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KEYNOTE PRESENTATIONS

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KEYNOTE 1

JOHN HALVER ORATION
ACCIDENTAL FISH NUTRITIONIST WHO CHANGED EVERYTHING
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Professor John E. Halver did not set out to become a fish nutritionist. Rather he was a biochemistry student at the University of Washington who was directed by his professor to look into a problem brought to him by a fisheries professor, namely widespread anemia in salmon fingerlings reared in regional hatcheries to enhance fisheries stocks. At this time, fish diets were not formulated to meet the nutritional requirements of fish because they were largely unknown. Halver’s involuntary involvement in the salmon anemia problem occurred 62 years ago and was a turning point in fish nutrition research. Based upon medical research finding, Halver suspected the fish were deficient in folic acid and vitamin B_{12}, which had only recently been discovered. By injecting anemic salmon fingerlings with both vitamins in the correct proportions, he found the fish recovered in 7-10 days. This experience changed Halver’s life. He switched from medical research to fish nutrition and, for his PhD thesis, he developed and tested the first vitamin-free test diet for fish. Using this diet, he determined the qualitative and later the quantitative dietary requirements for vitamins in fish, setting the stage for rationale fish feed formulation based on levels of required nutrients rather than on empirical testing of feed formulations. Halver was hired by the US Fish & Wildlife Service while still a graduate student to design and direct a laboratory in Washington State to conduct fish nutrition research in support of the region’s vast salmon enhancement hatchery system. He used this as a platform to elevate the visibility of fish nutrition research as a respectable nutrition topic. He expanded the capabilities of his laboratory by establishing a trout nutrition station in Idaho where the abundant, constant temperature spring water made it possible to do studies year round. He used the laboratory to solve a major, national problem with fish hepatomas in the USA in the early 1970s, finding that aflatoxins were the cause. For this work and his pioneering research in fish nutrition he was elected to the National Academy of Sciences in 1978. He authored hundreds of scientific publications and published the first edition of his well-known book, *Fish Nutrition*, in 1972. Halver retired from the Fish & Wildlife Service in 1978 and started a second career as a professor in the College of Fisheries, University of Washington. He served in this capacity until retiring at age 70 in 1992. He remained active as an editor, researcher and speaker at conferences until his sudden death in October of 2012 at 90 years of age. Halver led a successful, happy and charmed life full of adventure and accomplishments.
KEYNOTE 2

MODERN NUTRITION – SETTING THE TREND
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With aquaculture playing an increasing role in the supply of seafood for human food and nutritional security, there is due recognition of the importance of optimal nutrient and energy supply for improving productivity in aquaculture. We also witness increased interest for research in fish and shrimp nutrition over the recent decades. Recent concerted efforts in updating our knowledge on the nutrient requirements of fish and crustaceans are commendable. Yet, we are far from being capable of providing clear recommendations on nutrient requirements for different stages of a given species of finfish or shrimp generally having long production cycles. Compared to terrestrial livestock production relying on a relatively small number of species across the world, aquaculture involves the farming of a large number of species in a wide variety of environments and farming systems. This makes it indeed difficult to arrive at precision-farming of aquatic organisms all through their life cycles. We do have at our disposal a number of novel biological tools supported by strong information technology. In the absence of adequate quantitative data on requirements, meta-analyses of existing data with a systematic review approach can provide the necessary guidelines. But this can hardly replace dedicated studies using different response criteria beyond that of growth or nutrient utilisation. It is also clear now that knowledge gained in some species with regard to the management of feed supply in tune with the animals’ estimated requirements and behavioural needs can be extended to other species with proper adjustments. The diversity of digestive systems in fish and the physiological responses to nutrients supplied by the feed ingredients and to the associated xenobionts can be assessed using novel tools of integrative biology. Use of integrative ‘omic tools has become an established trend for assessing post-absorptive changes in different metabolic pathways as affected by dietary factors. We however need dedicated studies to separate the effects of specific nutrients from those due to ingredients themselves and the responses at the molecular level have to be complemented with responses at the biochemical level. Sustainable aquaculture development relies mainly on the efficiency of resource utilisation where nutrition research has already contributed much over the recent years. As nutritionists, we are also confronted with societal challenges in terms of exploring novel resources, nutritional value of products, food safety, sustainability and environmental performance of aquaculture. An inter-disciplinary approach is warranted now more than ever.
KEYNOTE 3

OMEGA-3 REFLECTIONS: WHERE WE’VE BEEN AND WHERE WE STILL NEED TO GO

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Since the first study over 40 years ago that defined the essentiality of polyunsaturated fatty acids (PUFA) in fish by determining the quantitative requirements for 18:3n-3 and 18:2n-6 in rainbow trout, essential fatty acid (EFA) research has gone through distinct phases. For the first 20-25 years the focus was primarily on determining EFA requirements of fish species. Nutritional and biochemical studies showed the major differences between fish species, with EFA requirements of salmonids and freshwater fish generally satisfied by C18 PUFA, whereas the n-3 or omega-3 long-chain (LC)-PUFA, EPA and DHA, were required to satisfy requirements in marine fish. In contrast, in the last 15-20 years, the research emphasis shifted to determining “optimal” requirements of EFA to support growth of fish fed diets with considerably increased lipid content and where growth expectations were much higher. This required a greater knowledge of the specific roles and functions of EFA in metabolism and physiology, and how this impacted on fish health and disease, particularly highlighting the different roles of C18 PUFA and the highly biologically active LC-PUFA. Requirement studies were more focussed on early life stages, in particular larval marine fish, defining not only levels, but also the balance between different EFA. Finally, a major driver in the last 10-15 years has been the unavoidable replacement of fish oil and fishmeal in feeds and the impact that this can have on omega-3 LC-PUFA contents of diets, the fish being fed them, and the human consumer. This led to considerable interest in endogenous pathways of LC-PUFA biosynthesis in fish, which has been the particular focus of studies in Stirling.

We now recognise that omega-3 levels in fish feeds can be defined in three ways. Firstly, the minimum level required to satisfy EFA requirements and thus prevent deficiency signs. Although defining this level is still a time-consuming and expensive process, we can extrapolate from existing data. Furthermore, this level is relatively small and easy to supply even with today’s current high demand for fish oil. The second level is that required to sustain maximum growth and optimum health in fish being fed modern high-energy diets. The balance between different PUFA and LC-PUFA (e.g. EPA/ARA and n-3/n-6 ratios) is important, and defining them is a more challenging scientific problem and so the ideal levels and balances are still not well understood, particularly in relation to fish health. The third level is the one that is driving much research; how can we supply sufficient omega-3 LC-PUFA to maintain the nutritional quality of farmed fish at the same level as 20 years ago, and similar or better than can be found in wild fish? This level far exceeds the biological requirements of the fish itself. To satisfy this requirement we need entirely new sources of omega-3 LC-PUFA. We cannot rely on the limited and finite marine resources that we can feasibly, and sustainably, harvest, or efficiently recycle. We need to produce n-3 LC-PUFA de novo. Ideally, in the future, this may be possible by mass culture of the primary producers, marine microalgae but, in the short to medium term, genetically modified, metabolically engineered oilseed crops are the only currently viable solution.
Two decades ago, fish meal and fish oil amounted to 90% of the Norwegian salmon feed. In 2013 less than 30% of the ingredients were of marine origin. The content of fish oil and meal in the feed was 11 and 18% in 2013 respectively. Around 25% of the fish oil and meal were produced from trimmings and fish silage. The main plant ingredients were soy concentrate (37% of the diet) and rapeseed oil (19% of the diet). From 2010 to 2012 there was a 14% reduction in marine ingredient content in the salmon diet concomitant with a 33% increase in the Norwegian salmon production. The limited supply of fish meal and fish oil makes this shift from marine towards plant ingredients necessary to be able to produce increasingly more salmon from a limited pool of marine ingredients. However, this shift may also affect how efficiently the raw materials in the feed are converted into edible product for human consumption. A higher content of plant ingredients may affect pellet quality, lower the digestibility of nutrients and increase the feed conversion ratio (FCR). The increased amount of plant ingredients also has consequences for the consumption of limited recourses such as fossil energy, phosphorous, land area and fresh water. The amount of wild caught fish used in the production of salmon has so far received most of the attention in a sustainability perspective. The fish-in-fish-out (FIFO) ratio is often used to estimate the amount of wild caught fish used to produce the amount of fish oil and meal required to produce 1 kg of salmon. From 1990 to 2013, the FIFO ratios for fish oil and meal in Norwegian salmon farming has decreased from 7.2 to 1.25 and from 4.4 to 0.73, respectively. The FIFO shows the dependency of marine resources on a kg to kg basis and does not consider the different nutrient content in the forage fish and in the salmon product. When the marine nutrient dependency ratios are calculated for marine oil and protein (amount of marine protein and oil from forage fish/marine protein and oil produced as salmon), it is shown that the Norwegian salmon is now a net producer of marine protein and oil. The reduction in marine ingredients from 2010 to 2012 only caused minor changes in the retention of protein, energy, phosphorous and the omega 3 fatty acids EPA and DHA in fillet and whole salmon. The retention of protein, energy and EPA/DHA was 24, 27 and 23% in the edible product in 2012, respectively. The major change in fillet nutrient composition from 2010 to 2012 was a decrease in fillet content of omega 3 fatty acids from 2.1 to 1.3 mg/kg. However, Norwegian farmed salmon is still an important source of the nutritionally important fatty acids EPA and DHA. The 7 billion salmon meals produced in 2013 cover the recommended daily intake of EPA and DHA for 123 million people for one year.
REPLACEMENT OF MARINE FISH OIL WITH HIGH-EPA OIL FROM TRANSGENIC CAMELINA SATIVA IN FEEDS FOR ATLANTIC SALMON (SALMO SALAR)

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The use of high levels of marine fish oil in aquafeeds is a non-sustainable practice. However, more sustainable oils sources from terrestrial plants do not contain long-chain polyunsaturated fatty acids (LC-PUFA). Consequently, feeds based on conventional vegetable oils reduce n-3 LC-PUFA levels in farmed fish. Therefore, the aquaculture industry desperately requires new, sustainable oil sources that contain high levels of n-3 LC-PUFA in order to supply the increasing demand for fish and seafood while maintaining the high nutritional quality of the farmed product. One approach to the renewable supply of n-3 LC-PUFA is metabolic engineering oilseed crops with the capacity to synthesize these essential fatty acids in seeds. In the present study, the oilseed Camelina sativa has been transformed with algal genes encoding the n-3 biosynthetic pathway and expression restricted to the seeds via seed-specific promoters to produce an oil containing > 20% eicosapentaenoic acid (EPA). This oil was investigated as a replacement for marine fish oil in feeds for post-smolt Atlantic salmon. In addition, this study with EPA-rich oil will contribute to our understanding of the biochemical and molecular mechanisms involved in the control and regulation of docosahexaenoic acid (DHA) production from EPA, and will thus better inform our understanding of this key part of the LC-PUFA biosynthetic pathway.

Materials and methods:
A total of 492 post-smolt Atlantic salmon with an average body weight of 82.5 ± 8.1 g (mean ± SD) were distributed between 12 tanks and fed one of three experimental diets in triplicate for 7-weeks. The diets contained fish oil (FO), wild-type Camelina oil (WCO) or EPA-Camelina oil (ECO) as the sole lipid sources. Growth performance, feed efficiency and fish health were determined along with nutrient and fatty acid composition. In addition, the metabolic/molecular consequences of this new ingredient were assessed by analysis of gene expression using oligonucleotide microarray technology.

Results:
At the end of the trial, fish from all treatments more than doubled their weight and no mortality was recorded. Salmon fed the ECO diet showed the highest growth in terms of weight gain compared to fish fed the FO diet. Tissue transcriptomic responses were determined in order to assess the influence the experimental feeds had on fish metabolism and health.

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This study assessed the effects of different levels of fish meal (FM) and fish oil (FO) replacement on growth, feed utilisation and survival of European sea bass (*Dicentrarchus labrax*) juveniles. Sea bass juveniles of 9.8±1.5 g and 9.1±0.5 cm were fed nine isoproteic (45%) and isolipidic (21%) diets containing graded and combined levels of FO and FM as follows: (58%FM/15%FO), (20%FM/6%FO), (20%FM/3%FO), (10%FM/6%FO), (10%FM/3%FO), (5%FM/6%FO), (5%FM/3%FO), (0%FM/0% FO) and (0% FM/0%FO+DHA+AA+EPA) during 3 months. Fish growth was very fast and SGR ranged from 2.5 in fish from 10 to 30 g, to 1.6 in fish from 30 to 60g. After 46d of feeding, when fish had tripled their weight, reduction of FM/FO levels down to 5%FM/6%FO did not significantly affect whole body weight or total length. The same results for fish length were also obtained after 88 d of feeding, when fish had a 5-fold increase in body weight. Feeding 5%FM/3%FO reduced whole body weight only by 1% after 46d of feeding and by 10% after 88d. Whereas fish fed FM and FO-free diet without addition of essential fatty acids (0%FM/0% FO) had the lowest body weight and SGR, supplementation with essential fatty acids (0%FM/0%FO+DHA+AA+EPA) significantly improved growth and the fish final body weight and total length were not significantly different from fish fed 5%FM/3%FO. Hepatosomatic index, intraperitoneal fat were low in this fish (0%FM/0%FO+DHA+AA+EPA).

Muscle lipid contents tend to increase by increasing levels of substitution of FM/FO, whereas protein content trend to decrease. FGRs were higher with diets without FM/FO than in the other groups during the first 46d of feeding, whereas after 88d there were no significant differences FGR of fish fed (0%FM/0%FO+DHA+AA+EPA) and (58%FM/15%FO), (20%FM/6%FO), (20%FM/3%FO) and (10%FM/6%FO), (10%FM/3%FO) diets.
USE OF CAMELINA OIL TO REPLACE FISH OIL IN DIETS FOR FARMED SALMONIDS AND ATLANTIC COD

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In this study growth and lipid composition of Atlantic salmon, rainbow trout and Atlantic cod were compared after feeding a diet containing camelina oil (CO) as a full replacement of fish oil (FO). CO is an interesting candidate for replacement due to its high total lipid content (40%) and a high amount of the ω3 precursor, 18:3ω3, its high levels of mono- and polyunsaturated fatty acids, as well as significant amounts of γ-tocopherol, a potent antioxidant. Diets were formulated to meet the nutritional requirements for each species, and either contained FO or CO as the main lipid source. Three separate experiments were conducted (triplicate tanks for FO and CO): Atlantic salmon (mean initial weight 242 ± 46 g) were fed experimental diets for 16 weeks in seawater at 14°C, rainbow trout (44.9 ± 10 g) were fed for 12 weeks in freshwater at 14°C, and Atlantic cod (14.4 ± 1.6 g) were fed for 13 weeks at 10°C. Growth performance of Atlantic cod was significantly affected by dietary CO in comparison to cod fed the FO diet (p < 0.05); however, growth performance of the salmonids was generally unaffected by consuming dietary CO. The lipid class composition of salmonid muscle tissue compared with cod muscle tissue had an important effect on tissue fatty acid composition for each species. As such, the fatty acid profiles of salmonids fed CO were different to those in cod fed CO based on results from ANOSIM, SIMPER, cluster analysis and principal components analysis. These results exemplify selective retention of long chain ω3 polyunsaturated fatty acids in the phospholipid membranes in cod muscle tissue compared to salmonid muscle tissue, which also has a lipid storage function. Regression analysis including all species showed significant linear relationships between the tissue concentration and dietary concentration of 22:6ω3, 20:5ω3, 18:3ω3 and 18:2ω6 (p < 0.001). The fatty acid biosynthesis capacities of these species fed CO was estimated by a fatty acid mass balance method, which found that Atlantic salmon and rainbow trout were able to desaturate and elongate ~25% and 23%, respectively, of their own long chain polyunsaturated fatty acids from dietary 18:3ω3. This was significantly greater than the elongation of 18:3ω3 in Atlantic cod (6.1%) (p < 0.001). In terms of both growth performance and utilization of CO toward fatty acid biosynthesis, it appears that dietary CO is more appropriate for salmonid species than gadids.
MARGINAL EFFICIENCIES OF LONG-CHAIN POLYUNSATURATED FATTY ACID USE BY BARRAMUNDI (LATES CALCARIFER) WHEN FED DIETS WITH VARYING BLENDS OF FISH OIL AND POULTRY OIL
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An experiment was conducted with barramundi (Lates calcarifer) juveniles to examine the marginal efficiency of utilisation of long-chain polyunsaturated fatty acids (LC-PUFA). A series of five diets with blends of anchovy oil and poultry oil (100:0, 60:40, 30:70, 15:85, 0:100) were fed to 208 ± 4.1 g fish over a 12-week period. The extruded diets were also formulated with minimal (15%) inclusion of fish meal. The replacement of fish oil with poultry oil had no impact on growth performance after a twelve week period. During this period the fish grew to an average weight of 548.3 ± 10.2 g. Feed intake (mean = 385.6 ± 10.8) and feed conversion (mean = 1.14 ± 0.02 feed/gain) were also unaffected by fish oil replacement. Analysis of the whole body fatty acid composition showed that these fatty acids reflected those of the diet. However it was also shown that there was a disproportional retention of LC-PUFA (notably DHA and EPA) by those fish fed the diets with the lowest levels of fish oil inclusion. This supports that there is an improvement in the marginal efficiencies of LC-PUFA use by this species when fed diets with reduced levels of fish oil. Further analysis of the muscle tissue fatty acids also allowed for the development of a strategy for fish oil replacement with defined impacts on meat LC-PUFA levels.
ASSESSMENT OF THE NUTRITIONAL VALUE OF LUPINS IN AQUAFEEDS: EFFECT OF PHYTASE ON THE HYDROLYSIS OF PROTEIN AND PHOSPHORUS IN DIFFERENT LUPIN VARIETIES

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Legumes are considered an important source of protein. Interest in this kind of ingredients for aquafeeds has intensified due to a rapid growth of the industry, increased demand for proteins, as well as the need to reduce dependence on fish meal. Lupin has been described as a feasible alternative for partial replacement of fish meal in diets for aquaculture in countries like Australia, Chile and the European Union. This legume also offers economic advantages over the use of other commonly used plant protein sources like soybean. Research efforts aiming to improve the available varieties of lupins have intensified. Evaluation methods to distinguish potential lupin cultivars with different nutritive values are essential to optimize their use in aquafeeds. Even among same fish species, comparison of results from different feeding studies is complicated by the fact that the nutritive value and nutrient composition of cultivars are variable. Differences in ANFs content among lupin varieties may also explain some of the contrasting results in the utilization of lupin seed meals. In this way, different lupin varieties (whole seed and dehulled seed) were evaluated in their nutrient composition, native phytic acid content, and solubility profile of protein and phosphorus (P) under wide pH range conditions (2.0-9.0). In addition, an in vitro gastrointestinal model (GIM) designed to simulate the digestive conditions of fish (pH, temperature and presence of fish digestive proteases from stomach and intestine) was used to evaluate the effect of a microbial phytase on protein and P bioaccessibility in two lupin cultivars with high (Lupinus mutabilis, E44) and low (Lupinus angustifolius, LILA) phytic acid content. Results indicated that both lupins have a similar protein solubility profile with minimum values between pH 4.0 and pH 5.0, near to the isoelectric point of the proteins. However, LILA cultivar showed higher protein solubility than E44 cultivar, in all the pH range evaluated. As expected the use of phytase increased P bioaccessibility in both cultivars, although this effect was more evident in high-phytate cultivar. A similar effect was observed in the soluble protein content that was higher after the enzymatic action of phytase, presumably due the action of the additive on the dephosphorylation of inositol phosphate and the disruption of insoluble phytate-protein complexes. This led to facilitating the access of fish digestive proteases to the substrate protein, resulting in a higher protein bioavailability measured by an increment in amino acids released during the GIM digestion. Based on these findings, the use of different lupin varieties and the possibility to improve their nutritive value using enzymatic additives such as phytase, represent a clear opportunity to supply in part the high demand for plant protein sources by aquaculture.
YEAST - A POTENTIAL SUSTAINABLE PROTEIN SOURCE WITH HEALTH BENEFICIAL EFFECTS
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There is a need to develop new high-quality protein ingredients to meet the demand from the rapidly growing aquaculture industry. Today plant ingredients make up a large proportion of fish feed. Feeding increasing levels of plant ingredients, however, increases the risk of developing inflammation of the intestine. The use of soybean meal (SBM) in diets for Atlantic salmon and several other fish species thus is known to induce enteropathy in the distal intestine, a condition often referred to as SBM induced enteropathy (SBMIE). Microbial products like yeast represent potential sustainable protein sources in aquafeeds, and in addition they may contain bioactive components with potential health beneficial effects. The potential of yeast as a protein source inclined to alleviate SBMIE in Atlantic salmon, and as a model of feed-induced intestinal inflammation, was therefore investigated.

