 2017). Through a transcriptome analysis (RNAseq), we recently revealed the molecular pathways at cellular, tissular and organism level by which VK might be required for early larval development and successful reproductive performance. Ongoing work on VK stability in feeds and its effect on fish microbiome will be presented. The results from this 6 years study will be reviewed and discussed.

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Choline supplementation improves growth performance of juvenile yellowtail kingfish (Seriola lalandi)

Ms. Angela Liu University of New South Wales Dr. Igor Pirozzi James Cook University Dr. Basseer Codabaccus University of Tasmania Dr. Cedric Simon Commonwealth Scientific and Industrial Research Organisation Mr. Barney Hines Commonwealth Scientific and Industrial Research Organisation Brisbane Australia(1) Prof. Jesmond Sammut Commonwealth Scientific and Industrial Research Organisation Dr. Mark Booth New South Wales Department of Primary Industries

Choline is an essential nutrient that is closely related to the B-complex vitamin group; it is required for the structure and function of cells in all animals. Common effects of choline-deficiency in aquaculture species include reduced overall growth performance, liver lipid accumulation and hepatic dysfunction. Dietary choline requirement is species specific and ranges widely between 0.27 – 3.0 g kg\(^{-1}\) diet. Choline is often supplemented in yellowtail kingfish (YTK, Seriola lalandi) aquafeeds as a precautionary measure to ensure optimal growth and health. However, there are no published studies quantifying the dietary choline requirement for YTK. Furthermore, YTK are commonly farmed in sea-pens where fish are exposed to fluctuating seasonal water temperatures that may alter choline utilisation.

The first objective of this study was to determine if choline supplementation is needed in a practical fishmeal-based formulation and to test whether the performance of YTK to residual or supplemented choline levels are temperature dependent. The second objective was to quantify the dietary choline requirement for juvenile YTK.

Two eight-week growth trials were conducted; in the first trial (initial mean weight 157.28 ± 11.88g) fish were reared at 16°C and 24°C and fed three practical diets supplemented with 0, 3 and 6g of choline chloride (CC) kg\(^{-1}\) diet with a residual choline level of 2.41, 4.55 and 6.41g choline kg\(^{-1}\) in the diet. In the second trial (initial mean weight 156.27 ± 15.27g) fish were reared at 16°C and fed five isonitrogenous and isocalorific semi-purified diets containing 0.70, 1.47, 1.83, 3.66, 3.88, 7.33g choline kg\(^{-1}\) diet by adding graded levels of CC.

YTK fed practical diets with added CC significantly improved feed conversion ratio (FCR, kg kg\(^{-1}\)) at 16°C and 24°C water, but only had a marginal improvement in specific growth rate (SGR, % BW day\(^{-1}\)). Based on SGR, FCR and carcass choline the dietary choline requirement for juvenile YTK was estimated to be 3.65, 2.83 and 3.43g choline kg\(^{-1}\) diet, respectively.

This study indicates that choline supplementation is required in a practical fishmeal-based feed for juvenile YTK. A formulation containing dietary choline of 3.65g kg\(^{-1}\) diet will provide optimal growth for the fish.
Effects of glucose administration on glucose and lipid metabolism in two strains of gibel carp (Carassius gibelio)

Dr. Junyan Jin Institute of Hydrobiology, the Chinese Academy of Sciences  Ms. Yunxia Yang (1) Prof. Xiaoming Zhu (1) Dr. Dong Han (1) Dr. Haokun Liu (1) Dr. Shouqi Xie (1)