Moderate levels of the yeasts Candida utilis (CU), Kluyveromyces marxianus (KM) and Saccharomyces cerevisiae (SC) were investigated as protein sources in diets for Atlantic salmon (Salmo salar) pre smolts, substituting 40% of the crude protein from high-quality fish meal. The CU and KM yeast was produced by biofermentation of sugars from soft or hard wood, while SC was produced from molasses. The results showed that the CU and KM containing diets gave similar growth rate, feed efficiency and nutrient retention as the fish meal based control, while the SC diet gave lower performance and nutrient retention. To further investigate the health beneficial effect of yeast, a negative control based on fish meal (FM), a positive control based on 20% SBM, and three experimental diets combining 20% SBM with either one of the three yeasts CU, KM or SC were fed to Atlantic salmon. Histopathologic examination of the distal intestine showed that all fish fed the SC or SBM diets developed characteristic signs of SBMIE, while those fed the FM or CU diets showed a healthy intestine. Fish fed the KM diet showed intermediate signs of SBMIE. Corroborating results were obtained when measuring the relative length of PCNA positive cells in the crypts of the distal intestine. Gene set enrichment analysis revealed increased expression of the pathways for NOD-like receptor signalling and chemokine signalling in the SC and SBM fish, while the CU and the KU fish was similar to the FM fish. Gene expression of antimicrobial peptides was significantly reduced in the groups showing SBMIE. Characterization of intestinal bacterial communities using PCR-DGGE showed an increased abundance of Firmicutes bacteria in fish fed the SC or SBM diets.

The results suggest that CU and KM were promising protein sources in diets for Atlantic salmon and CU was also highly effective to counteract SBMIE, while KM had less effect and SC no such SBMIE protecting effects.
TOTAL FISH OIL REPLACEMENT BY LINSEED OIL: DEVELOPMENT OF A NEW STRAIN OF CHARR (SALVELINUS. FONTINALIS, S. ALPINUS OR THEIR HYBRIDS) WITH HIGH OMEGA-3 CONTENT

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The aim of the project is to generate the tools to enable the development of new strains of Arctic charr (Salvelinus alpinus), brook trout (Salvelinus fontinalis), or their hybrids, that will advantageously metabolize plant oils (linseed oil) and easily synthesize long-chain, highly-unsaturated fatty acids (LC-HUFA’s). This approach will reduce production costs by substituting fish oils with lower cost vegetable oils. This approach will also increase the sustainability of the industry and support the diversification of the char industry in Eastern Canada. To this end, the variability of three key enzymes (delta-5-desaturase, delta-6-desaturase and elongase) responsible for the synthesis of LC-HUFA’s has been characterized.

Completed feeding trials demonstrated that total replacement of fish oil by linseed oil has no impact on growth performance or on feed intake for fish fed from 10 to 120g (figure 1). Hybrid demonstrated a more heterogeneous growth than Arctic and brook char commercial strain. Total replacement of fish oil by linseed oil modified LC-HUFA content but our results suggest a strong individual variability in LC-HUFA content that could be related to the effects of family and strain. The correlation between the expression of the key enzymes and the concentration of omega-3 LC-HUFA’s was evaluated.

Characterization of key lipogenic enzymes will allow a better understanding of the charr metabolism of DHA and EPA from ALA and the mechanisms involved. This information will allow us to identify strains or families showing a greater potential to deposit higher levels of LC-HUFA fatty acids. The presentation will include results of the feeding trial, fatty acid analysis, key enzyme expression and a description of the potential integration of the results in a breeding program.
A growth trial was conducted to evaluate growth and feed utilization of fish meal (FM) and soybean meal (SBM) diet in different strains of gibel carp (initial body weight 0.8 g). Four strains (CAS 3, D, F2 and Dongting) were tested. The trial lasted for 53 days and was divided into two periods (first 30 days, fed with FM or SBM, respectively; and last 23 days, both fed with FM). The results showed that, during the first period when fed with FM and SBM diet, all strains showed higher feeding rate and growth in FM diet compared to SBM and F2 was higher between strains. Feed efficiencies were higher in FM diet. Feed efficiencies in CAS 3 and D strains were higher compared to other strains when fed with FM diet. When fed with SBM diet, F2 strain showed higher feed efficiency between strains. Intestine length index was higher in F2 strain while not different between FM and SBM diet. Nitrogen retention was higher in FM diet while no difference between strains. Compared to SBM diet, phosphorus retention was higher in CAS 3, D, F2 while similar in Dongting strain when fed with FM diet. Carbon retention was higher in FM diet while no difference between strains.

During the second period, CAS 3, D and F2 but Dongting strain pre-fed with SBM diet showed compensatory growth when fed with FM diet. The compensatory growth was from higher feeding rate while feed efficiency was similar between pre-fed with FM and SBM diet. Specific growth rate in D strain was higher while Dongting strain was lower between strains. CAS 3 showed higher feed efficiency between strains. The differences in phosphorus, nitrogen and carbon retention were not significant.

Transcriptomics analysis showed that, compared to SBM diet, the upregulated genes in the muscle of the fish fed with FM diet were related to muscle development, carbohydrate metabolism and glycolysis. The downregulated genes were related to energy generating and metabolism and the pathway of DNA duplication. The responses in Dongting strain were different from other strains. In co-upregulated clusters, it was related to the pathway of glycolysis and muscle development. In co-downregulated clusters, it was related to the pathway of cytoskeleton.

The results suggested that fish strain selection could be helpful to improve the utilization of plant proteins.
MINIMIZING FISHMEAL USE IN YELLOWTAIL DIETS USING NON-GM SOYBEANS SELECTIVELY BRED FOR AQUAFEEDS
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A study was undertaken to examine the potential of two non-genetically modified (non-GM) soy products as potential fishmeal (FM) replacements. These products provide a sustainable base for aquaculture expansion, have consistent supply and quality and demonstrate superior nutritive value than other soy products, specifically with regard to commodity soybean meal and soy protein concentrate.

Dietary protein (DP) in the reference diet was replaced at 50, 60 and 70% using the NPFI-3010 meal or 40, 50 and 60% with the NPFI-3032 meal. Experimental diets (Table 1) were isonitrogenous (45%), isolipidic (13%), isocaloric (2.7 kcal of digestible energy/kg of diet) and were balanced for lysine (3.2%) and methionine + cysteine (2%). Taurine was supplemented to achieve a level of 1.5% total inclusion across diets. In addition, a commercial control diet containing 50% DP and 14% lipid was included for a total of eight test diets.

Juvenile yellowtails (Seriola lalandi) were obtained from eggs spawned by captive broodstock at Hubbs-SeaWorld Research Institute. Twenty, 4 g fish were stocked into 24 round 330L tanks arranged as semi-closed recirculating system with each diet randomly assigned to three replicate tanks. At the end of the 10-week feeding trial, growth (Fig 1), survival, feed conversion ratio, protein efficiency ratio as well as carcass proximate composition were compared between dietary treatments.

Results indicate that juvenile yellowtail fed diets in which the 3032 meal provided 50 and 60% of DP grew significantly better than those fed all other diets including the FM-based diet and the commercial control. Fish fed with the 3010 meal providing 50% of DP and those fed with the 3032 meal providing 40% of DP had similar growth performance and this was slightly superior as that attained by fish fed the FM reference diet. Only minor differences were found between any of the 8 diets in terms of intestinal histology. Fold height in the mid intestine was significantly higher for fish fed the diet with the 3032 meal providing 50% of DP and fold height in the distal intestine was also significantly higher for the fish fed the commercial diet. Taken together these data indicate that the improved non-GM soybean meals support better performance of juvenile yellowtail than fish fed the FM reference diet.
EFFECTS OF IN VITRO DIGESTION OF RAPESEED MEAL, SOYBEAN MEAL, MACROPHYTE, AND MARINE ALGA WITH CELLULASE, HEMICELLULASE, MULTI-ENZYME MIX, AND THREE SPECIES OF WHITE-ROT FUNGI EVALUATED BASED ON IN VIVO DIGESTIBILITIES OF PROTEIN, FIBER, ORGANIC MATTER, PHOSPHORUS, AND TRACE MINERALS IN RAINBOW TROUT

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We studied enzymatic and microbial methods to improve digestibility of low-cost feed ingredients, including rapeseed meal (Rsm), soybean meal (Sbm), macrophyte Egeria densa (Ege), and marine alga Gelidium crinale (Gel). For enzymatic digestion (Expt.1), we incubated above-mentioned test ingredients with either cellulase, hemicellulase, or multi-enzyme mix for 3 weeks at 35°C in hermetically sealed anaerobic containers. The ingredients were pre-treated with NaOH to facilitate enzymatic digestion, and were added with lacto-fermented rice paste to adjust the dough pH. For microbial digestion (Expt.2), test ingredients were inoculated with either of the following species of white-rot fungi: Trametes sp., Lentinus edodes, Pleurotus sp., and were incubated for 6 weeks at 28-38°C under semi-anaerobic conditions. Test ingredients digested in vitro as above were mixed with a casein-gluten based diet at a 3:7 ratio to make test diets, and evaluated by an in vivo digestibility trial using rainbow trout. Test ingredients, test diets and fecal samples were analyzed for organic matter (OM), crude protein (CP), and selected minerals (P, Ca, Mg, Fe, Zn, Mn, Cu). Test ingredients digested in vitro were also analyzed for neutral detergent fiber (NDF). In vivo apparent digestibilities of these nutrients were estimated based on the ratio of each nutrient and an inert marker (Y) in the diets and feces.

[Expt.1] Relative to non-processed ingredients (control); Cellulase, hemicellulase, and multi-enzyme mix decreased NDF content of Rsm and Sbm. Hemicellulase and multi-enzyme mix decreased NDF of Ege and Gel. Cellulase decreased NDF of Rem from 48% to 26%. Cellulase, hemicellulase, and multi-enzyme mix increased CP digestibility of Rsm. Multi-enzyme mix increased CP digestibility of Sbm from 66% to 87%. Cellulase, hemicellulase, and multi-enzyme mix increased DM and P digestibilities of Rsm. Hemicellulase increased DM digestibility of Rsm from 31% to 50%. Multi-enzyme mix increased P digestibility of Rsm from 46% to 69%. Multi-enzyme mix decreased fecal mineral content (Zn, Fe, Mn, Cu, Ca, Mg) of fish fed the Gel diet, where Zn content decreased the most from 7.7ppm to 0.98ppm. [Expt.2] Relative to non-processed ingredients (control); Trametes sp. and L.edodes decreased NDF content of Rem and Sbm. Trametes sp. and L.edodes and Pleurotus sp. decreased NDF of Gel. Trametes sp. decreased NDF of Rsm from 35% to 25%, and NDF of Gel from 62% to 20%. L.edodes increased P digestibility of Rem. Trametes sp. increased P digestibility of Rem and Gel, respectively, from 64% to 95% and from 72% to 95%. L.edodes decreased fecal Zn content of fish fed the Rem diet from 6.5ppm to 3.4ppm. These results suggest that both enzymatic and microbial methods increase digestibilities of fibers, protein, and phosphorus in low-cost plant ingredients.
Growth-hormone transgenic salmon (TG) grow more rapidly than their non-transgenic counterparts. Results of previous studies showed that TG salmon achieved the same target weight earlier and consumed 25% less feed, being more efficient in converting dietary protein into growth compared to the non-transgenic (NTG) salmon. The aim of this study was to determine whether TG and NTG diploid (DIP) and triploid (TRIP) Atlantic salmon could efficiently utilize a diet with a higher proportion of plant protein to sustain their needs for improved growth. To evaluate growth and feed efficiency, triplicate tanks of full-sibling, size-matched TG/DIP, TG/TRIP, NTG/DIP and NTG/TRIP Atlantic salmon reared in fresh water were hand-fed to satiation until achieving a minimum of 400% growth. Two iso-nitrogenous (50% crude protein) and iso-caloric diets (D1 and D2) were formulated, with D1 containing 36% dietary protein from plants (PP) and 64% fish meal (FM), and D2 containing 68% dietary protein from plants and 32% from FM. In parallel a digestibility study was conducted using size-matched TG/DIP, TG/TRIP, NTG/DIP and NTG/TRIP fish offered chromic oxide-labeled D1 and D2. The most recent results of this comparative study will be presented including assessment of growth rate, feed digestibility, feed utilization, and whole body composition.
PLANT PROTEINS IN FISH FEED – EFFECTS ON THE REGULATION OF CHOLESTEROL AND BILE ACID HOMEOSTASIS

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Replacement of marine ingredients with plant protein sources in feeds for farmed carnivorous fish may affect lipid and sterol metabolic pathways. Reduced feed efficiency and fish growth as well as compromised gut integrity are often seen in parallel to alterations in sterol metabolism. The long-term effects of these syndromes on fish health and welfare are currently unknown. In several recent feeding trials using Atlantic salmon, we have investigated the effects of dietary inclusion of plant protein sources (soybean, lupin, peas), purified plant antinutrients (saponins, phytosterols) and dietary supplementation with cholesterol and bile acids. Our main aim was to gain increased knowledge on the role of cholesterol and bile acids in fish and to understand how alterations in sterol metabolism may affect fish health in a situation of rapidly increasing use of plant ingredients in aquafeeds. A number of analytical tools have been utilized, spanning from classic measurements of fish growth performance and nutrient utilization, organ structure and function as investigated by histomorphological and biochemical analyses, as well as multiple transcriptome and metabolite profiling of different gut regions, liver and blood. The results clearly show that certain plant ingredients may disrupt sterol homeostasis and compromise essential gut functions such as lipid absorption. The transcriptional and biochemical signatures of gut and liver have revealed tightly regulated digestive functions that can rapidly be modulated by feed and feed additives. For example, plant and plant antinutrient inclusion typically resulted in body drain of bile acids and taurine, and the fish seemed to respond by increasing the hepatic capacity for cholesterol and bile acid synthesis. On the other hand, dietary cholesterol supplementation resulted in a typical atherogenic blood plasma profile similar to that of human patients with hypercholesterolemia. The fish seemed to respond to the dietary load of cholesterol by promoting induction of cholesterol and plant sterol efflux in the intestine, suppressing sterol uptake, and decreasing the hepatic cholesterol biosynthesis, whereas hepatic conversion from cholesterol to bile acids increased markedly. The studies have expanded our understanding of sterol metabolism and turnover that adds to the existing, rather sparse, knowledge of these processes in fish.
EVALUATING THE NUTRITIVE VALUE OF CORN STEEP LIQUOR TO YOUNG RAINBOW TROUT
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Wet milling of corn produces large amount of low value condensed steep water, also known as “corn steep liquor”. This product is a complex mixture of amino acids, carbohydrates, lactic acid, myo-inositol, and other organic compounds solubilised from corn or derived from bacterial fermentation. On a dry matter basis, corn steep liquor contains about 45% crude protein (CP), 2% lipids, 35% carbohydrates (nitrogen-free extract) and 16% ash.

Two feeding trials were carried out to examine the nutritive value of corn steep water to young rainbow trout (Oncorhynchus mykiss). Corn steep liquor samples were obtained from a state-of-the-art corn wet milling plant (Casco, London, ON, Canada). A first trial (Trial #1) examined the effect of increasing levels of corn steep liquor (0, 5, 10, 20% of a dry matter (DM) basis) incorporated at the expense of corn gluten meal (CGM) and wheat middlings in a commercial-type rainbow trout starter feed formulation (45% CP, 23% lipids, 21% herring fish meal) on growth, feed efficiency (gain: feed), and body composition of rainbow trout. The second trial also examined replacing CGM and wheat middlings by corn steep liquor but compared three diets: Diet 1 containing no corn steep liquor (control), Diet 2 with 10% standard corn liquor and Diet 3 with 10% corn steep liquor produced with a proprietary enzyme treatment. In both trials, each experimental diet was randomly allocated to three groups of fifteen rainbow trout fry (initial weight = 1 g/fish and 5 g/fish, for Trial #1 and Trial #2, respectively) reared at 15ºC for 16 weeks (Trial #1) or 12 weeks (Trial #2). The fish were carefully hand-fed three times daily to near-satiety in both trials.

The fish accepted all the experimental diets very well. In both trials, growth and feed efficiency observed compared favourably with what was observed in the past with this strain of rainbow trout (ARST) at this body weight range. The fish multiplied their body weight (and consequently their protein and gross energy contents) several fold. In Trial #1, weight gain and feed efficiency were not significantly affected by corn steep liquor incorporation. However, growth rate (expressed as thermal-unit growth coefficient) tended to decrease in a linear fashion with increasing steep liquor incorporation and the fish fed the diet with 20% corn steep liquor showed marginally less good performance. Results from the second trial supported the finding of the first feeding trial since incorporation of 10% corn steep liquor (on a DM basis) did not significantly affect growth and feed efficiency of the fish. No significant differences were noted in terms of the nutritional value of the two corn steep liquors (standard or enzyme-treated). The chemical composition of the fish fed the practical diets was not appear to be affected by incorporation of 10% corn steep liquor.

The results from the two feeding trials suggest that corn steep liquor is a relatively effective digestible protein and digestible energy source for high quality salmonid fish feeds. Its nutritive value appears comparable to that of CGM. More work is required to closely examine the nutritive value and functional properties of this ingredient.
The heterotrophic microalga (Schizochytrium sp.) product SP1 (Alltech Inc) is a novel oil and DHA rich feed ingredient. We tested SP1 in a recent experiment with Atlantic salmon smolts and currently present selected and commercially relevant results with respect to fish performance, fish health and fish-fillet product quality aspects. Four experimental extruded diets were produced containing increasing levels of SP1: 0% (0_SP1), 1% (1_SP1), 6% (6_SP1) and 15% (15_SP1) and decreasing levels of supplemental fish oil, down to 0% in diet 15_SP1. The experimental feeds were fed to triplicate salmon populations of 213 g initial body weight for a period of 12 weeks. The oxidative stability of the SP1 long chain n-3 polyunsaturated fatty acids (LC n-3 PUFA), especially that of DHA, during extrusion was very good. In the end of the feeding trial fish in all treatments more than tripled their initial body weight. SP1 had a positive effect in feed intake and at 1% incorporation level had a significant growth promoting effect. Plasma leptin levels adversely correlated with growth rate rather than feed intake rate. Average FCR values ranged between 0.78 and 0.88 but the differences were not statistically significant. Individual FCR values across all feeding treatments varied as a function of feed intake, growth rate and dietary energy digestibility. Faeces analyses revealed equally high apparent digestibility coefficient (ADC) of protein in all diets. ADC of lipid and energy was highest in diets 0_1SP1 and 1_SP1, but significantly lower in 6_SP1 and 15_SP1. Nevertheless ADC of DHA was higher in the SP1 rich diets, compared to those with only fish oil or combination of fish oil and SP1. Likewise, the levels of DHA, total LC n-3 PUFA in the salmon fillets were higher in the SP1 supplemented diets compared to those fed the control diet containing fish oil as sole LC n-3 PUFA source. Fillet quality was good in all treatments in terms of gaping, texture and liquid losses during thawing despite the high rapeseed oil levels present in 15_SP1 treatment. Fish liver size was unaffected and dress out percentage was significantly improved in the 15_SP1 treatment. Plasma creatine kinase levels were low in all treatments. Histological fluorescence staining and immunofluorescence analysis of salmon intestines (midgut: base of intestine and villi) revealed significant effects in slime, goblet cell production and iNOS activity with increasing levels of dietary SP1 supplementation. Nevertheless, microarray analysis did not reveal any signs of toxicity, stress, inflammation or any other significant effects from SP1 supplementation in diets for A. salmon. Mucin gene expression was not affected by the dietary treatments or sex. It seems thus feasible to on-grow Atlantic salmon and produce LC n-3 HUFA rich salmon fillets of high technical quality by partial or total substitution of supplementary fish oil in the diet by SP1.
FEED FIT FOR A KING: REPLACEMENT OF FISH MEAL WITH A MIXTURE OF REFINED PLANT PROTEINS IN THE DIET OF JUVENILE YELLOWTAIL KINGFISH (SERIOLA LALANDI)
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From the Seriola spp. or Amberjack s, the culture of yellowtail kingfish (YTK, Seriola lalandi) is gaining importance. However to fulfill their growth potential YTK seems to require high levels of marine or animal ingredients in their feed. Experiments in which fishmeal was replaced with single plant ingredients have not been very promising, although refined plant ingredients sometimes performed better. We therefore conducted an experiment in which fishmeal was replaced by a mixture of refined plant protein ingredients (corn- and wheat gluten and soya protein concentrate), with addition of amino acids and minerals. The control diet contained 60% fishmeal (D60). Fishmeal was replaced in 3 steps (D45, D30 and D15).

A 30d growth trial was conducted with triplicate tanks with 20 YTK of 43g assigned to each diet. Temperature was 21.2°C, Salinity 19.3 PSU and pH 7.7. The YTK were fed twice daily to apparent satiety. Voluntary feed intake and growth, and body composition at the start and end of the trial were determined. Further the oxygen consumption of the YTK was determined and faeces were collected by sedimentation for determination of digestibilities. After the growth trial the remaining YTK were fed for an additional 2 weeks, during which faeces were collected by stripping.

Growth and feed utilization of the YTK fed D60 was excellent with a thermal growth coefficient (TGC) of 0.42 and a feed:gain ratio (FGR) of 0.68. Fishmeal replacement resulted in a strong decrease in growth, to a TGC of 0.20 (FGR 0.87) for D15. This was the result of a strong decline in feed intake with decreasing fishmeal levels in the feed, already from the first day. The speed of the reaction, and the feed intake behaviour (spitting) suggested that physical feed properties (pellet hardness, water stability) might be involved in this feed intake decline.

The digestibility of energy and protein were high, 86-88% and 93-95% via sedimentation, and 78-85% and 86-90% via stripping. Digestibility of energy and protein increased with fishmeal replacement indicating that the used plant proteins, including corn gluten, were well digested. The utilization of digestible energy and protein were not influenced by fishmeal replacement, with marginal efficiencies for energy retention $k_E$ 0.7-0.77 and for protein retention $k_N$ 0.6-0.64.

Fishmeal replacement did influence the digestibility and retention efficiency of minerals. A strong decrease in digestibility, to highly negative values (-33% for Ca, -186% for Mg and -282% for Na), with little or no effects on mineral retention, indicated a substantial uptake of minerals from the water. This link between nutrition and osmoregulation was confirmed by a highly significant decrease in the dry matter content in the hindgut, from 144 g/kg in D60 to 96 g/kg in D15. We conclude that in the longer term also with equal feed intake effective fishmeal replacement might require reconsideration of mineral, and possibly other micronutrients, supplementation.
THE SHORT AND LONG-TERM EFFECTS OF PLANT PROTEIN ON FEED INTAKE REGULATION, GROWTH AND GH/IGF-I AXIS RESPONSE AND GENE EXPRESSION OF JAPANESE SEABASS (LATEOLABRAX JAPONICUS)

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A short (3-week) and a long-term (16-week) growth trial were conducted to evaluate the effects of replacing fish meal with plant protein blend (PPB) on the feed intake, growth performance and GH/IGF-I axis of Japanese seabass (Lateolabrax japonicus). Two experimental diets were prepared in Experiment 1. In control diet (FM), low temperature steam dried fish meal was used as sole protein source, and the fish meal was totally replaced by PPB (Soybean meal/wheat gluten meal=1:1.67) in the other diet (PPB100). In Experiment 2, five diets were designed as using PPB to replace 0, 25, 50, 75 and 100% of FM, with names of FM, PPB25, PPB50, PPB75 and PPB100, respectively. All the diets are isonitrogenous (CP, 40%) and isoenergetic (GE, 20KJ/g), and the PPB diets were supplemented with DL-methionine, Lysine-HCl, L-Threonine and Ca(H₂PO₄)₂ to balance the limiting amino acid and available phosphorus. The results showed that: 1) Feeding and growth were significantly inhibited when FM was totally replaced by PPB for 3-week. In which, plasma NPY and hepatic GH expression increased, while expression of GHR1, GHR2 and IGF-I gene were reduced significantly. Besides, we found 2057 genes in hypothalamus and 1126 genes in stomach differently expressed by analysis of transcriptomics for two groups, in which 22 genes directly regulate the feed intake of fish and most of them related with the mTOR signal pathway. 2) Growth was decreased when 50% or more FM was replaced by PPB. However, fish fed PPB25 diet showed highest growth performance; 3) Feeding and growth performance of Japanese seabass showed the inhibiting duration (0-12w) and adaptation/compensation duration (12-16w). Hepatic GH/IGF-I genes of PPB75 and PPB100 groups were accordance with the growth performance.

This study was conducted to evaluate the effects of rainbow trout silage oil on the immune function, growth performance and foot muscle fatty acid profile and proximate composition of South African abalone Haliotis midae. Rainbow trout silage oil was prepared from farmed rainbow trout processing waste, included at 25 g·kg⁻¹ in a formulated diet and compared to a control diet in a 153 day growth trial. It was found that rainbow trout silage oil addition significantly improved abalone cellular immune function by increasing hemocyte phagocytic activity from 48.8% in the control diet to 65.2% in the silage oil diet. However, production performance was negatively impacted: specific growth rate (SGR) and final animal weight were decreased 17% and 5.6% respectively, and feed conversion ratio (FCR) increased by 25.1%. Foot muscle fatty acid profile and proximate composition were unaffected. It is concluded that dietary fish silage oil inclusion can improve cellular immune function in Haliotis midae, but that the optimal inclusion level should be determined in order to negate the negative effects on production efficiency.
DIETARY PROTEASE SUPPLEMENTATION IN ATLANTIC SALMON

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The increasing use of alternative protein sources in aquaculture feeds has led to more relevance and application of dietary enzymes (phytase, carbohydrase and protease) in the aquaculture industry. Optimization of aquaculture diets using sustainable vegetable protein sources requires the use of phytase which hydrolyses plant phytic phosphorus making it available to the fish. While the benefit of utilizing carbohydrases such as xylanase in animal production has been recognized since many years, their application in aquaculture has so far focused in warm water fish such as tilapia mainly due to the adequacy of the raw materials as enzyme substrates. Dietary protease supplementation has developed in land-based animal production during the last decade and with regard to aquaculture, the first focus has been towards warm water fish and the economic value of reducing fish meal inclusion. However, the continuous reduction of fish-meal levels and the on-going search for alternative protein sources for cold water aquaculture application has raised interest in evaluating such enzymes in salmon diets despite key challenges such as lower temperature and different gastrointestinal conditions in comparison to warm-water fish as well as other salmonid fish.

The aim of this study was to evaluate the efficiency of using a protease product as feed supplement at graded inclusion levels (0, 400, 600 ppm of a protease (RONOZYME® ProAct) in the diet for Atlantic salmon fed either high plant protein or high poultry by-product diets, and by formulating diets with slightly sub-optimal dietary protein. Overall results were evaluated by growth, feed intake, feed conversion, nutrient digestibility (protein, lipid, energy, amino acids, minerals) and protein utilization.

Protease efficiency was evaluated in 215-g salmon fed plant-protein (PP) and poultry by-product (BP) based diets for 12 weeks. Fish from all dietary groups showed average specific growth rate (SGR) of 1.17-1.33 after 12-week of experimental feeding. Fish fed PP diets indicated a general negative trend for feed intake and growth, and little impacts on nutrient digestibility, while FCR was slightly better than in fish fed the BP diets (0.82 versus 0.80). In fish fed poultry by-product, dietary protease at 400 ppm significantly improved feed intake by 9 % and growth by 9.4 %. In fish fed 600 ppm protease, a similar trend was only observed in the last 4 weeks of feeding, leading to improved growth in the final 6 weeks. At the end of the trial, improved protein digestibility was observed in fish fed the BP diet with 600 ppm protease.

The present study demonstrate the potential of dietary enzymes such as protease, in Atlantic salmon aquaculture feeds.
MINING FOR SPECIES AND TISSUE SPECIFIC PEPTIDE BIOMARKERS FOR THE QUANTITATIVE DIFFERENTIATION OF PROCESSED ANIMAL PROTEIN IN AQUAFEEDS

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The worldwide growth of aquaculture of carnivorous fish species has lead to the development of more sustainable fish feeds in which traditional marine feed constituents such as fish oil and meal are being replaced with terrestrial ingredients. One of the largest sustainable low cost sources of readily available high quality protein is processed terrestrial animal protein (PAP) derived from the rendering industry. In the European Union (EU) however the use of PAP in animal feed was banned after the outbreak of Transmissible Spongiform Encephalopathy (TSE). Only recently, following a TSE risk assessment performed by the European Food Safety Authorities (EFSA), did the EU reauthorize non-ruminant PAP for use in aquafeeds. This introduces new challenges for risk assessment of both feed and food safety. In particular efficient control tools for the detection of ruminant proteins in compound feedingstuffs are required and in addition, tissue-specific PAP identification is desirable to truly identify and differentiate the different PAP groups currently on the market. In this context, peptidomics is thought a promising reliable monitoring tool, particularly when food matrices are very complex or have been processed at high temperatures. We have devised a peptidomics workflow for the detection of robust, reliable and sensitive peptide markers that will permit the species and tissue specific detection and quantification of bovine material in PAP mixtures destined for use in aquafeeds.
COMPARISON OF THE HUFA BIOSYNTHESIS CAPACITY BETWEEN THE EURASIAN PERCH (PERCA FLUVIATILIS) AND THE RAINBOW TROUT (ONCORHYNCHUS MYKISS) IN A CONTEXT OF FISH OIL REPLACEMENT BY PLANT OIL IN THE DIET

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Most fish species exhibit a low capacity of Highly Unsaturated Fatty Acids (HUFA) synthesis representing a bottleneck for the dietary substitution of fish oil by plant oil. Based on a previous study suggesting a relative high ability of Eurasian perch (\textit{Perca fluviatilis}) to synthesise HUFA, we assessed the regulation of this pathway through a long-term nutritional challenge with two formulated diets, a fish oil-based diet (FO) and a plant oil-based diet (VO). To strengthen this study, the potential of HUFA biosynthesis of Eurasian perch was compared to the one of rainbow trout (\textit{Oncorhynchus mykiss}), another fish species considered as having relative high abilities in HUFA synthesis. Eurasian perch juveniles of 6.9 g were reared at 23°C and 19°C for 16 weeks while rainbow trout juveniles of 8 g were maintained at 19°C and 15°C for 10 weeks.

Comparison of growth showed that specific growth rate (SGR) was not affected by fish oil substitution for both fish species when they were maintained at their optimal temperature (15°C for rainbow trout and 23°C for Eurasian perch). In contrast, both fish species exhibited significantly lower SGR at 19°C when fish oil was replaced by plant oil in the diet. The expression of fatty acid desaturase 2 (\textit{FADS2}) and fatty acid elongase 5 (\textit{ELOVL5}) genes that encode two key enzymes involved in desaturation and elongation steps of HUFA synthesis were investigated in two major metabolic organs, liver and intestine. Surprisingly, the dietary replacement of FO by VO did not affect \textit{fads2} and \textit{elovl5} gene expressions in both fish species. On the other hand, \textit{fads2} gene expression was significantly stimulated in both dietary treatments when fish were reared at the lowest temperature (15°C for rainbow trout and 19°C for Eurasian perch). Ongoing research will determine if \textit{fads2} gene expression profiles are directly associated with \textit{FADS2} activities. Additionally to the gene expressions and FADS2 activities in liver and intestine, the characterization of fatty acids composition in muscle and perivisceral fat will contribute to evaluate HUFA synthesis potential for both fish species when FO is replaced by VO.
NEW KNOWLEDGE AND COMPARATIVE ASPECTS OF BIOCHEMICAL AND PHYSIOLOGICAL CHARACTERISTICS ALONG THE DIGESTIVE TRACT OF ATLANTIC SALMON (SALMO SALAR)
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Research in the Gut and Health group of the Aquaculture Protein Centre over its ten years life time (2003-2012) has filled several knowledge gaps regarding physiology of the digestive tract of salmonids. The presentation will cover hitherto unpublished information collected from several of APC’s studies. Most of these studies had other foci than aspects of basic mechanisms and were mainly conducted to find and characterize new protein sources and their components for their production potential as well as their possible health effects on the gut and associated organs. However, the collected information evaluated together strengthens our general understanding also of basic functions and dynamics of the gastrointestinal tract and its role as, possibly, the most important organ system of an animal organism. General aspects as well as specific effects of diet ingredients and composition on function and conditions of the stomach, pyloric caeca and distal intestine, and associated processes in the liver will be presented and discussed. The following key aspects are representative for the content of the presentation: the whole length of the gastrointestinal tract from the stomach to the anus, ontogeny from hatching, adult fish, chyme pH, pH optima and characteristics of digestive enzymes (pepsin, trypsin, chymotrypsin, carboxypeptidase A and B, elastase, leucine aminopeptidase and lipase), bile salt concentration and profile, nutrient transport, effects of environmental salinity, ingredients and nutrient level of the diet.
EMERGING TOOLS FOR IN OVO DELIVERY OF NUTRIENTS AND REGULATORY PEPTIDES IN FISH: EFFECTS ON LARVAL GROWTH, FEED INTAKE AND PROTEIN METABOLISM

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There is an emerging trend to study early nutritional stimuli that may act as triggers of metabolic pathways in fish development. Currently, the opportunities to exert a nutritional stimulus during these early stages of high metabolic plasticity are restricted to maternal transfer and the onset of exogenous feeding. The present work comprises a series of trials relying on the use of microinjection and sonophoresis as tools to deliver nutrients (amino acids, vitamins) and selected regulatory peptides (ghrelin and follistatin) into zebrafish and gilthead seabream eggs. Batches of zebrafish eggs were microinjected with ghrelin (14 pmol/egg) and follistatin (0.58 pmol/egg) at 30% epiboly stage. Control (saline injected), GHR and FOL treatments were reared and fed under identical and standardized conditions until day 25. The efficacy of in ovo supplements was assessed in terms of growth, feed intake and protein accretion pathways, by means of zootechnical data, radiolabeled tracer studies, muscle histology and gene expression. At day 25 and in comparison to the CTRL treatment, FOL-injected larvae showed a significant enhancement of growth (+33% dry weight; +11% standard length) and increased muscle fiber diameter and density. Molecular data confirmed an up-regulation of GH1, IGF-1α, IGF-IRα and MYO-D1α genes in the FOL fish. No beneficial effects on growth were associated to ghrelin supplementation of eggs. Both FOL and GHR treatments showed a higher feed intake than CTRL, but failed to enhance protein accretion. Given the practical limitations of microinjection in delivering nutrients to a large number of fish eggs, subsequent studies targeted the use of low-frequency ultrasounds (sonophoresis) as a practical and non-stressful approach to enhance the transport of compounds across egg chorion in gilthead seabream. Preliminary results confirm that sonophoresis has the potential to increase the incorporation of selected nutrients (e.g. amino acids) into seabream eggs. Free aspartate had an increase of 400%, while free histidine, serine and glycine increased 20-30% when compared with seabream eggs without supplementation. With these tools for an efficient delivery of supplemental nutrients to fish eggs, we can contribute towards the generation of new knowledge, essential for tailoring the growth potential, skeletal development and protein accretion pathways in fish larvae.
EICOSANOIDS, STRESS RESPONSE AND FATTY ACID METABOLISM AND IN ATLANTIC SALMON, AS AFFECTED BY DIETARY ARA/EPA RATIO AND WATER TEMPERATURE

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Long chain polyunsaturated fatty acids are biologically active, with arachidonic acid (20:4n-6, ARA) and eicosapentaenoic acid (20:5n-3, EPA) being the two major omega-6 and omega-3 counterparts, respectively. The enzyme cyclooxygenase (COX-2) and 5-Lyposxygenase (ALOX-5) bioconvert ARA into the commonly termed “inflammatory eicosanoids”, including the 2-series prostaglandins (PG) and 4-series leukotrienes (LT), respectively. These hormone-like compounds play important roles as mediators and regulators of inflammation and immune functions. EPA, on the other hand, can decrease the production of these “inflammatory eicosanoids” directly, by replacing and reducing ARA as substrate, and indirectly, by altering the expression of COX-2, ALOX-5. Additionally, the eicosanoids derived from EPA (namely 3-series PG and 5-series LT) are commonly termed “anti-inflammatory eicosanoids”, as their activity is lower than those derived from ARA. The currently increasing utilisation of vegetable oils, used to substitute fish oils, in the formulation of salmon feed is responsible for increased dietary availability of omega-6/omega-3 ratio. Thus, concerns relative to the potential effects of the resultant modified eicosanoid cascades, which could ultimately affect aspects like stress response should be addressed. The modified dietary omega-6/omega-3 ratio, could enhance the incidence and severity of inflammatory responses to stressors, such as handling and sub-optimal water temperature, which are issues commonly encountered in the salmon industry. The objective of this study was to assess the effects of different dietary ARA/EPA ratios in Atlantic salmon at optimal (10 °C) and high water temperatures (19 °C). Three experimental diets were formulated varying only in the added lipid sources. Different vegetable oils and concentrated oils were blended so that the dietary fatty acid composition was identical across all diets including total SFA, total MUFA, 18:2n-6, 18:3n-3 and 22:6n-3 (DHA), with the only exception being ARA and EPA. Total content of ARA + EPA was fixed at 10 % (% total fatty acid) in all three experimental diets, but their ratio varied from D1 (ARA 7%, EPA 3%), to D2 (ARA 4%, EPA 6%), and finally D3 (ARA 1%, EPA 9%). Triplicate groups of juvenile Atlantic salmon (60/tank) were fed to apparent satiety at 10 °C in a freshwater RAS system. After 14 weeks, each group was split in two sub-groups: 20 fish were maintained in the same system, and another 20 fish were transferred into a separate system where temperature was gradually increased (1.5 °C/day) up to 19 °C. Fish were then maintained in the two systems for an additional 7 weeks. All growth performances, tissue fatty acid compositions, in vivo fatty acid metabolism, eicosanoids, and expression of key genes involved in ARA and EPA metabolism and stress indicators were evaluated. The results of this study will be presented and discussed with respect water temperature in the context of the potential development of functional feed for Atlantic salmon at high water temperature.
EFFECTS OF SOY PROTEIN CONCENTRATE AND TAURINE SUPPLEMENT ON INTERMEDIARY METABOLISM AND ANTIOXIDANT STATUS OF TOTOABA MACDONALDI JUVENILES
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Introduction. Soy protein is the most used ingredient by aquatic food industry to replace fish meal (FM) in diets. However, carnivorous fish fed soy protein based diets may require taurine supplementation to improve growth performance and food efficiency (1). The effect of dietary incorporation of soy protein concentrate (SPC) and the concomitant supplementation with taurine on hepatic intermediary metabolism and antioxidant system defense of Totoaba macdonaldi juveniles was assessed.

Methodology. Four isoproteic and isolipidic diets were formulated containing either 30 or 60% of SPC (SP30 and SP60), supplemented or not with 1% of taurine (SP30T and SP60T). A FM based diet, without SPC and taurine supplementation, was used as a control (CD). Triplicate groups of 32 organisms (7.5g) were fed these diets over 45 days. At the end, fish were fasted 6h, and 4 livers per tank were excised, immediately frozen and stored at −80°C. The activities of enzymes of intermediary metabolism, antioxidant system defense and lipid peroxidation (LPO) levels were measured according to the methodology described by Morales et al. (2004) (2). Statistical analysis of one- and two-way ANOVA were performed, where values of P<0.05 was considered significant.

Results and discussion. Dietary FM replacement by SPC reduced the activity of amino acids catabolism and gluconeogenesis key enzymes, whereas the concomitant dietary supplementation with taurine significantly increased its activity. Regarding lipogenesis, glucose-6-phosphate dehydrogenase (G6PD) was significantly increased by dietary taurine supplementation, but did not affect activity of malic enzyme and fatty acid synthetase, indicating that the NADPH generated by G6PD might be destined to different metabolic pathway. In this context, studies had been demonstrating that the variations in the intermediary metabolism of fish have been related to the imbalances in essential amino acid profile as well as the imbalances in the essential to non-essential amino acid ratio (3). Otherwise, the incorporation of the highest level of SPC significantly increased hepatic lipid peroxidation and the activity of superoxide dismutase. Concomitant taurine supplementation restored the activity of amino acid catabolic and gluconeogenic enzymes and hexokinase to levels similar of those of the control diet. Taurine supplementation also led to a significant increase of G6PD and catalase (CAT) activity, as well as to a significant reduction of liver LPO. Taurine is recognized to prevent LPO by increasing activities of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and CAT. Also, taurine may improve the antioxidant system defense via inhibiting the LPO process and through osmoregulation and membrane stabilization (4). Otherwise, in non-supplemented taurine diets, the increase of LPO in totoaba liver was correlated with the lowest activities in CAT and G6PDH. These results suggest that taurine may play an important metabolic modulation action on totoaba fed SPC based diets, contributing to the enhancement of the overall metabolism and to the reduction of liver oxidative damage.

THE IMPACT OF HIGH WATER TEMPERATURE ON FATTY ACID DIGESTIBILITY AND METABOLISM OF EUROPEAN SEA BASS (DICENTRARCHUS LABRAX) FED VEGETABLE-OIL BASED DIETS
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Studies have shown that marine fish species can be cultured to marketable size using diets with up to 60% replacement of added fish oils (FO) by vegetable oils (VO). Commercial aquafeeds having high levels of vegetable oil have been used throughout the production cycle. High water temperatures of 28-30°C are commonly encountered in the Mediterranean basin in the warmer months. In addition, global warming is expected to pose increased physiological challenges on lipid metabolism of farmed fish. The objectives of the present study were to evaluate the interactive effects of dietary vegetable oils and water temperature on fatty acid digestibility and in vivo lipid metabolism in European sea bass.