We compared the glucose clearance ability of gibel carp CAS III (A strain) with Dongting (DT strain). A previous study suggested that these two strains responded to insulin differently. As insulin plays an important role in glucose utilization, we hypothesized that the ability to eliminate excess glucose after a glucose load would differ between A strain and DT strain. To test this hypothesis, fasted specimens of both strains of gibel carp were injected with glucose. As expected, glucose induced hyperglycemia in both strains. In both strains, mRNA levels of the glycolytic enzyme 6-phosphofructokinase (6PFK) increased in the white skeletal muscle post-injection, while expression of the gluconeogenic enzymes glucose-6-phosphatase (G6Pase), fructose 1,6-bisphosphatase (FBPase) and phosphoenolpyruvate carboxykinase (PEPCK) decreased in the liver post-injection. DT strain exhibited enhanced muscular glycolytic processes as reflected by elevated mRNA levels of pyruvate kinase (PK) and returned to basal glycemia more quickly than A strain (within 6 h versus more than 12 h). DT strain had a higher glycogen concentration in the liver along with an up-regulated expression of hepatic lipogenic enzymes sterol regulatory element binding protein 1 (SREBP1) and acetyl-CoA carboxylase (ACC) indicating that DT strain was better able to store glucose than A strain. Overall, DTs train was better able to clear excess blood glucose after the glucose tolerance test than A strain.
SEASONAL VARIATIONS IN KINETICS PARAMETERS OF DIGESTIVE PROTEASES IN FRESHWATER FISH MAY HELP TO MAXIMIZE THEIR EFFICIENCY IN PROTEIN DIGESTION

Dr. Mikhail Solovyev Institute of Systematics and Ecology of Animals SB RAS. Dr. Eugene Rogozhin Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry RAS. Dr. Elena Kashinskaya Institute of Systematics and Ecology of Animals SB RAS. Dr. Fco. Javier Moyano

Dpto. Biologia y Geologia. Universidad del Almería. Campus CEIMAR

The objective of the present study was to assess if there is some kind of functional adaptations to seasonal changes in water temperature affecting the efficiency of protein digestion performed by freshwater fish. These adaptations should be evidenced through variations in the kinetic parameters of the main enzymes involved, as well as in their net ability to hydrolyze substrates. For this purpose, a three-step approach was considered:

1. Sampling of two fish species Carassius gibelio and Perca fluviatilis at two different seasons (spring and summer) at the basin of Chany Lake (Siberia, Russia).

2. Characterization of the main kinetic parameters of trypsin, a key enzyme involved in protein digestion, performed on fish sampled at each season. After being sacrificed, guts were used to obtain enzyme extracts on which trypsin activity was quantified using BAPNA as substrate. Apparent kinetic parameters (Km and Vmax) were determined for trypsin activity in samples obtained in each species and season at the actual pH values measured in fish guts, as well as at temperatures measured in water either in spring (pH 8.0, 5 °C) and summer (pH 6.5, 25 °C).

3. Assessment of the comparative ability of the proteases obtained from each species and sampling season to digest a given protein under controlled conditions provided by an in vitro assay. The different enzyme extracts were used to assess the effects of three different factors (time of hydrolysis, pH and temperature) on the net hydrolysis of a standard protein (hemoglobin). The hydrolysis was evaluated both quantitatively (total release of amino acids) and qualitatively, using reversed-phase HPLC, N-terminal sequencing, and LC/MS analysis in order to characterize the products of digestion.