Over a period of 120 days, three practical diets with either added fish oil (FO) or a blend of canola/cotton seed oil (w:w) at 30% (CACS30) or 60% (CACS60) replacement of the added FO were formulated and fed to triplicate groups of sea bass maintained at water temperatures of 24°C (optimum) or 30°C (high) in a block factorial design. Twenty-five fish (initial weight of 32.2 g) were randomly allocated to each of the 18 tanks within respective temperature block. Specific growth rate and thermal-unit growth coefficient of seabass showed no significant difference among the treatments. Deposition of 18:2n-6 and 18:3n-3 were significantly higher in fish kept at 30°C than those kept at 24°C. The fish fed CACCS60 diet had highest 18:2n-6 compared to the fish fed FO diet. In particular, eicosapentaenoic acid in the whole body was more evident at 24°C than at 30°C but this was not significant. In terms of whole body docosahexaenoic acid, there were no significant effects of temperature and dietary treatments. The highest saturated fatty acid content was observed in fish kept at 24°C. There were no significant main effects of dietary VO or water temperature on unsaturated fatty acids. Irrespective of water temperature, there were significant effects of diet and interaction between diet and temperature on lipid digestibility, which increased with VO substitution levels. The absorption of individual dietary fatty acids decreased with chain length and increased with the degree of unsaturation, though there was no significant effect of temperature.

In conclusion, the results showed that the elevated temperature had no affect on fatty acid digestibility. The major results of neutral/polar fatty acid fractions and in vivo fatty acid metabolism will be presented and discussed towards providing information on diet formulation suitable for European sea bass in high water temperatures.
DIGESTIBLE ENERGY, GROWTH AND APPETITE REGULATION FOR ATLANTIC SALMON UNDER LOW OXYGEN CONDITIONS
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Oxygen is integrally linked with fish metabolism and growth potential, and its variation poses a widespread limitation to farmed fish growth. Low dissolved oxygen levels often occur in warm, shallow or stagnant water, and can to some extent be seasonally predicted in open farming systems. Such farming systems are subject to environmental change, with widely accepted climate change theory indicating warming global seawater bodies, with concomitant decreases in saturation oxygen levels. It is generally accepted that fish grow less under low oxygen conditions, however little data exists as to which extent feed intake and growth regulation are differentially affected by such conditions, or whether feed formulation plays a large role in regulating this growth depression.

A study was therefore designed to elucidate the combined effects of low oxygen and digestible energy on feed intake and growth for Atlantic salmon at 12°C. Two experimental diets were formulated to two digestible energy contents. Diets were evaluated in a three month growth study with 1.3 kg salmon, at ideal dissolved oxygen (90-110% saturation, 7-9 mg/L) or a simulated daily oxygen profile with a period of low dissolved oxygen (50-80% saturation, 4-7 mg/L). A third treatment was evaluated for each diet, which was subject to ideal oxygen conditions, but pair-fed the food ration from the equivalent low oxygen group.

Salmon exposed to low oxygen had a reduction in growth, feed intake and retention of protein and fat and a poorer FCR, but were not affected by DE content. Low oxygen also had a negative impact on blood gases and osmoregulation, while increasing haemoglobin concentration and haematocrit. The pair feeding groups at optimal oxygen conditions had approximately 50% of the growth decline exhibited by the low oxygen groups. This shows that growth reduction caused by low oxygen cannot be explained feed intake alone, and indicates that growth is down-regulated by factors other than appetite. Endocrine factors controlling growth and appetite regulation will be discussed further.
RESEARCH TOWARDS THE DEVELOPMENT OF NOVEL TRIPLOID ATLANTIC SALMON AQUAFEEDS

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Currently, the European Atlantic salmon industry is considering the implementation of triploidy within the sector. Although research has shown that triploids can grow faster than their diploid siblings, it is the higher incidence of vertebral deformities and occurrence of cataract which has prevented their adoption to date. Recent research has shown that conventional diploid diets may be nutritionally inadequate to support the inherent faster growth of triploids. We present a series of dietary trials conducted in both fresh and saltwater stages of production with the specific aim of reducing spinal deformity and the occurrence of cataract while maximising growth. In both pilot and commercial studies, supplementation of higher dietary histidine inclusion (1.2% vs. 1.7%) during periods of rapid growth and high water temperature in seawater arrested the development of cataracts. Further seawater studies have also examined the effect of different “nutrient packages” on growth of triploid salmon, and by elevating certain dietary components we have been able to enhance triploid growth in seawater by +10% compared to their diploid siblings, whilst simultaneously reducing spinal malformation (50% reduction).

Spinal malformation has remained an inherent culture problem of triploid salmon, where prevalence increases during seawater stages. As such, most research has focused on addressing the problem at sea, however, we have provided evidence that spinal malformation may result mainly from inadequacies of the diet during freshwater. A series of experiments have been conducted feeding elevated levels of dietary phosphorous, and for different durations from first feeding, with results indicating a reduction in radiological spinal deformity with increasing P supplementation. Current research aims to optimise P feeding duration in order to minimise environmental discharge. Studies are also focusing on the molecular mechanisms of spinal development in relation to dietary P, and characterising nutrient digestibility in the triploid gastrointestinal tract. Finally, with the growing emphasis on sustainability of aquafeeds, and subsequent replacement of FM/FO, a series of experiments have examined how triploids respond to low FM/FO diets. Trials will determine the micronutrient requirements of triploids in response to increased VM/VO substitution and subsequent effects on growth and deformity.

Collectively, results of the various trials are discussed in terms of triploid dietary requirements in relation to spinal development and environmental discharge, and work towards the development of specific triploid salmon aquafeeds to facilitate commercial viability of triploidy within Europe.
Gastrointestinal pH is a relevant factor involved in several processes such as solubilisation of proteins and minerals (Ca, P, Fe), enzymatic hydrolysis, and modulation of microbiota. These processes have clear consequences in growth and health. Nevertheless, there is very scarce information on the luminal pH through the digestive tract in fish and only in few species. This review will examine the problems associated to luminal pH analysis and the information currently available to estimate the real enzymatic capacity and the consequent availability of ingested nutrients.

Anatomical and physiological interspecific diversity is a characteristic in teleosts, with gastric and agastric fish having different digestion patterns. In addition, gastric fish may exhibit different acidification strategies either maintaining a low gastric pH between meals or a neutral pH that decreases only after food ingestion. The most common postprandial response found is characterized by a decrease of gastric pH from neutral values followed by a short increase of the intestinal pH being the initial values recovered with the progressive evacuation of the different sections of the digestive tract. Nevertheless, besides the specific characteristics and feeding habits, other important factors may affect the pH along the digestive tract and consequently the digestive capacity. The first one is age; most gastric fish lack of stomach at hatching while acidification and alkalinisation capacity appears progressively during the transition to juvenile. The second one is feeding schedule; food accessibility, meals time and frequency affect feed transit and the postprandial response. The third one is food quality; the dietary ingredients may exhibit variable buffering capacity.

While many studies determined the optima pH for activity of main digestive enzymes, few studies examined the actual working pH during the digestive process. Causes, consequences and regulation of gut pH are still poorly understood and there are many unexplored questions. For instance, the use of acidifiers did not give yet conclusive results. More accurate pH measurements from larvae to adults and more research at molecular, biochemical and behavioural level are needed to have an understandable picture of the complex processes occurring during the feed transit. This knowledge is necessary to define adequate feeding protocols and feed formulations resulting in an improvement of nutrients availability.

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EVIDENCE OF EXTENSIVE CORI CYCLING ACTIVITY IN EUROPEAN SEA BASS (DICENTRARCHUS LABRAX L.) FOLLOWING AN INTRAPERITONEAL GLUCOSE CHALLENGE

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The existence of Cori cycling activity in carnivorous fish is disputed. To determine if Cori cycling is active in European seabass, we applied ¹³C-NMR isotopomer analysis to plasma glucose following intraperitoneal injection of [U-¹³C]glucose. This method resolves intact [U-¹³C]glucose molecules from partially-labeled ¹³C-isotopomers that are formed via Cori cycling (Figure 1). Thus, the fraction of glucose molecules derived directly from the [U-¹³C]glucose load or following passage of ¹³C label through the Cori cycle may be estimated.

Methods: 8 fish (88±7 g) were fasted for 21 days, anesthetized, injected intraperitoneally with 2000 mg kg⁻¹ 50% [U-¹³C]glucose. Basal blood glucose was assayed by glucometer. Fish were allowed to recover for 48h and blood was sampled for analysis by glucometer and by ¹³C-NMR. Contributions of load and recycled glucose to total plasma glucose were calculated from their respective ¹³C-isotopomer enrichments divided by precursor load enrichment.

Results: 48 hours after glucose injection, plasma glucose levels were significantly elevated compared to baseline (60±12 vs. 263±22 mg.dl⁻¹, p < 0.01). The fraction of plasma glucose derived directly from the load was 24.6 ± 1.8% while the fraction representing Cori cycling of the glucose label was 11.9 ± 0.4%. Hence, approximately one-third of the ¹³C-label recovered in blood glucose had undergone Cori cycling.

Conclusions: In fasted European seabass given an intraperitoneal glucose challenge, a significant fraction of the glucose load was found to have been metabolized through the Cori cycle. This indicates that the Cori cycle is active and is a significant contributor to glycemia under these conditions.
NMR SPECTROSCOPY BASED METABOLOMIC ANALYSIS OF TURBOT FED WITH FISH PROTEIN HYDROLYSATE
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A Nuclear Magnetic Resonance (NMR) based metabolic profiling approach was used to profile metabolite changes in turbot female half-sib families fed with fish protein hydrolysate (FPH). Low molecular weight fish hydrolysate was produced by enzymatic (alcalase and flavourzyme) treatment and obtained by ultra-filtration (UF). Five isonitrogenous and isoenergetic experimental diets were formulated and fish meal (FM) was replaced by 0%, 2.7%, 5.4%, 8.1% and 10.8% FPH. The growth trial lasted for 68 days. At the end of the trial, ¹H-NMR spectra identified 34 intracellular metabolites in liver and 33 intracellular metabolites in muscle of turbot, including amino acid, sugars, organic acid ect. Compared to the FM group, cholate, valine, isoleucine, leucine, arginine, aspartate, N,N-dimethylglycine, dimethylamine were significantly increased and Alanine, glutamine were significantly decreased in liver of turbot fed with 10.8% FPH. More dramatic changes were found in muscle of turbot fed with FPH. Histidine was significantly increased and lactate, alanine, proline, glutamate, glutamine, fumarate, tyrosine were significantly decreased in turbot fed with 5.4%, 8.1% and 10.8% FPH. Taurine was significantly increased and cystathionine was significantly decreased in turbot fed with 8.1% and 10.8% FPH.

These results demonstrate that NMR-based metabolomics is applicable for the discovery of metabolic biomarkers which could be used to elucidate the mechanisms of fish meal replacements.
NEAR-INFRARED SPECTROSCOPY AS A TOOL FOR THE NON-INVASIVE ASSESSMENT OF SPINY LOBSTER CONDITION
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Spiny lobsters are the target of Australia’s most valuable wild fishery worth over $400 million. Demand for lobsters is increasing at a time when the fishery is fully exploited, with the only prospect of further production arising from aquaculture development. A non-destructive technique to analyse lobster condition has considerable potential for the established spiny lobster fishery as well as promise for use towards an aquaculture industry. Few techniques are available to accurately assess the nutritional condition of juveniles and adults without killing them. Digestive gland index, an approximation for digestive gland lipid content, is considered the most reliable technique to assess nutritional condition but its measurement requires euthanizing and dissecting lobsters. This practice cannot be applicable to the live lobster market or to assess the condition of important broodstock in aquaculture. Blood protein level has been used as a non-destructive index for condition but its interpretation is complicated by the moult cycle. The blood protein concentration increases during intermoult because of a decrease in blood volume and a corresponding increase in tissue mass, this renders condition assessment of spiny lobsters problematic because of the long intermoult stage.

This pilot study reports on the application of a novel non-invasive technique to assess lobster condition based on near-infrared spectroscopy (NIR) analysis of lobster tails. Preliminary multi-species NIR models were developed to predict abdomen muscle dry matter (AMᵩᵥ), nitrogen (AMᵩ₅) and carbon (AMᵩ₇) content in three commercial spiny lobster species: cultured Eastern spiny lobster juveniles (17–55 g), Sagmariasus verreauxi, cultured Tropical spiny lobster juveniles (104–377 g), Panulirus ornatus, and wild caught Southern spiny lobster juveniles and adults (107–1484 g), Jasus edwardsii. Lobsters (n = 92) were held in laboratory-controlled states of feeding and starvation for up to 42 days to achieve a large range in nutritional status. The NIR spectra were recorded on a Bruker MPA FT-NIR spectrometer using a 8 cm⁻¹ resolution between 12500 and 4000 cm⁻¹. A first derivative of the spectral region of 9400–4250 cm⁻¹ with 25 smoothing points was used for the partial least square regression of AMᵩᵥ and AMᵩ₅ and AMᵩ₇.

The results indicate that abdomen muscle composition correlates significantly ($r^2 = 0.62$-$0.73$) to important indices of nutritional condition (i.e., digestive gland index, digestive gland lipid content, and blood protein). The composition of the abdomen muscle was successfully predicted with a satisfactory degree of accuracy (i.e., average error of prediction for AMᵩᵥ = 1.34%, AMᵩ₅ = 0.22% and AMᵩ₇ = 0.72%) using the separate regression models with a random cross-validation (20 segments). The $r^2$ for these models ranged from 0.77 to 0.85. This innovative research paves the way for the development of single-species condition models using hand-held portable non-invasive devices for use in the lab or in the field. The method developed has a direct application in the management of wild stocks, the live lobster market, and for aquaculture R&D of spiny lobsters, and is potentially transferable to other crustacean species of commercial interest.
A moderate inclusion of protein hydrolysates (PH) in diets for altricial fish larvae has been reported to enhance feed intake, improve maturation of the digestive tract, improve larval quality, improve larval capacity for processing dietary proteins, improve growth and increase survival. However, a high inclusion of PH leads to poor growth and often low survival rates. The poor ability of early larval stages of fish to efficiently digest inert microdiets, when compared to live feed, may be attributed to their limited ability to denature and enzymatically cleave complex proteins. A confounding factor in many studies is that low molecular weight (MW) dietary nitrogen, such as PH, can easily leach into water. The main challenge in protein nutrition of fish larvae is to develop a highly digestible microdiet while controlling leaching losses, as encapsulation techniques often make microdiets difficult to digest.

The present work reviews recent findings adding to the discussion on the ideal form of presenting dietary nitrogen in feeds for altricial fish larvae. Including high levels of low MW PH in sparid larvae microdiets that led to poor performance, higher amino acid (AA) catabolism, and a strong impact on larvae whole body proteome, including energy and AA metabolism, and protein chaperoning and degradation. This detrimental effect could have been caused by protein overloading of larval digestive and metabolic capacities, due to a fast influx of AA shortly after the meals. However, when 0.5-30 kDa hydrolysates were included at high levels in a seabream diet, in an attempt to overcome this, poor growth was still observed. Even if protein digestion and catabolism did not seem to be affected, an augmented expression of proteins related to sarcomeric protein degradation and energy metabolism point to impaired anabolic efficiency induced by a diet rich in moderate MW PH. Moreover, tracer studies with model peptides of different MW not only confirm that larval digestive capacity for more complex peptides increases with development, but further suggest that MW is not the only factor affecting the digestibility of peptides. In addition, progress in encapsulation of PH allows for controlled release of peptides and AA in the gut, which may improve protein retention.

The combination of zootechnical performance data, with effects on nutrition physiology arising from tracer studies and omic’s data, and improvements in microdiet technology, have improved our understanding on the ideal form for supplying dietary nitrogen in feeds for altricial fish larvae. Still, further work on PH is needed to develop a high performance inert microdiet to feed the early stages of marine fish larvae, in particular concerning the effects of bioactive peptides in PH, and their role in modulating the gut microbiota, immune function, antioxidative status, appetite and digestion.


INTTEGRATED METABOLIC, PROTEOMIC AND TRANSCRIPTOMIC PROFILING OF GILTHEAD SEABREAM LIVER TO TRACK INTERACTIONS BETWEEN DIETARY FACTORS AND SEASONAL TEMPERATURE VARIATIONS
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Gilthead seabream is sometimes affected by a metabolic syndrome, known as the “winter disease”, which has a significant economic impact. It is caused, among other factors, by the thermal variations that occur during colder months and there are signs that improved nutritional status can mitigate the effects of this thermal stress [1-2].

For this reason, a rearing trial was undertaken where the effect of a fortified diet on the metabolic and immunological impact of thermal stress was assessed through metabolomic, transcriptomic and proteomic analysis of gilthead seabream hepatic tissue, following a co-measurement strategy. The aim of this trial was, on one hand, to validate the possibility of mitigating the impact of environmental stress on gilthead seabream through a nutritional strategy and, on the other hand, to help understand which biological processes are affected by the chosen nutritional supplementation, in order to develop molecular tools for evidence-based diet formulation tailored to the specific needs of aquacultured fish during particularly challenging situations.

For the trial, four groups of 25 adult gilthead seabream were reared for 8 months, being fed either with a control diet (CTRL, commercial formulation) or with a diet dubbed “Winter Feed” (WF, fortified formulation containing a higher proportion of marine-derived ingredients, as well as phagostimulants, marine phospholipids, antioxidant vitamins, taurine and soy lecithin). Fish were sampled at two time-points (at the end of winter and at the beginning of summer), with liver tissue being taken for metabolome (FTIR spectroscopy), transcriptome (Agilent microarrays) and proteome analysis (DIGE).

This presentation will focus on the liver metabolome results, which show that seasonal temperature variations constitute a metabolic challenge for gilthead seabream, with hepatic carbohydrate stores being consumed over the course of the inter-sampling period. Furthermore, results point towards a positive effect in terms of performance and improved nutritional status when using the WF diet. This diet seems to have a mitigating effect regarding the seasonal challenge, not only in terms of carbohydrate depletion, but also in terms of the observed accumulation of lipids in the later sampling. These results are confirmed by the transcriptomic and proteomic results, which display a significant overlap in terms of affected pathways, suggesting that, despite the non-redundant and complementary nature of metabolome, transcriptome and proteome data, they appear to be generally consistent for the purpose of nutritional profiling.

References:
EFFECT OF DIETARY OMEGA-3 FATTY ACIDS ON VIRAL INFECTION AND TOLL-LIKE RECEPTOR ACTIVATION IN MACROPHAGES FROM ATLANTIC SALMON

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Fish oil is the major source of the essential fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Due to increasing demands for EPA and DHA as ingredients in human health products and fish feed, there is now a serious shortage of these fatty acids on the international markets. This has led to increasing substitution of fish oil by plant oils in Atlantic salmon feed and thereby reducing the EPA and DHA content of salmon. This reduction is probably unavoidable. However, the minimum required levels of these fatty acids in fish diets for securing fish health are unknown.

The main objective of this project is therefore to determine if EPA and DHA deficiency can influence the immune system causing infections or reduced ability to respond to vaccines. There are numerous studies documenting a role for essential fatty acids on immunity in both experimental animals and humans.

We have analyzed primary macrophage cultures in vitro from the following dietary groups: 0%, 1% and 2% EPA and DHA alone and in combination + a control commercial diet group. The cell cultures were isolated from the fish and thereafter infected with pathogenic salmonid viruses in order to study if the different diets have affected the innate immune response against viral infection. In addition, cells were also stimulated with Toll-like receptor ligands to study the expression of these pattern recognition receptors and signaling status of the cells. Resistance to infection and ability to respond to immunostimulants was be quantified by real-time PCR analysis of important cytokine transcripts. This study will present new knowledge on minimum dietary levels of essential fatty acids necessary for the fish to be able to respond to infections and vaccines.

The work was supported by Norwegian Research Council (NRC).
REGULATION OF GENES INVOLVED IN FATTY ACID BIOCONVERSION IN EUROPEAN SEA BASS (DICENTRARCHUS LABRAX) FED VEGETABLE OIL DIETS: EFFECTS OF ALTERNATE FEEDING

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An intensive global research effort has been focused on finding viable strategies to replace fish oil (FO) in aquafeed. Vegetable oils are the favored candidates for FO replacement up to 60% in marine species. However, the requirement to use these oils for feed formulations makes it necessary to determine genetic capacities and metabolic possibilities of cultivated fish. Our recent findings showed that the n-3 HUFAs levels in European sea bass fingerlings fed vegetable oil diets and FO diets in cycles were identical to those of fish continuously fed FO diets. This prompt us to further investigate the nutritional regulation of hepatic expression of PPAR-α, SREBP-1c, D6D and Elovl5 genes controlling the regulation of fatty acid bioconversion activities in sea bass.

The present study aimed to elucidate and clarify the effects of alternating feeding schedules in which dietary lipid source is routinely alternated on fatty acid profile and lipid metabolized genes in European sea bass juveniles (24.3-g). Five feeding schedules were administered to fish: control (FO, continuously fed fish oil diet), canola (CO, continuously fed canola oil diet), blend diet (BLD, continuously fed with a diet containing 50% FO/50% CO), three weeks alternate (3WCO, fish fed for 3 weeks with CO and the following 3 weeks BLD), and finishing group (9WCO, fish fed for 9 weeks with CO and the following 3 weeks FO) for 12 weeks. Significantly higher SGR and lower FCR were detected in fish fed the FO, BLD and 9WCO diet compared to the fish fed the CO diet and the 3WCO diet. The n-3 LC-PUFA deposition (mainly EPA and DHA) were generally decreased in whole body, fillet and liver in fish fed CO diet compared to fish fed the FO diet. Fish fed 9WCO diets had an intake of FO and CO identical to that of the fish fed 3WCO diet, whereas n-3 LC-PUFA deposition of fish fed 9WCO diet were significantly higher than fish fed 3WCO diet. Although statistically non-significant, PPAR-α and SREBP-1c were slightly up-regulated, in some individual samples among the replicates in 3WCO and 9WCO treatments. However, there was no clear trend in D6D expression among the groups and Elovl5 gene expression was not detected.

In conclusion, the results show that by utilizing an alternating dietary lipid source feeding schedules where CO and FO based diet were routinely alternated, European sea bass were able to increase their efficiency of n-3 LC-PUFA deposition. However, due to high individual variability it is not possible to conclude about a positive effect of alternate feeding on the genes involved in FA bioconversion. Further studies are needed to precise the mechanisms leading to deposition of n-3 LC-PUFA in sea bass.
Ballan wrasse (Labrus bergylta) has proven to be an efficient cleaner fish against the ectoparasite, sea louse (Lepeophtheirus salmonis) on Atlantic salmon (Salmo salar). The salmon industry still has to rely on wild catches to serve them with cleaner wrasses. For B. wrasse to be a part of the lice solution, the industry must have access to reliable delivery of B. wrasse at the desired size when needed. To provide this in large scale B. wrasse farming is the solution.

The digestive tract of B. wrasse distinguishes itself from that of more traditional farmed species by possessing no stomach and being very short, only ~2/3rd of the total body length. For a best possible designed feed and feeding regime it was therefore a need to describe the functionality of the digestive tract. In addition to analysis of passage time and macronutrient digestibility of a moist and dry diet, the transcriptome of four sections (anterior, anterior in mid gut, posterior of mid gut and hind gut) was analyzed. Apparent digestibility (in %) was as follows: protein: dry feed: 86, moist feed: 86, carbohydrate: dry feed: 76, moist feed 86, saturated lipids: dry: 72, moist: 84, mono-unsaturated fatty acids: dry: 72, moist: 86, poly-unsaturated fatty acids: dry: 83, moist: 93.

The passage time of a dry and a moist diet was measured at continuous feeding. The passage time for the dry diet varied between 9 and 13 hours, whereas it was 13 hours in the fish fed the moist diet. Apparent digestibility of macronutrients were higher in the fish fed the moist diet compared to dry diet fed fish, with the exception of protein (see table). Most of the digestion took place in the anterior part of the intestine, 76 ± 6 (% ± SD) of the total digestion. The transcriptome of the intestine indicate that regardless of being short and in lack of a stomach in front, the distribution of intestinal functions is fairly similar to that known in other species. The anterior part (bulbus) and anterior mid-gut have the highest expressions of genes involved in lipid metabolism and transport, protein digestion, catabolic processing of carbohydrates. These two sections of the intestine also had a higher mitochondrial activity than further posteriorly. The hind-gut was dominated by immune activity, lysosomal activity as well as sphingolipid metabolism.
The culture of the common octopus (Octopus vulgaris) is hindered by massive mortalities encountered during early paralarval stages. Polyunsaturated fatty acids (PUFA) have been identified as essential compounds for paralarvae, but precise dietary requirements have not been determined. Our primary objective is to gain insight into the essential fatty acid requirements of paralarval stages of the common octopus through characterisation of the enzymes of endogenous biosynthetic pathways. Here we present recent studies on the molecular and functional characterisation of a stearoyl-CoA desaturase (Scd) and an elongase of very long-chain fatty acids (Elovl) from the common octopus. We report the isolation of a desaturase-like cDNA whose coding sequence encompassed a 326 aa protein. Phylogenetic analysis grouped the newly cloned O. vulgaris desaturase into a cluster with greatest similarity to Scd orthologues from both vertebrates and invertebrates, and separated from the group of fatty acyl desaturases (Fad) including the octopus ∆5-like Fad recently identified by our group. The function of the octopus Scd was determined by complementation assays of the Saccharomyces cerevisiae strain L18-14C, which contains a disruption of the yeast ∆9-desaturase gene, OLE1. Consistent with the function described in other organisms, the octopus Scd showed ∆9-desaturation specificity and it is thus implicated in the biosynthesis of monounsaturated fatty acids such as oleic acid (18:1n-9). Interestingly, no apparent ability to produce 16:1n-7 from 16:0 was exhibited by the octopus Scd, similar to the Scd previously described in the nematode Caenorhabditis elegans.

Elongases of very long-chain fatty acids (Elovl) are rate-limiting enzymes in fatty acid elongation pathways. We now also report the complete molecular cloning of an Elovl-like cDNA with homology to vertebrate Elovl4, elongases involved in the biosynthesis of very long-chain (>C24) PUFA. Heterologous expression of the octopus Elovl4 in the S. cerevisiae strain InvSc1 confirmed that the octopus Elovl4 was able to produce polyenes of up to 34 carbons. Further studies are required to confirm the presence of very long-chain PUFA in O. vulgaris tissues that preferentially express Elovl4.
APPLICABILITY OF BEAN PROTEIN CONCENTRATE FOR ATLANTIC SALMON FEEDS: II HEPATIC TRANSCRIPTOME RESPONSES

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Background: As aquaculture continues to expand, so does the requirement for high-quality and sustainable protein sources for feed production. At present, European feed manufacturers rely upon fishmeal (FM) and imported soybean protein concentrate (SPC) which poses a significant threat to sustainability and supply of these ingredients. Thus, there has been a growing demand for alternative protein sources and a product with good potential has been identified from faba bean, a crop widely grown in the UK and Europe. Bean protein concentrates (BPC) with > 55% protein content can be produced at relatively low cost through the process of fine grinding and air classification. A large nutritional trial, whose detailed results are presented separately at this conference, was conducted in collaboration with EWOS Innovation utilizing Atlantic salmon parr to establish the effects of dietary BPC and to estimate the optimum inclusion level for this ingredient. Dietary BPC levels of up to 20 % improved growth compared to SPC, while high levels reduced growth and caused mild enteritis. The present study investigated the changes in hepatic transcriptome that occurred in salmon when dietary SPC was replaced with BPC.

Materials and Methods: Six dietary treatments were investigated. Five diets had identical content of FM (22%) and progressively higher inclusion of BPC (0%, 11%, 22%, 34%, 45%) substituting SPC referred to as diets B0, B20, B40, B60, B80, while a high FM diet was included as a reference. Transcriptomic analysis was performed on pooled liver samples using a custom-made 44K salmon oligomicroarray (Agilent). Expression data were analysed for functional pathways significantly affected within biological processes including metabolism, genetic information processing, cellular processes, organismal systems and environmental information processing.

Results: Pathways of metabolism and genetic information processing were those most affected by BPC dietary replacement. Overall, fish fed diets B0, B20 and B40 had similar metabolic profiles while the majority of differences occurred in fish fed B80, with B60 generally expressing intermediate values. Specifically, lipid metabolism was the most affected in B80-fed fish with overall increased expression of > 4-fold compared to diets with low BPC content, followed by carbohydrate (~ 4-fold increase) and amino acid metabolism (~ 3-fold increase). In contrast, pathways involved in processes such as RNA transcription & translation and protein processing were down regulated in fish fed B80 compared with the other treatments. It was particularly noteworthy that there was correlation between pathways of hepatic protein synthesis and growth with diets supporting best performance (B20 and B40) also showing the highest expression levels.

Outcomes: In agreement with growth outcomes, liver transcriptomic analyses indicated that moderate inclusions of BPC in salmon diets were beneficial in increasing hepatic protein synthesis while not negatively affecting metabolic pathways when compared to diets using only FM and SPC. These data support the viability of BPC as a partial replacement for SPC and FM in feeds for Atlantic salmon.

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CLONING AND CHARACTERIZATION OF CYSTEINE SULFINIC ACID DECARBOXYLASE (CSD) FROM YELLOWTAIL SERIOLA QUINQUERADIATA AND RED SEA BREAM PAGRUS MAJOR AND EXPRESSION ANALYSIS OF CSD IN SEVERAL MARINE FISH SPECIES

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Marine carnivorous fish species require taurine in a diet because they possess limited taurine synthetic ability. There are several taurine synthetic pathways that are identified in fish and it has been thought that hypotaurine production via cysteine sulfonic acid by activity of cysteine sulfinic acid decarboxylase (CSD) is the rate limiting step of taurine synthesis in marine fish species. If CSD activity can be manipulated in marine carnivorous fish species, it is possible to reduce or eliminate additional taurine in low fishmeal diet. However, CSD in marine fish is not well characterized. Therefore, here the present study was conducted to clone and characterize CSD from two marine carnivorous fish species, yellowtail Seriola quinqueradiata and red sea bream Pagrus major. We also analyze gene expression of CSD in tissues of red sea bream, yellowtail, Japanese seabass Lateolabrax japonicus, and barfin flounder Verasper moseri.

Total RNA was isolated from liver of yellowtail and red sea bream. Primers were designed according to highly conserved region of CSD reported previously. Partial sequence of yellowtail and red sea bream CSD were obtained by PCR and full length sequence of CSD gene were determined by RACE. Homology analysis of the CSD sequence and phylogenetic analysis by neighbor joint method was conducted. Tissue distribution of CSD expression in yellowtail, red sea bream, barfin flounder Verasper moseri and Japanese seabass Lateolabrax japonicus was also examined by PCR.

Full length CSD (1882 bp and 1821 bp) was cloned from red sea bream and yellowtail. Homology of red sea bream and yellowtail CSD sequences was 88.8% each other and more than 83% for CSD of medaka Oryzias latipes and Nile tilapia Oreochromis nilotichus. Domain analysis of red sea bream and yellowtail CSD suggested existence of pyridoxal phosphate binding site in the CSD. CSD expression was detected in various tissues examined but strongly expressed in liver and pyloric caecae in all fishes examined. CSD also expressed in heart in all fishes except Japanese seabass. The present study suggests that red sea bream and yellowtail CSD require pyridoxine as a co-enzyme and expressed in various tissues of fish.
LC-PUFA BIOSYNTHESIS IN SENEGALESE SOLE AND ITS MODULATION BY DIETARY LIPID LEVEL AND FATTY ACID COMPOSITION

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The Senegalese sole, Solea senegalensis, has long been regarded as an unusual marine fish species as it can be successfully reared on diets with very low levels of n-3 long chain polyunsaturated fatty acids (LC-PUFA), in particular docosahexaenoic acid (DHA, 22:6n-3), during larval and post-larval stages. More recently, high levels of fish meal or fish oil replacement have been tested with no major detrimental effects on fish performance or flesh DHA levels. A recent molecular study described and functionally characterized a fatty acyl elongase (elovl5) and an unusual desaturase with ∆4 activity (∆4fad). The in vitro results suggested capability to synthesize DHA from eicosapentaenoic acid (EPA, 20:5n-3) in this species, further supported by the transcriptional up-regulation of ∆4fad in response to LC-PUFA-poor diets. However, the in vivo activity and biological relevance of this pathway remained to be determined and this was the objective of the present study.

Methods
Senegalese sole juveniles (start weight of 5±1g) were fed one of 4 experimental diets in triplicate tanks over a period of 91 days. The experimental diets were isoproteic (57% crude protein) and contained either 8% or 18% total lipid supplied by either 100% FO (diets 8FO and 18FO) or with 75% of the FO replaced by a vegetable oil blend (diets 8VO and 18VO). At the end of the experiment livers and intestines of 2 fish/tank were excised, isolated hepatocytes and enterocytes prepared by collagenase digestion, and then incubated for 1h with [1-14C]18:3n-3 and [1-14C]20:5n-3.

Results
There was no ∆6 desaturation of 14C-labeled 18:3n-3 in either tissue and only elongation to 20:3n-3. In contrast, substantial amounts of radioactivity from 14C-labeled EPA were recovered in 22:5n-3, 24:5n-3 and DHA, and the levels of these metabolites were higher at lower dietary lipid level and clearly stimulated by raising dietary VO levels particularly in hepatocytes.

Conclusion
This study presents results that are consistent with the previously reported substrate specificities of the LC-PUFA biosynthesis enzymes characterized in vitro and confirms the existence of a true capacity to biosynthesize DHA from EPA in Senegalese sole which, furthermore, is modulated by dietary composition both in terms of lipid level and fatty acid composition. The activity and regulation of this pathway can hence partly explain the low dietary requirement for n-3 LC-PUFA in this species during early development and ongrowing.
Adequate nutrition is vital for fish brain development, and nutrient deprivation has been implicated in disease and aging. Despite the fact that growth control is exercised through the precise regulation of stem and progenitor cell lineages, little is known about how this regulation is affected by impaired nutrition. Adult neural stem cells (NSC) are scarce in humans (and mammals in general), but other vertebrates, as zebrafish, contain several populations of continuously proliferating cells in their brain, which could be used as models to study post-embryonic neurogenesis. Indeed, neurogenesis has been extensively studied in the adults of several teleost fish species and previous research has described multiple neurogenic proliferating cell populations in the anterior central nervous system. The aim of the present study was to determine the effect of nutrient-sensitive restriction on NSC proliferation and preservation in optic tectum (OT) and telencephalon (TL) in zebrafish. In the first experiment, four months old wild-type zebrafish were either fed with a commercial diet (Gemma Micro) or starved for 3 weeks and then re-feeding (Gemma Micro) for 3 weeks. Body weight was significantly lower in starved group than in fed controls throughout the experiment. Proliferating cell nuclear antigen (PCNA) immunostaining revealed a positive cell population found in the lateral, medial and caudal margins of OT and ventral part of TL in control animals. PCNA-negative immunostaining was found in OT and TL in starved group. However, after 3 weeks of re-feeding those starved fishes showed interesting PCNA-positive proliferating cells in OT and TL. In the second experiment, four months old wild-type zebrafish were either fed with a commercial diet (Gemma Micro). To mark cycling cells 1mM BrdU was bath applied to adults for 6 days and the label was chased for 3 weeks following nutrition deprivation. Under normal conditions, slowly dividing stem cells give rise to rapidly proliferating progenitors that eventually exit the cell cycle and differentiate. However, after 3 weeks of starvation the active proliferation stopped and BrdU immunostaining revealed positive stem cells among the positive stained cells suggesting stem cells are preserved. Nutrition deprivation showed a decrease in stem cell proliferation and a reduction in stem cell number; however, a small pool of active stem cells remained. Upon refeeding, stem cell number increased dramatically, indicating that the remaining stem cells are competent to respond quickly to changes in nutritional status. These results suggest that re-feeding after immediate starvation period is not critical for NSC proliferation and the ability zebrafish to regenerate NSC after acute conditions. This study paves the route to future nutritional studies investigating particularly the role of long chain-polyunsaturated fatty acids.
DIVERSIFICATION OF SUBSTRATE SPECIFICITIES IN TELEOSTEI FADS2 FATTY ACYL DESATURASES: CLONING AND FUNCTIONAL CHARACTERISATION OF Δ4 AND BIFUNCTIONAL Δ6Δ5 DESATURASES IN THE FRESHWATER Atherinopsid CHIROSTOMA ESTOR

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Mexican silverside (Chirostoma estor Jordan, 1880) from lake Pátzcuaro, known locally as “pez blanco”, is a highly valued freshwater fish in Mexico. However, this species is endangered due to over-fishing, environmental degradation and introduction of exotic species, prompting efforts to preserve the species through aquaculture. The zooplanktrophic C. estor is one of the few representatives of freshwater atherinopsids, found only in some lakes of the Mexican Central Plateau. However, C. estor displays many characteristics of marine species including improved survival, growth and development when cultured in saline conditions, producing small eggs, and having high docosahexaenoic:eicosapentaenoic acid (DHA:EPA, 30:1) ratios in tissue lipids. Unlike marine fish though, we previously showed that C. estor had the capacity for endogenous biosynthesis of long-chain polyunsaturated fatty acids (LC-PUFA) from C18 PUFA, at least in saline. In the present study we investigated the molecular mechanisms of LC-PUFA biosynthesis in C. estor, namely the characterisation of desaturase and elongase genes involved in the biosynthetic pathways. The results indicated that C. estor expressed two Fads2 fatty acyl desaturases displaying Δ4 and Δ6/Δ5 specificities, and thus all the desaturation reactions required to convert C18 PUFA to the physiologically essential LC-PUFA. Moreover, C. estor expressed an Elovl5 elongase with the ability to elongate C18 and C20 PUFA substrates to the corresponding C20 and C22 products, thus also securing the enzymatic capabilities required for LC-PUFA biosynthesis. These results are consistent with this species having the ability to endogenously synthesise LC-PUFA. Interestingly, this pattern of LC-PUFA biosynthesis genes was previously demonstrated in the herbivorous euryhaline marine fish Siganus canaliculatus, or rabbitfish. This indicates that a similar molecular mechanism for LC-PUFA biosynthesis is present in teleost species occupying different trophic levels but with similar environmental (salinity) tolerances. The data provide further insight into the diversification of substrate specificities in teleostei Fads2 fatty acyl desaturases and reveal that some practical environmental conditions can be aimed to naturally enhance LC-PUFA biosynthesis in cultured fish.
FATTY ACID METABOLISM IN A SOUTHERN BLUEFIN TUNA (THUNNUS MACCOYII) CELL LINE

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Southern Bluefin tuna (SBT, Thunnus maccoyii) aquaculture is a highly valuable industry but research on these fish is hampered by strict catch quotas and the limited success of captive breeding. To address these limitations, we have developed a SBT cell line (SBT-E1) to investigate fatty acid metabolism in SBT. The SBT-E1 cells proliferated well in standard Leibovitz's L-15 cell culture medium and this was dependent on the concentration of foetal bovine serum (FBS) as the source of fatty acids. Addition of the C₁₈ polyunsaturated fatty acids (PUFA) α-linolenic acid (ALA, 18:3n-3) or linoleic acid (LNA, 18:2n-6) to the culture medium had little effect on the proliferation of the cells, whereas addition of the long-chain PUFA arachidonic acid (ARA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3) or docosahexaenoic acid (DHA, 22:6n-3) significantly reduced the proliferation of the cells, especially for DHA. Addition of vitamin E to the culture medium overcame this effect suggesting that it was due to oxidative stress. The fatty acid profiles of the cells reflected those of the respective culture media with little evidence for desaturation or elongation of the supplied fatty acids. The only exceptions were EPA and ARA which showed substantial elongation to 22:5n-3 and 22:4n-6, respectively. These results were confirmed by experiments with [1-¹⁴C] labelled fatty acids which allowed us to investigate the short-term dynamics of fatty acid metabolism. As expected, cells supplied with [1-¹⁴C] EPA showed the greatest level of elongation which was significantly higher than that seen in cells supplied with [1-¹⁴C] ALA or [1-¹⁴C] LNA. Interestingly [1-¹⁴C] ALA and [1-¹⁴C] EPA showed significantly higher levels of β-oxidation, compared to saturated, monounsaturated and polyunsaturated [1-¹⁴C] C₁₈ fatty acids. This research highlights the unique metabolism of fatty acids by the SBT-E1 cells; however, further research is needed to elucidate the underlying molecular mechanisms of this metabolism.
ANOTHER GREAT BARRIER – INTESTINAL BARRIER FUNCTION IS DISRUPTED BY PLANT MEALS AND THEIR COMPONENTS
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In addition to and seemingly despite its role in nutrient assimilation, the intestine also functions as a barrier between the external and internal environments. To secure optimal health, it must protect the organism from potentially deleterious compounds present in food and water, including antigens, toxins, and pathogenic organisms. If these enter, it must mount an appropriate response. Disruption of the physical and/or immunological barrier can have serious consequences, both regarding susceptibility to infectious disease, as well as development of chronic diseases. The study of tight junction regulation between epithelial cells in mammalian models has revealed that this can be physiologically altered by e.g. nutrient transporters, but also disrupted by pathological processes. Involvement of barrier dysfunction in the pathogenesis (cause) of various diseases is not, however, completely understood.