Results showed a significant effect of both the fish species and sampling season on the apparent kinetics parameters of trypsin. In agreement to this, the mathematical model derived from the in vitro assays revealed a significant (p<0.05) effect of the three studied factors on protein hydrolysis for both species and seasons. This suggests a sort of functional adaptation of trypsin to seasonal changes, oriented to maximize protein digestion under variable conditions.
The objective of this study was to evaluate the effect of water temperature on gastric pH values and the activity of pepsin. For this purpose, two hundred rainbow trouts were equally distributed among twenty four cubical 2000 L tanks. Then, fish were divided on two experimental groups that were exposed for 20 days at 13 °C and 20 °C respectively. Fish were hand fed twice per day during a trial. At the end of the trial, before determination gut pH and activity of digestive enzymes, fish were fasted for 72 h. Then, fish was given a food and fish were sacrificed in 2, 5, 9, 13, 18, 26, 36, 48, 60, 84, 96, 120 hours after feeding. Fish were euthanized by a blow on the head and their guts were removed. The values of pH were measured with a portable pH meter with a microelectrode. Then, the guts were lyophilized and stored at -80 °C. The activity of pepsin was quantified using standard procedure with 0.3 % hemoglobin as substrate and at actual physiological pH values in fish stomach and temperature in tanks. The pH (Glycine-HCl buffer for pH range 1.5-3.5, acetate buffer for pH range 4-5, and phosphate buffer for pH 6 and 7) and temperature optimums (temperature ranged from 10 to 70 °C), as well as Km and Vm parameters from Michaelis-Menten equation (substrate concentration ranged from 0.004% to 0.6%) were determined. The temperature and pH optimums for pepsin was found between 35 and 42.5 °C (inactivated at 50 °C) and pH 2.0-2.5 (at pH higher than 5.0 the activity was absent) for both groups of fish respectively. Km value was lower at 20 °C (0.02%) than at 13 °C (0.06%) whereas the Vm values were similar. The time after feeding, actual pH values in stomach, and water temperature were significantly influenced on the activity of pepsin (ANOSIM, p ≤0.05). This study showed that the water temperature in 20 °C is more effective for acid stage of digestion in stomach of trout. The relationship between optimal pepsin activity and the optimal rearing temperatures for this species are discussed.
Development of digestive enzyme activity in larvae of Sphoeroides annulatus feeding with different protocols

Dr. Mario Galaviz Universidad Autónoma de Baja California Mr. Julio Mercado (1) Dr. Leonardo Ibarra Av. Sábalo-Cerritos s/n. Estero del Yugo Mazatlán Sinaloa Dr. Lus M López (1) Dr. Juan M Brown Centro de Investigación en Alimentación y Desarrollo A.C

The bullseye puffer fish Sphoeroides annulatus is a marine fish with a high value in the national and international market, which has high tolerance to environmental variations, however, one of the main factors influencing the survival and growth of larvae of this species and other marine fish, is the quality and availability of the food during the first exogenous feeding and weaning (change of food live to micro-diets). The aim of this study was to evaluate the activity of digestive enzymes during ontogenetic development of the S. annulatus fish larvae fed under different protocols. Larvae sampling was conducted on the premises of the Laboratory of Reproduction and Marine Fish Farming of the Research Center for Alimentation and Development, A. C., Mazatlán (CIAD. All larval rearing protocols were performed using the green water technique, thus, after the transfer and up to 40 days post hatching (DPH) depending on treatment there was added to the culture tanks a mixture of 80,000 cells ml⁻¹ of Nannochloropsis oculata and Isochrysis sp, adding also enriched rotifers and Artemia with an enrichment with a mix of fatty acid. Five treatments were performed (T14, T18, T22, T26 and T30) DPH in which each one was performed in triplicate and the number indicates the day of weaning (change of live food to formulated feed), samples of larvae were sacrificed and lyophilized for further analysis. The enzymes tested were trypsin, chymotrypsin and leucine aminopeptidase (LA). Results show that the activity of trypsin and chymotrypsin increase as the larval develops, which could indicate a maturation of the digestive system during the ontogenetic development, however, the larvae fed with T22 were those that showed highest level of trypsin activity and chymotrypsin. LA activity did not show significant differences during the first DPH, but from the first change of rotifer to Artemia (14 DPH) the activity of this enzyme was raised and remained constant until day 35 DPH. According to the results obtained in the present study, it is recommended that bullseye puffer fish larvae may possibly start their weaning from DPH 22 without affecting their growth and development.
The Physiological and Metabolic Differences between Visceral and Subcutaneous Adipose Tissues in Nile Tilapia (Oreochromis niloticus)

Prof. Zhen-Yu Du Laboratory of Aquaculture Nutrition and Environmental Health (LANEH), School of Life Sciences, East China Normal University, 200241 Shanghai, China Ms. Ya-Wen Wang (1)

Visceral adipose tissue (VAT) and subcutaneous adipose tissue (SCAT) have different structures and metabolic functions, and play different roles in the regulation of mammal systemic endocrine. However, little is known about morphology and physiological and metabolic functions between VAT and SCAT in fish. We compared the morphological, physiological and biochemical characteristics of VAT and SCAT in Nile Tilapia and measured their functions in energy intake flux, lipolytic ability, and gene expression patterns. The results indicated that SCAT contained more large adipocytes and non-adipocytes than VAT in Nile tilapia. VAT had a higher lipid and was the primary site for lipid deposition. Conversely, SCAT had higher hormone-induced lipolytic activity. Furthermore, SCAT had a higher percentage of monounsaturated and lower polyunsaturated fatty acids than VAT. SCAT had higher mitochondrial DNA, gene expression for fatty acid β-oxidation, adipogenesis and brown adipose tissue characteristics, but it also had a lower gene expression for inflammation and adipocyte differentiation than VAT. Taken together, SCAT and VAT have different morphological structures, as well as physiological and metabolic functions in fish. VAT is the preferable lipid deposition tissue, whereas SCAT exhibits higher lipid catabolic activity than VAT. The differences between VAT and SCAT should be considered in future nutrition and metabolism studies in fish.

Key words: Nile tilapia; Visceral adipose tissue; Subcutaneous adipose tissue; Metabolism; Gene expression pattern;
FEEDNETICS: a tool to assist fish farms to plan feeding strategies and manage feed stocks

Mr. Filipe Soares SPAROS Lda. Mr. Tomé Silva SPAROS Lda. Mr. Luís Conceição SPAROS Lda. Olhão Portugal

This work presents a framework for the application of the FEEDNETICS model as a decision-support tool in the planning of feeding strategies and management of feed stocks, in a “typical” fish farm. Representing usually 20-50% of operational costs, feeding is one of the major expenses of a fish farm production. However, many farms use non-optimized feeding strategies which is reflected in additional feeding costs. This result either from poor feed conversion rates (e.g. due to overfeeding or use of inadequate diets) or from the need to increase water quality control (e.g. excessive nutrient loads may require more aeration to maintain a proper level of dissolved oxygen). A modelling approach, by allowing a holistic approach to the overall production system, can be applied for several scenarios aiming the optimization of feeding strategies.

The FEEDNETICS model can be used by the farmer as a day-to-day decision-support tool for multiple purposes. Herein is presented a framework for its application, aiming at guiding the planning of feeding strategies and the management of feed stocks, which is divided into two phases:

(i) Definition of feeding strategy, in which the FEEDNETICS model is used for screening different diets and feeding regimes, aiming at the optimization of one or more indicators (i.e. economic, performance and environmental);

(ii) Periodical predictions of feed needs, in which the FEEDNETICS model is used in the day-to-day management in order to estimate the feed needs for the overall production. The drivers for the model are supplied by forecasts of water temperature, after the model being adjusted taking into account the recorded parameters to date (e.g. real amount of given feed, real water temperature, observed mortality).

A hypothetical application for a “typical” fish farm, following the abovementioned framework, is presented, showing the adaptability for different systems and the flexibility to include different levels of input data.
T. macdonaldi is one of the most important endemic finfish species in the Gulf of California, Mexico. The production of this species is a well-controlled process done at the Autonomous University of Baja California. Nutritional research on totoaba has been a key issue to further the feasibility of a natural restocking program and development of a commercial marine grow out.

To support developing of totoaba and other marine carnivore fish husbandry and culture, a proper amino acid profile from alternative feed ingredients is needed. Taurine deficiency occurs in fish species possessing a limited ability to synthesize it and also when natural taurine sources are excluded from the diet. A taurine-deficient diet induces physiological abnormality, namely green liver syndrome as previously observed in totoaba. Based on 4-SKM kinetics it has been concluded that increased taurine levels result in improved normal physiological condition when compared to the basal diet (0.23% taurine).