In intestinal tissue samples from numerous recent feeding trials and other experiments in Atlantic salmon, effects of alternative plant ingredients, as well as their antinutritional factors, on physical barrier function and regulation have come to light. The data includes results from:

1) Transcriptional studies on intestine from salmon fed diets containing up to 20% extracted soybean meal
2) Transcriptional studies on intestine from salmon fed diets supplemented with different levels of soyasaponins
3) Short term *ex vivo* exposure of intestinal tissue to purified lectins, Kunitz' trypsin inhibitor and saponin from soybeans – individually and in combination – on tissue permeability for various markers
4) Challenge trials from salmon fed an experimental diet containing predominantly plant ingredients, but only 5% extracted soybean meal, and subsequently exposed to various bacterial and viral pathogens

The results indicate that various genes directly involved in the epithelial barrier functions are differentially expressed in salmon with developing inflammatory changes due to soybean meal or to high levels of soya saponins in the diet. A leaky barrier also appears to be caused by other antinutrients almost immediately upon exposure, and may therefore also be involved in the development of soybean meal-induced inflammation in the distal intestine. However, antinutrient involvement in the induction of inflammation may depend on the presence of endogenous components in the intestinal lumen. Also, a diet containing a large proportion of plant ingredients, but that does not elicit inflammation, may still alter the intestine’s barrier function, increasing susceptibility for at least some disease pathogens. Thus on the one hand, a disrupted intestinal barrier can be caused by antinutrients and other components in the intestinal lumen, which may lead to inflammation. On the other hand, the intestine’s barrier is disrupted by manifested inflammation, but inflammation may not be necessary for a disrupted epithelial barrier.
MODULATORY EFFECTS OF A PROBIOTIC ENRICHED DIET IN THE ENERGY METABOLISM OF JUVENILE FISH EXPOSED TO CHRONIC STRESS
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In intensive aquaculture, conditions are not always ideal and fish respond to deleterious situations with metabolic adjustments, providing energy to cope with the conditions and maintain homeostatic balance. Dietary supplements, namely probiotics are potential health management tools in aquaculture, as they can aid fish coping. The aim of this study was to investigate the effect of a Lactobacillus rhamnosus GG (LGG) enriched diet on the energy metabolism of crowded juvenile fish. Juvenile Nile tilapia, Oreochromis niloticus (8.1±3.7 g) were distributed by 4 treatment tanks as follow: i) control group fed with commercial feed and held at normal density conditions (CT); ii) a probiotic group fed with probiotic supplemented diet and held at normal density conditions (CTP); iii) a group fed with commercial feed and crowded after 21 days (CR); and iv) a probiotic group fed with probiotic supplemented diet and crowded after 21 days (CRP). All groups were tested in duplicate and crowding conditions were kept for 14 days. A blood biochemical panel and the activity of key metabolic enzymes in liver were investigated, and these were: hexokinase and pyruvate kinase from the glycolysis, fructose 1,6-diphosphatase and phosphoglucone isomerase from the gluconeogenesis, citrate synthase and malate dehydrogenase from the citric acid cycle, ß-hydroxyacyl-CoA dehydrogenase and ß-hydroxybutyrate dehydrogenase from the fatty acid metabolism, and alanine transaminase and aspartate transaminase from amino acids metabolism. In the end of the trial, fish from CTP and CRP groups gained more weight whereas fish from CR group presented the lowest growth performance. After 14 days of stress exposure, fish from CRP group showed higher levels of plasma glucose, triglycerides and total cholesterol and presented stable levels of plasma electrolytes, while fish from CR group presented depleted glucose levels and impaired ion regulation. Probiotic dietary enrichment modulated metabolic enzymes towards energy production even when fish were under stressful conditions. In this study, the chronic nature of the inflicted stressor led to maladaptation in fish where the energy substrates were depleted, but the enrichment of the diet with probiotic bacteria increased substrate availability and the activity of enzymes from energy related pathways. Thus, fish from CRP group presented better overall condition and adaptive response under deleterious conditions. This study provides information on the potential of probiotic dietary supplementation as a strategy for health and nutrition management under intensive aquaculture conditions.

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DOES PLANT-INDUCED INTESTINAL INFLAMMATION IN SALMONIDS LEAD TO A LEAKY GUT? - A SURVEY OF METHODS FOR TESTING IN VIVO GUT WALL INTEGRITY
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The lumen of the gastrointestinal (GI) tract can be considered as an external environment to the host and contains a wide diversity of microbes, nutrients, metabolites, anti-nutrients, bioactive molecules and signal molecules. The gut barrier plays a pivotal role in protection against luminal microbiota (both commensals and pathogens), bacterial substances and toxins, and other antigenic molecules from the diet. A dysfunction in the gut barrier function can thus lead to passage of both unwanted molecules and microbes.

Under normal physiological conditions, the prevention of bacterial translocation and uptake of unwanted molecules occurs through both transcellular and paracellular pathways, and the integrity of the gut barrier is also protected by e.g., secretion of mucus, defensins and other antimicrobial chemicals produced by the body. In several inflammatory bowel diseases in humans, failure of one or more of these mechanisms has been demonstrated, resulting in bacterial translocation, immune activation and deleterious effects on health.

The increased use of plant ingredients in aquafeed for carnivorous fish can result in GI diseases such as soybean meal-induced enteritis, which shows similarities to human inflammatory bowel diseases. Thus in the present experiment we have performed a survey of markers that are commonly used to assess gut wall integrity and permeability. Plasma samples from salmonids with healthy and inflamed intestines have been analyzed for bacterial translocation by PCR-based methods targeting the 16S rRNA gene, and for the content of bacterial products and toxins such as D-lactate and endotoxins. We have also analyzed the uptake of orally administered test molecules with limited absorption in a healthy gut, as their circulating levels may indicate the severity of damage to the intestinal integrity. The most promising permeability markers in salmonids will be presented.
The exact causative agent of soybean meal induced enteritis (SBMIE) in Atlantic salmon (Salmo salar) has not been conclusively identified, but the presence of antinutrients, especially saponins has been suggested as the main responsible agent for the observed effects. The aim of the present study was to find if saponins alone may be responsible for SBMIE. A dose-response experiment was carried out in post smolt Atlantic salmon (mean weight 442 g) fed one of two basal diets, one based on fish meal (FM) without bean meals, and one with lupin meal (LP) as an important protein source. These diets were supplemented with purified (95%) soyasaponins at inclusion levels of 2, 4, 6 and 10 g/kg. Effects on growth performance, gut health, digestive physiology and intestinal gene expression were observed. The feeding trial lasted 10 weeks. Growth performance was affected by the basal diet with a lower final body weight in fish fed LP-based diet. Low doses of soyasaponin (2-4 g/kg) showed a trend to increase final body weight in fish fed FM-based diets whereas high soyasaponin doses tended to decreased final body weight in both diets. High levels of dietary soyasaponin (6-10 g/kg) induced inflammation in fish fed both basal diets. Regardless of the basal diets, soyasaponin inclusion decreased brush border enzyme activity and increased the trypsin activity in the distal intestine, with a dose-dependent response. For fish fed the FM diet, saponins caused a dose-dependent decrease in blood plasma cholesterol levels, blood plasma bile acids, and bile acids in the distal intestine digesta. Analyses of a proliferation marker (PCNA) showed that soyasaponin increased the height of PCNA protein staining in the mucosal folds of the distal intestine in both basal diets with a dose-response trend. Gene expression profiling of the distal intestine showed that high levels of soyasaponin produced marked expression changes for a number of previously suggested candidate genes for intestinal inflammation. Among immune related genes, IL-17a, IL-1b, MYD88, CD4a and collagenase 3 were all up-regulated whereas a dose-dependent depression of genes related to metabolism (aquaporin 8 and fatty acid binding protein 2) was observed. This study showed that high levels of dietary soyasaponin are capable of inducing similar responses as seen in SBMIE in the distal intestine of Atlantic salmon whether the diet contains a legume ingredient or not. In general, strongest effects were observed for fish fed the FM diet for most of the studied parameters.

Keywords: Atlantic salmon, soyasaponin, fish meal, lupin meal, enteritis.
Inosine, a purine nucleoside containing the base hypoxanthine and the sugar ribose, which occurs in transfer RNAs. Inosine is considered as a functional nutrients. The amberjack, *Seriola dumerili*, is a marine pelagic carnivorous species and has a great potential for the global aquaculture industry owing to its rapid-growing and adaptive characteristics. Hence, it became one of the most important aquaculture species in Japan as well as in the Mediterranean Region. Information is not available on the inosine requirement and its effects on immune responses and gut morphology of amberjack. Therefore, a feeding trial was conducted for 50 days to determine the effects of dietary inosine on growth performance, immune responses and gut morphology of juvenile amberjack *S. dumerili*. Six isonitrogenous (50% crude protein) and isolipidic (12% crude lipid) diets were formulated. Diets 1 to 5 were semi-purified and supplemented with 0% (D1, negative control), 0.1% (D2), 0.3% (D3), 0.6% (D4) and 0.9% (D5) dietary inosine. Diet 6 (D6) was fishmeal based diet without any supplementation of inosine (positive control). Triplicate groups of fish with initial average weight of approximately 25.9 g were randomly stocked in eighteen 200L polycarbonate tanks at a stocking density of 20 fish per tank. The fish were fed twice daily by hand to satiation level. After 50 days of feeding trial, fish fed with D4 showed the highest final weight, specific growth rate (SGR, %/day), weight gain (%) and survival (%) followed by groups fed with diets D5, D6 and D3; and the lowest in fish fed with inosine free negative control diet (D1). No significant difference was found in terms of feed conversion ratio, feed efficiency ratio and protein efficiency ratio between the treatments fed with diets D4 and D6; and D4 and D5. All the growth and feed utilization parameters were significantly lower (P>0.05) in D1 (negative control). Whole body proximate composition and somatic parameters were also not influenced (P>0.05) by the dietary treatments except in hepato somatic index (HSI) where, inosine supplemented diets showed significantly higher (P<0.05) values than the negative control group. A wide variations in some of the blood parameters were observed. In case of oxidative stress parameters, fish fed inosine supplemented diets showed best condition because of the least oxidative stressed condition as well as the highest tolerance against oxidation. Serum lysozyme activity tended to be higher (P>0.05) for fish fed diets supplemented with inosine. In terms of intestinal health condition, enterocyte height, fold height and microvillus height of the anterior and posterior enteric sections increased significantly (P<0.05) in fish fed diets D3 and D4; and was lowest in the negative control group. It is therefore recommended that dietary inosine supplementation at 0.3 to 0.6% is beneficial to promote growth, immune responses and intestinal health condition of Juvenile Amberjack (*S. dumerili*).
Deoxynivalenol (DON) and ochratoxin A (OTA) are mycotoxins of importance for animal and human health. They may occur at considerable levels in fish feed ingredients of plant origin. The knowledge on health effects and toxicokinetics of these toxins in fish is lacking.

Post-smoltified Atlantic salmon (Salmo salar) were fed DON (0.5, 1.0, 2.0, 4.0 or 6.0 mg/kg diet), OTA (0.2, 0.4, 0.8, 1.6 or 2.4 mg/kg diet) or none of them for 8 weeks. Fish were sampled during and after the toxin feeding trial to determine effects on fish health as well as toxicokinetics, with focus on the potential risk for mycotoxin residues in the fish products. The sampled fish were weighted and length measured, and several tissues were collected to determine effects on clinical chemistry, immunology and pathology, and tissue concentrations of the mycotoxins. DON at 4 and 6 mg/kg reduced feed intake and condition factor, and increased relative liver weight in a dose dependent way. Furthermore, DON at 6 mg/kg reduced total protein, albumin and hematocrit in the blood. OTA produced no significant clinical or pathological effects. As a conclusion on toxic effects, our results indicate rather low sensitivity of salmon for adverse effects of DON and OTA in feed.

DON and OTA did not show any tissue accumulation, with residue concentrations at all samplings below the daily dose (µg/kg body weight). DON showed a wide and relatively even tissue distribution whereas OTA was predominantly found in liver and kidney. As a conclusion on toxicokinetics, no accumulation of DON and OTA seems to occur in salmon, and residues of these mycotoxins or metabolites in fish meat do not seem to imply any risk for consumers.
DIETARY VEGETABLE OILS: EFFECTS ON THE EXPRESSION OF IMMUNE-RELATED GENES IN SENEGALESE SOLE INTESTINE. PRO-INFLAMMATION INDUCED BY SOYBEAN OIL AND THE RESPONSE OF IMMUNE SYSTEM TO A STRESSOR

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The decreased availability of fish oil, traditionally used as oil source in marine aquafeeds, has lead to the search for alternatives oils. Vegetable oils (VO) are being extensively used as lipid sources in marine fish diets, inducing an imbalance on certain dietary fatty acids. Alteration on the dietary ratio of n-6/n-3 has been described to have detrimental effects on fish immunity. Senegalese sole has high susceptibility to certain stressful conditions and diseases, and little is known on the effects of dietary VO on its immunity.

Senegalese sole were fed diets (56% crude protein, 12% crude lipid) containing linseed (LO), soybean (SO) or fish (FO) oils as unique oil source. After 90 days of feeding, a stress test, (5 min of net chasing) was applied. Fish intestine were collected for biochemical analysis and expression of immune-related genes using Open-array RT PCR, including genes involved in the antiviral defense (9), interleukins and receptors (15), complement pathway (8), pathogen-associated molecular patterns (PAMPs) recognition (5), lysozymes (5), prostaglandin synthesis (2), chemokines (6) and differentiation Antigens (3). Also, 3 housekeeping genes were amplified for normalization.

The use of vegetable oils did not induced changes in fish growth, but affected fatty acid profile of intestine being linoleic acid (18:2n-6) increased in fish fed SO diet and linolenic acid (18:3 n-3) in those fish fed LO diet. Six complement genes were highly expressed in fish fed SO including the complement components c2 and c4a from classical pathway, the genes from the alternative pathway c3, c5 and c9, and the complement regulatory gene cfh. The interleukins il1b, il10, il11a and il12b and the interleukin receptors il1rl1 and cxcr2 also showed higher mRNA levels in fish fed SO than FO and LO. Hierarchical clustering analysis based on the 50 genes studied demonstrated expression signatures that set the SO diet apart and clearly differentiated of FO and LO diets. Also, most of the significant genes related with complement pathway, interleukins and c-type lysozymes clustered together and exhibiting similar expression profiles in the samples analyzed.

This general over-expression could indicate a chronic activation of inflammatory processes in fish gut. However, when a stress was applied, most of those genes are sub-expressed. These results are discussed in terms of the deleterious effects of a chronic inflammation induced by n-6 dietary fatty acids and the subsequent low immune potential to react when and alarm situation, such as stressful conditions, appears.
DIETARY MICROENCAPSULATED ORGANIC ACIDS ENHANCED GROWTH, IMMUNITY, HEPATOPANCREATIC INTEGRITY AND RESISTANCE AGAINST VIBRIOSIS IN PENAEID SHRIMP

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It is common practice in the aquaculture industry to use large amounts of antibiotics following the discovery of its growth promoting and disease fighting capabilities. In view of the current greater restrictions or ban on antibiotic use, there is a critical need to evaluate alternatives. One potential substitute for antibiotic growth promoters in feeds is organic acids. Organic acids such as benzoic, formic, lactic and propionic acids have traditionally been used as storage preservatives for food and feed ingredients due to their antimicrobial properties. Not much is known about the beneficial effects of these functional additives in shrimp feeds.

Laboratory-based and pond-based feeding trials were conducted on white shrimp (Litopenaeus vannamei) and tiger shrimp (Penaeus monodon), respectively. White shrimp were fed diets supplemented with a microencapsulated organic acids blend (OAB) at 0% (control), 1%, 2% or 4% of diet. Results showed that shrimp fed the 2% OAB diet had significantly higher (P<0.05) growth and more lipid storage cells in the hepatopancreas compared to shrimp fed the control diet. Dry matter and phosphorous digestibility was significantly improved in shrimp fed the 2% OAB diet. When challenged with pathogenic Vibrio harveyi, shrimp fed the 1% or 2% OAB diet showed significantly higher survival compared to control. In the Vibrio challenged shrimp, phenoloxidase activity was significantly higher for shrimp fed the OAB diets.

Due to these encouraging laboratory results, we conducted a field trial in a commercial tiger shrimp farm. Shrimp were stocked at a density of 160,000 post-larvae per pond (0.6 ha). The commercially manufactured shrimp feeds contained either no added OAB or 2% OAB. The mean total bacterial count and Vibrio spp. count in the pond water of shrimp fed the 2% OAB feed were generally lower compared to the control pond. After 16 weeks, adult tiger shrimp (19 - 21 g) were collected from the ponds using a cast net and transferred into a series of glass aquaria to be challenged with V. harveyi. Shrimp fed 2% OAB showed significantly higher survival due to enhanced immune response and less hepatopancreatic damage. Hepatopancreas of shrimp fed the control feed showed hemocyte infiltrations, substantial reduction of R-cells and tubule necrosis. After 20 weeks, another random sample of live shrimp (30 - 32 g) were collected from the ponds and transferred into a series of tanks for nutrient digestibility determination. Protein, dry matter, ash and phosphorous digestibility were significantly enhanced in the OAB supplemented feed. Total bacterial and Vibrio counts were significantly lower in the hepatopancreas and gut of shrimp fed the OAB feed. After 22 weeks, shrimp growth was similar between treatments.

This is the first study to demonstrate the potential growth promoting and antimicrobial efficacy of dietary organic acids in shrimp. The ability of organic acids to protect hepatopancreatic integrity during a Vibrio bacterium infection is noteworthy.
The present study aimed to investigate the effects of different dietary sustained-release microencapsulated sodium butyrate (MSB) (0: non supplement, 1.5, 3.0 h) for control or oxidised soybean oil (oxi-oil) diets on fish production, intestinal mucosa condition, immunity and bacteria in juvenile common carp (Cyprinus carpio). Dietary MSB considerably increased the weight gain (WG) and reduced the feed conversion ratio (FCR) within the control or oxi-oil groups despite no statistical significance. Fish gut mucosa was damaged in the oxi-oil group without MSB, but normal appearance was present when fish were fed MSB1.5 and MSB3.0 in the oxi-oil group. Microvilli density was increased in fish fed the MSB1.5 and MSB3.0 treatments in the oxi-oil group ($P < 0.001$), and microvilli density was affected by different pre-fed diets in the mid-gut (MG) ($P < 0.001$) while it was affected by different sustained-release time of MSB in the distal gut (DG) ($P = 0.003$). Interaction between pre-fed diet and dietary sustained-release time of MSB was significant for the relative expression levels of gut HSP70, pro-inflammatory cytokines (IL-1β and TNF-α), and anti-inflammatory cytokines (TGF-β) genes within each segment besides HSP70 in the DG and IL-1β in the fore gut (FG). However, modulation of gut adhesive bacteria within each segment investigated was not obvious when common carp were fed diets with MSB, as similarity coefficients of > 0.79 were observed. These results indicated that MSB can be used as dietary supplement to repair or prevent damage to the intestine of carp fed oxidised oil.

With the increasing awareness of the importance of the gut microbiota in influencing aquatic animal health and digestive function, feed additives, such as probiotics, have received great interest. Probiotics are applied in an attempt to fortify and modulate the host microbiome to provide benefits to the host and fish farmer. One of the most well documented probiotics in aquatic animals is *Pediococcus acidilactici* MA18/5M. For example, the dietary application of *P. acidilactici* MA18/5M to Nile tilapia (*Oreochromis niloticus*) or rainbow trout (*Oncorhynchus mykiss*) can elevate lactic acid bacterial (LAB) levels, out-competing certain bacterial phylotypes (such as *Aeromonas* spp. for instance) and modulate microbial diversity and richness in the gastrointestinal tract. An increased similarity of the gut microflora of the anterior segment of the intestinal tract between fish from a same population (tank) has also been reported.

*P. acidilactici* MA18/5M populations in the gastrointestinal tract, and the microbial modulations which they induce, can improve intestinal morphology and integrity. Across multiple studies with fish elevated microvilli length, endocytic activity, villi length, and abundance of goblet cells and intraepithelial leucocytes have been observed as well as the reduction of *Vibrio anguillarum* induced intestinal damage in *ex vivo* experiments. The mechanisms behind these benefits are partly described: the probiotic application reduced the expression of genes related to cellular level stress and apoptosis (e.g. Hsp70, PCNA and Casp3), which could lead to better integrity and increased abundance of mature and active enterocytes. As an end point, a better absorption of minerals leading to a measurable better mineralization has been reported in larval fish fed *P. acidilactici* MA18/5M in adequate amount.

In addition, a number of studies have demonstrated improvements of immune parameters or so called immune priming. Elevated expression of proinflammatory genes have been reported in salmonids, marine fish species (TNFα, IL1β) and tilapia (TNFα) intestine. In addition, an elevated expression of the mucosal antibody IgT has often been observed and recently parameters involved in viral defenses were shown to be modulated. The expression of these genes improves the immune-responsiveness of the intestine and may help to explain the mechanisms behind the observed increases in intraepithelial leucocytes and the improved enteric resistance to certain pathogenic bacteria. These localised changes can subsequently modulate the systemic immune response and ultimately affect the survivability of the fish challenged by various pathogens. Specifically, elevated peripheral leucocyte levels and serum lysozyme activity have been observed after dietary *P. acidilactici* MA18/5M applications with salmonids. In similar fashion, elevated peripheral neutrophil and monocyte proportions and serum lysozyme activity have been observed in tilapia.