Environmental conditions and improper handling are the main stressors that affect marine finfish during husbandry. Stress is the physiological state of an organism when environmental changes threat their health, followed by a physiological response to regain homeostasis. Tryptophan has been used in stress treatment in a large number of organisms for its role as a serotonin precursor and hypothalamic-pituitary-interrenal axis (HHI) modulator, which regulates stress response. We evaluate the effect of dietary tryptophan on the serotonergic system during acute stress response in totoaba. Our results indicate that tryptophan supplementation modulated cortisol response and mediated negative feedback of 5-HT system in telencephalon after acute stress.

Our current knowledge has placed this species as a warm-water carnivore with a low lipid and high protein requirement, and also a taurine dependent, and tryptophan supplementation modulated cortisol response and mediated its stress. This kind of research has proven fundamental in understanding not only diet requirements but also the mechanism by which specific nutritional elements promote or effect this species.

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The WorldFish mission is to strengthen livelihoods and enhance food and nutrition security by improving fisheries and aquaculture. In WorldFish Strategy 2017–2022, the Sustainable Aquaculture Flagship aims to enable sustainable increases in livelihoods from aquaculture production without creating adverse socio-economic or environmental impacts. To achieve this objective, WorldFish will capitalize on synergies within the broader CGIAR portfolio and work collaboratively with multiple research and delivery partners on 1) fish breeds and genetics, 2) aquaculture systems, and 3) fish health, nutrition and feeds. Research on fish feed and nutrition will lead to the development of cost-effective and sustainable aqua-feeds, in order to spur the sustainable growth of aquaculture in Africa and Asia. In this presentation, the authors will first characterize the aqua-feed industry and its context in some African and Asian countries, then describe WorldFish research plans for innovation in aquaculture nutrition, and finally provide an update on action research and outreach activities for sustainable aqua-feed development.
Grass carp (Ctenopharyngodon idellus), one of the major herbivorous cyprinid fish, are now widely cultivated in China as well as in many other countries as edible fish or as biological control agents for aquatic weeds. This study was conducted to examine the systemic metabolic strategies of grass carp to maintain glucose and lipid homeostasis when fed with low- or high-carbohydrate or -fat diets. The isonitrogenous diets with different carbohydrate levels (15.90, 21.81 and 45.45 %) and fat levels (0.93, 4.87 and 10.79 %) were fed to grass carp for 8 weeks. After the feeding trial, the maximal growth performance and feed utilization of grass carp were observed in 21.81 % dietary carbohydrate group and 4.87 % dietary fat group.

Gene expression data indicated that the expression of G6Pase was down-regulated and the expression of GS was up-regulated in fish fed with low dietary glucose, with reducing glucose consumed. In high glucose intake group, glycolysis was increased by enhanced expressions of G6Pase and GK genes. Meanwhile, FAS and ACC gene expressions were significantly up-regulated, and LPL and CPT1 genes expressions were down-regulated, bringing about enhanced lipid accumulation. Subsequently, serum cholesterol and total lipid contents were significantly increased in fish accepted high glucose diet, and then induced appetite suppression through up-regulation of leptin expression.

In addition, fish increased hepatic GK and PK expressions to elevate glycolysis, and enhanced ACC and FAS expressions to accelerate biosynthesis of fatty acid to adapt to low lipid intake. Meanwhile, fish fed with low lipid diet decreased hepatic cpt1 expression to depress lipolysis, leading to low contents of serum triglyceride and body fat. In contrast, excess lipid intake increased G6Pase, PEPCK and PPARα expressions to stimulate gluconeogenesis and β-oxidative, while decreased ACC and FAS expressions to reduce lipid synthesis in fish liver. Moreover, increased β-oxidative-induced fatty acid or gluconeogenesis-induced serum glucose might induce the appetite suppression by high dietary fat through modulation of leptin expression. The study could be a reference in the systemic adaptation of metabolic strategies in response to inappropriate carbohydrate or fat diets.
Severe dietary phosphorus deficiency induces alterations in the hepatic transcriptome of puffer fish (Takifugu obscurus)

Prof. Chaoxia Ye South China Normal University

Food is the main source of phosphorus (P) for fish because the concentration of P in freshwater and seawater are low. While it is clear that phosphorus deficiency affects growth and lipid metabolism, an experimental overview of underlying processes has not been presented and the extent of changes induced by P deficiency have not been investigated at the transcriptome level. In the present study, two experimental diets supplemented with 0 and 0.6% P from Ca(H2PO4)2 were fed to T. obscurus for 60 days. Compared with fish fed diet supplemented without P, fish fed diet supplemented with 0.6% P showed significantly higher final body weight, weight gain, feed efficiency, serum P content and cholinesterase activity, while significantly lower total cholesterol, triglyceride and lipoprotein a, alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase activity in the serum. The contents of EPA and DHA in liver of fish fed diet supplemented with 0.6% P were significantly higher than that in fish fed diets supplemented without P. As a result, n-3 PUFA, the sum of PUFA and the ratio of n-3/n-6 significantly increased in fish fed diet supplemented with 0.6% P. In order to systematically comprehend the molecular response in puffer fish (T. obscurus) fed P deficiency diets, we investigate genes that are differentially expressed in liver of fish between P0 group (diet without P supplement) and P6 group (diet supplemented with 0.6% P). A total of 106 million clean reads (150bp per) was obtained, and approximately 81% of these reads were successfully mapped to the genome of Takifugu rubripes. A total of 374 differential expression genes (DEGs) were identified which including 157 up-regulated and 217 down-regulated genes. The results of the GO enrichment analysis of differential expression genes were classified into three categories: biological process (164 subclasses), cellular component (44 subclasses) and molecular function (94 subclasses). 348 DEGs were enriched into 231 pathways. The DEGs were scrutinized in 8 fatty acids metabolism-related categories and 6 of them were further validated by qRT-PCR. These results provide further insights into the influence mechanism of dietary P to the molecular response in puffer fish.
The California yellowtail (Seriola dorsalis) is a species with considerable potential for aquaculture, and as such, research has been initiated to develop the basis of husbandry and care protocols for its culture. Although recent progress have markedly improved larval rearing, size and quality of spawns remain variable, thereby increasing risk in overall hatchery production. Much of the embryonic survival is determined by broodstock factors in addition to environmental conditions; variability in survival rates is often seen between spawn, female brooders, and years. The larval stages are also influenced by broodstock factors, and appropriate nutrition is quintessential to a successful run.

Taurine is now widely recognized as an essential nutrient in many fish species for juvenile growth. Although beneficial effects have been reported, there is a great dearth of information concerning the role of taurine in reproductive output, i.e. egg production and subsequent larval quality. Consequently, a trial was conducted where broodstock of California yellowtail received taurine supplemented or unsupplemented feed (2.67% and 0.28% dietary taurine, respectively, dry matter basis), and the resulting eggs and larvae were divided and raised on a taurine-supplemented or unsupplemented protocols. The trial was repeated in two consecutive years.

Discriminant factor analysis of spawn metrics indicate significant differences in the reproductive output from fish fed the taurine-supplemented feed. The difference was primarily driven by significant increases in total eggs per spawn, relative fecundity, percent floating and percent viability, as well as the odds of survival of larvae to 1st feeding. Egg measurements contributed little to treatment separation. All larvae from the control broodstock and control larval group died by 20 days-post-hatch. ANOVA analysis revealed no significant differences between other treatment groups in terms of live weight or notochord length. However, the larvae in the TBCL and CBTL groups were numerically longer and heavier.

These results highlight the importance of dietary taurine for broodstock maturation in California yellowtail. Moreover, taurine supplementation in larvae from unsupplemented broodstock did not restore larval survival. In conclusion, we recommend supplementing broodstock diet with taurine to promote reproductive output, embryonic development, and subsequent larval quality.