**Conclusion:** These microbial modulations and improvements in host digestive and immunological functions help to explain some of the production level benefits of *P. acidilactici* MA18/5M applications in multiple fish species.
Aquaculture is becoming increasingly important to supply the global population with fish due to the decline of the fisheries industry. In order to decrease the use of antibiotics and chemotherapeutants, the immunomodulatory effect of various functional feeds has been explored. Functional feeds are used extensively in the aquaculture and agriculture sectors in order to protect livestock from pathogens and parasites, as well as a means to improve growth and feeding efficiency. Yeast cell wall (YCW) extracts are ingredients which cannot be digested by the host but modify the gut environment. These supplements have been shown to improve growth and feeding efficiency of farmed fish, and there is evidence that they also reduce the abundance of sea lice in Atlantic salmon. In this study, the effect of YCW extracts on the skin mucus proteome of Atlantic salmon using two-dimensional gel electrophoresis was carried out, with the main aim of identifying a biomarker of mucosal modulation. Results show that 49 spots showed a statistically significant change in normalised volume between the control and YCW diets. All but five of these spots had a lower expression level in the experimental fish. Twelve spots were successfully identified by LC-MS/MS and they belonged to different functions and pathways. The gene expression of a selection of these proteins was studied in skin mRNA from three different feeding trials using yeast cell wall extracts supplements. Consequently, calreticulin-like protein (CALRL) was identified as a possible biomarker for prebiotic diets since it showed the most consistent change in expression in both the mucus proteome and skin transcriptome. The discovery of a biomarker is highly welcomed by the industry since it is expected to quicken the pace of research into functional feed ingredients.
THE APPLICATION OF THE BACTERIAL STORAGE COMPOUND POLY-B-HYDROXYBUTYRATE AS A FEED ADDITIVE IN AQUACULTURE

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Recently, a biological strategy in the form of poly-β-hydroxybutyrate (PHB) was suggested for the control of bacterial diseases in aquaculture. PHB is a compound that is accumulated by a wide variety of micro-organisms as an internal carbon and energy reserve. It was found that this compound could protect the aquaculture model organism *Artemia franciscana* against vibriosis (Defoirdt et al., 2007). The release of the PHB monomer, β-hydroxybutyric acid, was suggested to inhibit the growth and/or the activity of the pathogens, although the exact mode of action was not known. Since then several researches have been conducted in the Laboratory of Aquaculture & Artemia Reference Center that investigated the effects of including PHB in the feed for a variety of aquaculture organisms such as juvenile European sea bass (De Schryver et al., 2009), juvenile Siberian sturgeon (Najdegerami et al., 2010), juvenile Nile tilapia (Situmorang et al., unpublished), giant freshwater prawn larvae (Nhan et al., 2010), Chinese mitten crab larvae (Sui et al., 2010), and giant tiger prawn larvae (Laranja et al., unpublished). Remarkably, it seemed that in addition to having a protective effect against pathogens, PHB also supported an increased growth performance and larval survival of treated animals. The results indicate that PHB acts in a multitude of ways after ingestion to improve the health status of the host which may include modification of the intestinal microbial community composition, a direct antipathogenic activity, an immunostimulatory effect, and an energy delivery effect. This presentation will provide an overview of the currently available published and unpublished results that indicate the health improving effects of PHB and that focuses on the efforts that have been performed to elucidate the mode of action of PHB after ingestion. The studies presented have all contributed to the development of the bacterial storage compound PHB as a sustainable feed additive for aquaculture purposes.


NUTRIENT DEFICIENT DIETS CAN SUPPRESS THE GROWTH OF JUVENILES OF THE WHITE SHRIMP, LITOPENAEUS VANNAMEI, IN A MICROBIAL FLOC, FED-BASED, ZERO-WATER EXCHANGE SYSTEM

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In zero-water exchange intensive systems, bioflocs are thought to be a source of nutrients and to contribute to the growth of marine shrimp. The present study was conducted to evaluate if nutrient-restraining diets could affect the growth of L. vannamei raised in a microbial floc, fed-based, zero-water exchange culture system. Shrimp with 3.04 ± 0.43 (n = 6,144) were stocked under 128 shrimp/m³ in 48 outdoor tanks of 1 m³ and fed 12 diets for 10 weeks. Diets contained 30.0 ± 1.3% crude protein (CP) and 7.8 ± 0.5% fat (as dry matter basis). Dietary levels of essential amino acids (EAA) and long-chain polyunsaturated fatty acids (LC-PUFA) were restrained by reducing fish meal from 12.0 to 8.0, 4.0 or 0% and fish oil from 2.0, 1.0 or 0%, respectively. Contribution of microbial flocs to shrimp growth was measured through the natural stable carbon isotope (δ¹³C) mass spectrometry technique. In tanks, biofloc volume increased from a mean of 8 mL/L in the 1st week to 54 mL/L in last week of culture. Biofloc nutrient composition reached 14.7 ± 2.7% CP, 12.4 ± 5.7% fiber, 1.7 ± 0.32% fat, 0.16 ± 0.22% Σn-3, 0.22 ± 0.53% MET and 0.63 ± 0.11% LYS. Except for THR, no other correlation was found in the individual and total EAA and LC-PUFA profile of bioflocs and feeds. At harvest, no statistical difference among feed treatments (P >0.05) were observed in final shrimp survival (74.8 ± 6.4%), yield (1,339 ± 183 g/m³), FCR (2.01 ± 0.32) and feed intake (20.6 ± 0.2 g/stocked shrimp). However, shrimp weekly growth and final body weight (BW) differed statistically among feed treatments (P< 0.05). Both dietary EAA and LC-PUFA significantly impacted final shrimp BW. While final BW did not differ between diets containing 0% (11.8 ± 0.7% ΣEAA and 0.65 ± 0.11% Σn-3) and 4% fish meal (12.7 ± 0.3% ΣEAA and 0.71 ± 0.12% Σn-3; as % of the diet, DM basis), there was a significant improvement when levels were raised to 8% (13.5 ± 0.3% ΣEAA and 0.89 ± 0.19% Σn-3) and 12% (14.4 ± 0.3% ΣEAA and 1.01 ± 0.12% Σn-3). Similarly, increases in fish oil from 0% (0.67 ± 0.11% Σn-3, 12.8 ± 1.3% EAA) to 1% (0.83 ± 0.18% Σn-3, 13.3 ± 1.1% EAA) and 2% (0.95 ± 0.18% Σn-3, 13.1 ± 1.11% EAA) all resulted in progressive increments in final shrimp BW. Individual δ¹³C examination of shrimp caudal tissue at stocking (~20.71) and harvest (~22.31 ± 0.33), and of experimental diets (~23.80 ± 0.47) and microbial flocs (~16.60 ± 0.59) indicated that shrimp derived from 78.9 to 84.7% of its carbon growth from experimental diets. This study has shown that under an intensive fed-based culture system, microbial flocs had a limited nutrient contribution to L. vannamei growth, and that nutrient deficient diets negatively depressed shrimp growth performance.
FLOATING FISH FAECES - A NOVEL APPROACH TO OPTIMIZING SOLID REMOVAL IN AQUACULTURE SYSTEMS

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The rapidly growing aquaculture sector faces some serious challenges in addressing the environmental impacts of intensive fish farming. Demand for fish continues to increase, putting pressure on the limited resources of water for production and regulations governing the discharge of effluents are increasingly stringent. One main issue is the management of suspended solids which can cause severe environmental problems in open aquaculture systems and have the potential to impact whole system efficiency in closed facilities. Since faecal waste is the main source of suspended solids, the removal of faeces before nutrient leaching or fragmentation processes can take place is much to be desired. Based on previous extensive lab studies (Unger and Brinker, 2013) we used dietary manipulation to reduce the density of faeces, resulting in floating faecal casts. In a field study we compared the removal efficiencies of floating and high-density faeces and examined the consequences for water quality parameters under commercial conditions.

A study tested the value-added by the modification of faecal buoyancy under commercial conditions in a semi-recirculating trout farm in Germany. A commercial diet resulting in faeces in the upper density range (1.0489 ± 0.0012) was used as a control. Based on previous lab studies, intact and stable floating faeces (0.96103 ± 0.002) that accumulated consistently at the water surface were achieved by the addition of cork to the control diet. 135.9 kg d⁻¹ of the commercial diet and 155.5 kg d⁻¹ of cork diet were supplied to systems with comparable water exchange rates. Histological assays of fish tissues indicated no pathogenic alterations (liver, intestine) associated with the cork supplement, and growth, survival and feed conversion appeared not to be affected. A special surface-only waste separator removed 35.4 % of the faeces produced, significantly reducing the solid and organic load on the system, as a consequence of which single-pass biofiltration efficiency for TAN and nitrite was improved by about 16 % and 10 %, respectively. TAN concentration of the production units was halved and overall biofilter performance was distinctly more stable and robust. Leaching from solids was minimized and lower levels of nitrogen and phosphorus were maintained over the whole study period. Moreover a shift in the profile of solid particulates from fines to larger particles, which can be more easily removed, was observed. Dry matter content of the floating faeces recovered in the surface separator was about 18 %.

This study showed that the modification of faecal buoyancy facilitates rapid and cost-effective removal of suspended solids, removing the need for sludge thickening. This is of utmost importance in maintaining healthy stock and system stability and in addressing environmental concerns.

References: Unger, J. & Brinker, A.
Floating feces: A new approach for efficient removal of solids in aquacultural management
Aquaculture, 2013, 404-405, 85-94
The main objective of this study was to verify the impact of the pellet physical quality on the biological value of the feed fed to salmon and trout. The sub goals were to (1) identify the effect of recipe and process conditions on pellet physical quality and (2) investigate the effect of pellet physical quality on appetite, digestibility, FCR, growth and fish gut health.

Six different experimental diets were prepared using different recipes and process conditions. The first four recipes were optimized with a fixed blend of plant protein sources, North Atlantic fishmeal and micronized pea starch at three levels. Of the two diets at the lowest starch level one diet was produced with increased oil addition before the extruder step. Increasing starch for the two next diets led to a slight reduction in protein and energy in the diets. The following diets were optimized as the diet with the highest starch level with dehulled beans as starch source. In the latter some of the fish meal was exchanged by vegetable protein. A combination of different recipes and other process conditions for the three high starch diets gave 6 diets with increasing pellet strength and reduced solubility in water.

The diets were fed to 6 duplicate groups of salmon and trout for 54-56 days at an average temperature of 11.2 ºC. Start weight for salmon were 2401±58g (120 fish per tank) and start weight for trout were 3021±69g (91 fish per tank). Experimental data were subjected to single factor analysis of Variance using Kruskal-Wallis 1-way ANOVA for k samples (nonparametric test) and Duncan and Tukey were used as post hoc tests to find difference between the diets. Both t-test and two-way ANOVA were used to compare groups for histological analysis.

For salmon, a significant difference for feed intake (FI), feed efficiency ratio (FCR), growth (SGR) and digestibility (ADC) between the 6 diets was observed (p<0.001). In addition, both fat belching (FB) and distal intestine undigested pellet (UDP) value were significantly different between the codes. In trout significant differences for FI (p<0.001), FCR (p=0.006), SGR and ADC (p<0.001) between the 6 diets were observed. Also, fat belching (FB) was significantly different between the codes. Fish groups from two diet codes were chosen for histological analysis and significant effects of species and diet were found.

In conclusion, the results illustrate interesting connections between pellet quality, digestive problems and general performance in both salmon and trout.

PHYTOGENIC SUPPLEMENTATION CAN IMPROVE THE PERFORMANCE OF COMMERCIAL SHRIMP FEEDS WITH REDUCED FISH MEAL LEVELS

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Phytogenics feed additives are plant-derived substances which are added to the feed in order to improve animal performance. These plant active ingredients (e.g. phenolic and flavanoids) can exert multiple effects on the animal, such as antimicrobial action and direct reduction of gut bacteria, stimulation of gastric juices, support of liver function, anti-inflammatory and anti-oxidant properties. Due to their effects on improving feed efficiency, phytogenics could be an important tool to reduce feed cost in the context of high priced feed ingredients such as fish meal (FM). A feeding trial was conducted to evaluate whether a phytogenic feed additive (Digestarom® P.E.P. MGE) could be used as a tool to reduce the level of FM in shrimp diets. The treatments consisted in 5 isoproteic diets (40 % CP) with a positive control diet with 25% FM inclusion, and four test diets with two lower levels of FM (22% and 19 %) with or without supplementation with a phytogenic feed additive as follows: Positive control 25% FM; T1 22% FM; T2 22% FM + Digestarom®; T3 19% FM; T4 19% FM + Digestarom®. Each diet was randomly assigned to 5 replicates of 30 juvenile white shrimp (approximately 0.33±0.00g ) and fed over 8 weeks. The results indicated that the reduction in FM significantly (P<0.05) reduced shrimp performance with the control diet having the best performance. Nevertheless, weight gain, specific growth rate, feed efficiency and protein efficiency ratio were significantly improved (P<0.05) for shrimp fed diets supplemented with phytogenic when compared to the lower FM diets non-supplemented with phytogenic. Protein, lipid, ash and moisture content in whole body and muscle were not significantly affected by the treatments. Dietary treatment did not significantly affect total protein, glucose, triacylglycerol and cholesterol concentrations in shrimp serum. Enzyme activity (ALT, AST, AKP and LDH) in serum were also not significantly influenced by the dietary treatments. Analysis of midgut ultrastructure by transmission electron microscope indicated that shrimp fed diets supplemented with phytogenic had an improved midgut microvilli structure compared to those fed the lower FM diets. The improvement in performance of the lower FM diets when supplemented with the phytogenic feed additive can be important as part of a strategy to reduce costs by balancing the reduction of FM with the inclusion of phytogenics.

Keywords – Phytogenics, feed efficiency, feed, fish meal, white shrimp.
DEVELOPMENT OF A FACTORIAL BIOENERGETIC MODEL FOR FEED MANAGEMENT OF COBIA, RACHYCENTRON CANADUM

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There is little data on the nutrient requirements or feed management options for cobia, Rachycentron canadum. To address both of these deficiencies, a series of empirical studies were undertaken to derive a set of key parameters to allow the development of a factorial model for growth and feed management of cobia. Growth models can provide a useful basis for examining many complex, interrelated nutritional issues, but should not be considered to the exclusion of independent empirical data to validate them. A growth model using parameters of fish live-weight and water temperature to predict growth rate (g/d) was developed using regressive modelling of data collected from commercial farms, scientific literature and laboratory studies. During this data collection period samples of fish ranging in size from ~100 g to ~1000 g were collected and analysed for their moisture, protein, lipid and energy densities. Carcass protein content (% of live weight) was observed to be constant (18%) over the whole range of fish examined but moisture and energy \( y_{kJ/g} = 3.207^* (x)^{0.167} \) varied as the fish live-weight \( x \) increased.

Two growth studies were undertaken. One study examined the protein and energy gain of cobia in response to increasing ration levels of a diet of known digestible protein and energy content. From this study it was possible to calculate the utilization efficiencies of digestible protein and energy at a range of intake levels by this species. In a second experiment, cobia of various sizes were starved to calculate the protein, lipid and energy lost due to their respective metabolic demands. From this it was possible to calculate the relationship between fish live-weight and metabolic demands for protein, lipid and energy, and from this study, and earlier farm growth data, it was then possible to develop a model of metabolic energy demands by cobia as a function of live-weight and temperature. Table 1. Optimal protein to energy specifications for cobia based on iteratively determined requirements from the factorial model.

<table>
<thead>
<tr>
<th>Fish weight (g)</th>
<th>10</th>
<th>50</th>
<th>100</th>
<th>500</th>
<th>1000</th>
<th>2000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expected Growth (g/d) @30⁰C</td>
<td>1.5</td>
<td>3.3</td>
<td>4.6</td>
<td>9.9</td>
<td>13.8</td>
<td>19.2</td>
</tr>
<tr>
<td>Digestible Energy Demand (kJ/fish/d)</td>
<td>12</td>
<td>34</td>
<td>55</td>
<td>161</td>
<td>258</td>
<td>414</td>
</tr>
<tr>
<td>Ideal Diet Protein to Energy Ratio (g/kJ)</td>
<td>43.2</td>
<td>32.4</td>
<td>28.7</td>
<td>21.5</td>
<td>19.0</td>
<td>16.8</td>
</tr>
</tbody>
</table>

Each of the empirically determined parameters was combined into a factorial model using Microsoft Excel as a simple operating platform. This model allows the prediction of growth of cobia over a range of production sizes and temperatures for this species. The model also provides the user with a feeding table that includes the amount to be fed, target FCR, expected fish weights based on the energy density of the feed being used. The model can also be used to predict optimal diet specifications, such as the ideal protein to energy ratios in the diet at any fish live-weight.
OPTIMISING PIGMENTATION IN CRUSTACEANS: COMBINING DIET, GENETICS AND ENVIRONMENT
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Colour of cooked seafood is a major factor in consumer desirability and acceptability, with darker more consistently coloured crustaceans demanding premium market prices. Essential components to producing this colour are the dietary incorporation of the carotenoid astaxanthin, the response of the animal to the colour of their surroundings, and the functional genetics of how the colours and patterns are produced. In aquaculture, colour variation at harvest has generally been observed in a number of crustaceans, but this variability has not been measured and nor is the mechanism behind it well understood.

Our recent research has focussed on the Giant Tiger Prawn, Penaeus monodon, and understanding the interactive beneficial effects of dietary carotenoid supplementation in combination with dark substrates and genetics to enhance prawn colour. Astaxanthin is present in pigment structures beneath the shell, known as chromatophores, and they respond in different ways to diet and environment. This must combine with another critical element to producing optimal shrimp colour, a protein called crustacyanin (CRCN), which specifically binds to astaxanthin to produce the colours we see in crustacean shells. Two CRCN genes have been identified from a range of crustaceans, and CRCN sequence composition and gene expression levels vary between species.

This presentation will detail a series of experiments that have assessed mechanism of pigmentation regulation in prawns, both under controlled conditions and on commercial farms. Significant improvements in colour grade score can be achieved and used to maximise quality and price in farmed shrimp. In summary, we now have an excellent understanding of how dietary nutrition, environmental adaptation and genetics combine to create and modify crustacean colour.
This experiment intended to assess the effect of different dietary lipid sources on the growth performance, fatty acid composition of fillet and liver or flesh quality traits of rainbow trout (Oncorhynchus mykiss). Four iso-nitrogenous (approximately 50% crude protein) and iso-lipidic (approximately 15% crude lipid) experimental feeds were formulated. The control feed contained only fish oil as the primary lipid source. In the other three dietary treatments, fish oil was replaced by 100% sesame, sunflower and linseed oils, respectively. Duplicate groups of 30 rainbow trout (~34 g) held under similar culture conditions were fed to apparent satiation by hand per day for 70 days. At the end of feeding trials, there was no difference in feed utilization efficiency or growth performance between the experimental groups (P > 0.05). However, fish fed feed with SFO had the highest VSI values compared to the other fish groups (P < 0.05). Results showed that fillet lipid level of fish was the highest in fish fed with control feed (P < 0.05). However, crude lipid levels were similar in fish fed with sesame, sunflower and linseed oil. The fillet EPA and DHA levels were the highest in fish fed the control feed (P < 0.05). In contrast, the liver EPA level was the highest in fish fed the LO feed. Fish fed SFO feed had the highest level of total n-6 fatty acids in fillet and liver. Furthermore, the 20:3n-3 fatty acid, not detected in feeds, was existent in both the fillets and livers of fish. During the 12 days refrigerated storage period at 1 °C thiobarbituric acid (TBA), trimethylamin nitrogen (TMA-N) and pH values gradually increased in all dietary groups (P < 0.05). However, the chemical indicators of spoilage, TBA, TMA-N and pH values were in the limit of acceptability for human consumption. Fillet of fish fed feeds including sesame, sunflower and linseed oils were resistant to oxidative rancidity during the refrigerated storage period. Results of the present study indicated that sesame, linseed or sunflower oils could be a potential used in feeds for rainbow trout.

Keywords: Rainbow trout, nutrition, FAs, growth, refrigerated storage, flesh quality.
IODINE VALORISATION OF RAINBOW TROUT FLESH BY DIETARY SUPPLEMENTATION WITH THE SEAWEED GRACILARIA VERMICULOPHYLLA

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This study evaluated the effects of increasing dietary inclusion levels (0, 5 and 10%) of Gracilaria vermiculophylla in rainbow trout growth performance and flesh quality. Three isonitrogenous (42%) and isoenergetic (17 kJ/g) diets (CTRL, G5 and G10) were fed to triplicate groups of 25 fish (67 g) reared at 16 °C for 91 days. Sensory attributes, instrumental colour and iodine content (ICP/MS) were analysed in the muscle. Carotenoids were determined and identified (HPLC-DAD) in the skin and muscle. All groups of fish triplicated their initial body weight, and fish fed G10 showed a significantly lower final body weight than all other treatments. Feed efficiency and protein efficiency ratio in G10 group were significantly lower than CTRL but similar to G5. Protein intake of G10 diet was similar to CTRL and G5 treatments, but lipid and energy intake were significantly higher. Whole body protein content of fish fed G10 diet was significantly lower than the CTRL. G10 diet induced the lowest protein retention, whereas fish fed G5 had the highest lipid and energy retention. The inclusion of Gracilaria significantly increased the skin carotenoid content of fish fed G10 (16.7 μg g⁻¹). The two major carotenoids identified in the skin, β-carotene and lutein esters, showed similar levels among treatments, but zeaxanthin (0.1 μg g⁻¹) and astaxanthin (0.2 μg g⁻¹) increased significantly in the skin of fish fed G10 compared to CTRL (0.05 and 0.09 μg g⁻¹ respectively). In the muscle, total carotenoid content was lowest in fish fed G10 (1.0 μg g⁻¹) and a significant reduction of lutein and zeaxanthin was observed in these fish. Instrumental colour showed that raw and cooked fillets were more luminous (L*), less yellowish (b*) and more redish (a*) with seaweed inclusion. G10 samples yield the lowest chrome intensity (C*), confirming the lowest carotenoid content in these fish. The sensory evaluation of cooked fillets showed that fish fed seaweed diets are juicier than the CRT with G5 presenting the most intense (pinkish) colour. The instrumental differences in terms of a* and b* values are balanced in such a way that the sensory panel perceived G5 as the sample with higher colour intensity. Iodine levels in the flesh increased with the seaweed inclusion, with fish fed G5 doubling its iodine content (214.5 μg kg⁻¹) in relation to the CTRL (111.7 μg kg⁻¹). The inclusion of Gracilaria meal in diets for rainbow trout is possible up to 5%, as higher inclusion levels reduced growth performance of the fish. The sensory panel perceived G5 as the sample with higher colour intensity and juicier than the CTRL. Flesh iodine levels doubled in this fish which can greatly contribute to the valorisation of rainbow trout flesh.
Escaping of farmed Atlantic salmon is a serious problem in Norwegian aquaculture, due to the effects this may have on our stocks of wild salmon in our many rivers along the coast. Labeling of the farmed fish is thus of high interest, and several different principles have been investigated, including internal electronically markers, different genetic markers and DNA fingerprints. The main object of our experiment has been to develop a method that, both easily and cheaply, can distinguish farmed from wild salmon, and importantly: at the same time track the salmon back to the farm!
We decided to study the possibilities of adding chlorides of rare earth elements (REEs) to the feed. Based on the general “boneseeking” characteristic of REEs these elements would accumulate in bone structures such as scales. In our feeding studies with 1 and 0+ salmon smolt (start weight about 100 grams) we used 5 different REEs, alone or in combination: Lantanium (La), Cerium (Ce), Prasodynium (Pr), Neodunium (Nd) and Dysprosium (Dy). The labeling feeds contained 125 or 250 mg REE/kg feed and were used in only 5 to 10 weeks after transfer to seawater. Scales from the fish were analyzed by ion-coupled mass spectrometry (ICP-MS) before start and after 5 and 10 weeks of feeding, and then after 2 or 4 months of feeding this salmon ordinary commercial feed (“Washout” period. End weight about 500 grams). Growth and feed factor were not influenced, and no mortalities were observed. Scales turned out to be labeled by all five elements tested. The concentration in scales increased gradually during the feeding period, and no principal differences were found between the two smolt types. Combining two REEs in the same feed did not significantly affect the incorporation of either of the two elements. After a “washout” period of two to four months the concentrations of the used elements were still significantly higher than the background. The best results were obtained with the elements where the background levels were lowest: With Dysprosium the concentration in scales after 10 weeks of feeding on the label feed were 30 times higher than the background!
Scales are easy to collect, so sampling can be done by unskilled personnel. They are removed non-lethally and hence allow for multiple sampling during a fish’s life. Scales also require minimal preparation before analysis.

As a conclusion, we have demonstrated that by adding small amounts of Rare Earth Elements to the feed during a short period, salmon smolt from different farms can be differently labeled, and that this label will “survive” for several months. The method is quite simple, and no extra handling of the fish is needed.
Atlantic salmon (Salmo salar) with mean weight 1131 g was force fed one single meal (approximately 0.8% of body weight) of dry or soaked (2 h in sea water) feed in triplicates. The dry feed was of commercial-like extruded quality. The measured pellet hardness was 52.3 and 54.3 N, respectively, in dry and soaked feed. Pellet size was 10 mm. The fish was fasted for 2 to 3 days prior to the trial and was not fed after the single meal. Mean temperature was 13.4 °C. Samples from stomach and small intestine were collected 2, 6, 12, 18, 24 and 48 h after the feeding. Two hours after feeding, the gastric evacuation rate was significantly higher in salmon fed soaked diet than in those fed the dry diet. In a separate trial, quadruplicate groups of Atlantic salmon with mean weight 1047 g was fed two extruded feeds with different physical quality to satiation. Each feed quality was produced in three batches to contain three different markers (La, Yb and Y). The measured hardness for Diet 1 was 128.5, 140.5 and 152.1 N for the batches added La, Yb and Y, respectively. For Diet 2, the corresponding figures were 148.2, 172.0 and 153.3, respectively. The pellet size was 10 mm. The salmon was fed one meal daily. The feeds added La was fed for a period of 26 days. On day 27, feeds containing Yb was given, and on day 28 and thereafter, the salmon was fed feeds with Y added. Temperature was 11.5 °C. Faeces was collected from the outlet water during 30 minutes intervals at 8, 16, 24, 32, 40 and 48 hours after the feeding on day 27, and analyzed for La, Yb and Y. The ratio of the markers was calculated as concentration of each marker divided by concentration of sum of markers. For both feed groups, some Yb appeared in faeces 8 h after feeding diets containing this marker, and amount of Yb peaked around 24 h and almost no Yb was left after 48 h. Sixteen hours after feeding, at the time when the change in marker concentrations had happened fastest, the relative concentration was lowest for La, and highest for Yb, in faeces from fish fed Diet 1, indicating a higher gastrointestinal transit rate for this feed.

Our data strongly suggest that physical feed quality and moisture content of feed affects the rate at which feed is digested in salmon, which may, at least in part, explain the effect of physical quality and moisture content on feed intake observed in previous studies (Aas et al., 2011; Oehme et al., 2013).

ADDING FLAVOUR COMPLEXITY TO FRESHWATER FarMED BARRAMUNDI, LATES CALCARIFER, THROUGH DIETARY MANIPULATION

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Flavour quality of aquaculture products is rapidly becoming the most important factor affecting the profitability of aquaculture industries in the developed world. Nutrition plays an important role in the organoleptic properties (flavour and aroma) of fish as key flavour compounds are passed into culture organisms from the diet. Conventional dietary ingredients are often devoid of critical flavour compounds resulting in products that are considered bland in comparison to wild caught seafood. Many marine algae are known to be rich in a wide array of important flavour compounds such as dimethylsulfide (DMS) and bromophenols. Ulva ohnoi is a widely cultivated marine algae revered for its culinary appeal and is known to be rich in a wide array of these potent flavour compounds. The effect of feeding U. ohnoi on the organoleptic properties of cultured freshwater barramundi was assessed. Sensory assessment was used to evaluate the organoleptic properties of fillets of freshwater barramundi reared on diets containing 0%, 10%, 20% and 30% of dried U. ohnoi. The sensory profile was assessed following 7, 14 and 21 days of feeding. The flavour of U. ohnoi-fed barramundi was described as being rich and complex in comparison to barramundi reared on a standard commercial diet and was sweeter and more desirable. Feeding with increasing U. ohnoi content resulted in barramundi developing a cooked crab aroma and a crab-like/seafood flavour. Feeding with a diet of 30% U. ohnoi for 21 days resulted in a 140% increase in the intensity of crab-like/seafood flavour when compared to barramundi reared on a standard commercial diet. A more than 7 times increase in the concentration of DMS was also observed in the flesh of barramundi fed a 30% inclusion of U. ohnoi for 21 days. Diets containing up to 30% U. ohnoi were readily accepted by the barramundi with changes to flavour being observed within seven days of feeding. Feeding cultured freshwater barramundi a diet containing the marine algae U. ohnoi was effective in altering the flavour profile of the fillets, imparting a rich and complex seafood flavour. The potent flavour compound DMS was associated with this change in flavour and is suggested as a key flavour compound in fish flesh. The use of specifically formulated finishing diets for the purpose of flavour enhancement has the potential to increase the value of many culture organisms providing new economic opportunities for aquaculture producers globally.
Atlantic salmon minimum EPA+DHA requirement determined by fish health and lipids in brain, retina, red blood cells and liver during long term feeding

Nini Sissener¹, Bente E. Torstensen¹, Ingunn Stubhaug², Grethe Rosenlund²

¹NIFES, Bergen
²Skretting, Stavanger, Norway

Aquaculture production of salmonids is increasing whereas availability of fish oil is relatively stable. The natural consequence is a decrease in dietary concentrations of EPA (eicosapentaenoic acid, 20:5n-3) and DHA (docosahexaenoic acid, 22:6n-3). Hence, it is critical to determine the minimum requirement of Atlantic salmon (Salmo salar) for these fatty acids to sustain high growth rates and fish health.

In two long term feeding trials during the seawater production cycle, Atlantic salmon were fed low dietary EPA and DHA in graded levels, from about 1.3 to 7.5 % of fatty acids (0.4-2.4 % of the diet). The first trial was run at two temperatures, 6 and 12 °C, while the second one was run at 12 °C.

The level of EPA provided in the feed appeared to be sufficient in all diet groups, as no differences were seen in polar lipid tissue concentrations of neither brain, retina, red blood cells (rbc) nor liver. For DHA, a reduction in tissue levels was observed with lower dietary supply. Dietary effects on brain and retina DHA were only observed in fish reared at 6 °C and not in fish reared at 12 °C, suggesting that requirement is temperature dependent. Tissue levels of omega-6 fatty acids, on the other hand, were affected in both brain and retina at both temperatures. The same was the case for DHA levels in red blood cells and liver. Hence, DHA appears to be the limiting n-3 fatty acid in diets where EPA and DHA are present in the ratios found in fish meal and fish oil.

To assess the physiological significance of the differences in tissue fatty acid status, the osmotic resistance of red blood cells was tested, hematological analyses were done, and a handling stress-test was conducted at the end of both trials. There were no differences in hematology, and rbc did not appear to be more fragile during osmotic stress despite the changes in fatty acid composition. In the first trial, fish fed the lowest level of EPA+DHA had elevated plasma cortisol concentrations compared to all other diet groups, both before and after the stressful treatment was applied, while this was not the case in the second trial.

Based on these results, it seems that EPA+DHA can be reduced from current commercial levels without affecting fish health. However, it is still uncertain what the exact minimum requirement level of EPA+DHA is. Despite no classical signs of essential fatty acid deficiency, results on the tissue levels corresponded well with growth results, and together indicate that 1-2% EPA+DHA of fatty acids may not be sufficient for Atlantic salmon, possibly depending on temperature.

References: Financial support from RFF-Vest (#217478) and NRC (#225086/E40) is gratefully acknowledged.
EFFECTS OF DIETARY ARACHIDONIC ACID ON LIPID METABOLISM AND HEALTH STATUS IN JUVENILE GRASS CARP, CTENOPHARYNGODON IDELLUS
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Arachidonic acid (ARA) plays important roles in regulating lipid metabolism, biological processes and immune responses. This study aimed to elucidate the effects of ARA on lipid metabolism and health status in juvenile grass carp. A 60-day feeding experiment was conducted using three isonitrogenous and isoenergetic semi-purified diets containing 0.03% (control group), 0.30%, 0.60% ARA. The FCR and IPF ratio in the 0.30% group were significantly lower than those in the control group (P<0.05), and the 0.30% group showed significantly higher protein efficiency compared to the control group. Meanwhile, dietary ARA decreased the fat content in hepatopancreas, and the whole body lipid content was significantly lower in the 0.30% group than in the other groups (P<0.05). The levels of LA, C18:3n-6, C22:4n-6, total n-6 PUFA and LNA in hepatopancreas, whole body, kidney and IPF were significantly higher in the ARA groups (P<0.05). The lowest serum ALT and AST activities were found in the 0.30% group, and showed significant difference compared with the 0.60% group (P<0.05). The serum T-chol and LDL-chol concentrations were significantly higher in the ARA groups (P<0.05). The serum TNFα and IL-6 concentrations decreased with the increased dietary ARA content and the 0.60% group showed significant lower TNFα concentration compared with the control group (P<0.05).

In the hepatopancreas, the mRNA expression of PPARγ showed significantly lower levels in the ARA groups (P<0.05), whereas only in the 0.30% group, it showed significant lower level in the IPF (P<0.05). Both in the hepatopancreas and IPF, the mRNA expression levels of FAS, FAD and ELO were significant lower in the ARA groups (P<0.05). The mRNA expression of TLR22 and MyD88 in kidney showed the highest levels in the 0.30% group (P<0.05).

However, in the hepatopancreas, the ARA groups showed significant lower mRNA expression levels of these two genes compared to the control group (P<0.05). Overall, the appropriate dietary ARA inclusion (0.30%) effectively suppressed the lipid accumulation and altered the key genes expression of lipid metabolism, improved the health status by decreasing the aminotransferase activities and pro-inflammatory cytokines concentrations in serum and increased the expression of immune-related genes expression in kidney of juvenile grass carp.

Keywords: arachidonic acid; Ctenopharyngodon idellus; PPAR; Cytokines; gene expression
MINIMUM REQUIREMENTS OF ESSENTIAL OMEGA-3 FATTY ACIDS IN ATLANTIC SALMON IN SEA WATER

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The very long chain (Vlc) n–3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are essential components in salmon diets. Due to shortage of fish oil on the international markets, the level of EPA and DHA are reduced in fish feed. There is therefore an urgent need to know the minimum required levels of essential fatty acids (EFAs) in fish diets for securing good growth, health and product quality.

The aim of the present study was to define the minimum requirements for EPA and DHA in Atlantic salmon. Fish were fed experimental diets with either five dietary levels of EPA, DHA or a 1:1 mixture of EPA plus DHA (0%, 0.5%, 1%, 1.5% and 2% in all diet groups), as well as a commercial control diet. Atlantic salmon of approximately 40g were kept in indoor tanks with sea-water for 27 weeks. The fish were sampled at start (40g), 200g and at 400g. There were no major differences in growth or mortalities between the different dietary groups, except for a reduced growth in the 0% EFA deficient group. The fatty acid composition of the diets was reflected in the fatty acid composition of different tissues and influenced the apparent retention of EPA and DHA in the whole body.

The time course of changes in fatty acid composition of muscle, liver and heart tissues was differently affected by the diets; with the heart responding more slowly to the essential fatty acids depleted diet than the liver and muscle fillet and thus showing a high capacity to conserve the level of DHA. A reduction in the Vlc n-3 fatty acids level of salmon in aquaculture is unavoidable. This study will present new knowledge on minimum dietary levels of EFAs necessary to prevent negative effects on fish growth and health.

The work was supported by Norwegian Research Council (NRC).
Due to sustainability issues and stagnating global levels of fish meal and fish oil, further growth in production of Atlantic salmon necessitates increasing use of other feed ingredients, in Europe primarily based on plant raw materials. A consequence of this is reduced dietary levels of the long-chain omega-3 fatty acids EPA (eicosapentaenic acid, 20:5n-3) and DHA (docosahexaenoic acid, 22:6n-3). Such diets have been tested in controlled tank experiments; however, fish in small-scale experiments are not exposed to the variety of different stressors and fluctuations in environmental conditions that occur during an actual farming situation. Hence, there was a need to test this out in full scale commercial conditions, during a complete sea water production cycle.

The trial was performed at the Centre of Aquaculture Competence (CAC), which is located at the site Langavika in Gardsundfjorden, Hjelmeland (Western Norway), 200m offshore in deep waters (250-300m). The site has a System Farm concrete barge and 12 steel cages (24 x 24m) from Marine Construction (Os, Norway), fitted with 20 m deep net pens. Six of these cages were included in the feeding trial. Initial stocking density was 50,000 fish/cage, and all fish were fed Skretting Supreme for 4 weeks after transfer, before introducing the experimental diets. Diets were formulated at two different levels of EPA+DHA: 7.5% of fatty acids, which at the time was a commonly used level in commercial feeds, and about 4.5% of fatty acids (decreasing with increasing pellet size), and fed to triplicate cages. An initial sampling was conducted (fish weight 150g), with further samplings of whole fish and fillet for fatty acid composition at 600g and 1200g. Additionally, extensive samplings were conducted at 2500g and just before slaughter (around 5000g), where blood and organ samples were collected for study of fish health and lipid metabolism. Vaccine side effects and cataract scores were also recorded.

During the trial, fish have been exposed to normal farming conditions, including handling and transportation stress, fluctuations in temperature, oxygen levels etc. The fish experienced moderate gill problems (proliferative gill inflammation), and bath treatment for sea lice has been applied several times during the production. Furthermore, there was an outbreak of pancreas disease (PD) in the farm during the summer months of 2013, making this a trial with natural disease challenges.

Production performance data including growth rate, FCR and mortality throughout the trial will be presented, as well as data on fatty acid composition, routine evaluation of slaughter quality and sensory evaluation by a trained panel. Additionally, selected results on fish health and lipid metabolism will be presented and discussed.

Financial support from NRC (#225086/E40) is gratefully acknowledged.
DO ATLANTIC SALMON NEED MARINE OMEGA-3 FATTY ACIDS DURING GROW OUT IN SEA?

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The nutritional requirement for marine omega-3 fatty acids (FA) in fast growing Atlantic salmon (Salmo salar) during grow out in sea is not well documented. A requirement around 0.5-1% of diet dry weight has been suggested (Sargent et al., 2002; NRC, 2011), but the requirement can possibly be covered by dietary 18:3n-3. Availability and sustainability concerns have caused reduced inclusions of both fish meal and fish oil in commercial salmon diets, resulting in lower dietary levels of marine omega-3 FAs. Thus it is important to document whether salmon have a minimum requirement for these FAs or if it can be covered by 18:3n-3.

Diets were formulated with only 10% fish meal and graded levels of fish oil (0-35% of added oil) resulting in dietary levels of EPA (eicosapentaenoic acid, 20:5n-3) and DHA (docosahexaenoic acid, 22:6n-3) ranging from 1.3 to 7.5% of FAs (0.4-2.4% of the feed in the 9mm pellets). The rest of the added oil was a blend of plant oils.

Two trials were conducted. In the first trial the experimental diets were fed to fish at 12°C from 150 to 1500g, before the dietary groups were split and continued at both 6 and 12°C until they reached a final weight of 3500g. In the second trial, fish were first fed one diet low in EPA and DHA (3% of total FA) before they were fed the experimental diets from 1450 to 3500g.

Mortality was low and independent of diet in both trials. In the first trial, growth was similar in all dietary groups until 1500g. During the final stage, specific growth rate was significantly lower in fish fed the lowest EPA+DHA diet (1.3% of total FA) compared to the group receiving 5.3% EPA+DHA. Results were similar at both temperatures. In the second trial growth was significantly lower in fish fed 1.3-2.7% compared to 4.4-7.5% EPA+DHA of total FA.

FA-composition in the fish reflected diet composition, but it took 6 months and a 7-fold increase in body weight before the FA-profile of the fish stabilized according to dietary FAs. The retention of DHA (1500-3500g bw) was 120-190% increasing with decreasing dietary levels in trial 1, whereas DHA retention only reached 90-100% in trial 2. The retention of EPA was lower (40-130%) and values >100% was only achieved at the lowest dietary level in trial 1. Temperature did not affect FA retention.

These results suggest that Atlantic salmon have a specific requirement for marine omega-3 FAs for optimal growth in sea at around 3% of FA, and that short term growth trials would not have shown these effects. Furthermore, salmon can be a net producer of DHA dependent on the initial status in the fish and dietary supply.

Financial support from RFF-Vest (#217478) and NRC (#225086/E40) is gratefully acknowledged.
Salmonid aquaculture is the single largest consumer of marine fish oil. Pressure is building on the industry to move towards aquafeed formulations that use this resource more efficiently while maintaining a product of high nutritional quality. This requires not only a strong understanding of fishes real requirements for omega-3 long chain polyunsaturated fatty acids (n-3 LC PUFA), but also knowledge of the metabolic fate of these fatty acids and resulting tissue levels. Though commonly grouped together as n-3 LC PUFA, we know that eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have considerably different physiological functions and that the availability of one likely influences the requirements of the other. In order to optimise the use of these critical fatty acids in salmonid diets we need to determine requirements for these fatty acids individually as well as combined and the relationship between the two. Furthermore, examining the resulting impacts on lipid metabolism and n-3 related gene expression will contribute towards better understanding the underlying biological mechanisms involved.

Eight experimental diets were formulated and fed twice daily to Juvenile Atlantic salmon for 14 weeks at 10°C. Diets consisted of a Fish oil based positive control diet (PC), a Tallow based negative control diet (NC) and experimental diets based on Tallow supplemented with EPA, DHA, or both at 50% or 100% of the concentration observed in PC (Tallow + 50% EPA, Tallow + 100% EPA, Tallow + 50% DHA, Tallow + 100% DHA, Tallow + 50% BOTH, Tallow + 100% BOTH). Growth performance results revealed weight gain in EPA 50% and EPA+DHA 100% was significantly reduced compared to NC, EPA+DHA 100% was also reduced compared to DHA 50%. Food conversion ratio was significantly higher in EPA 50% compared to PC. Tissue nutrient and fatty acid composition, apparent in vivo fatty acid metabolism and hepatic gene expression will be presented and discussed. It is envisaged this study will contribute towards increased understanding of salmonid n-3 requirements and metabolism and more efficient aquafeed formulations.
Taurine has been reported to be efficacious in supporting growth of carnivorous fish species, particularly when supplemented to diets primarily containing plant feedstuffs. Although taurine may be destroyed to some extent by heat and moisture, and is susceptible to the Maillard reaction with reducing sugars, information is unavailable at this time on the stability and bioavailability of taurine subjected to extrusion processing in the manufacture of compounded fish feeds. Therefore, a feeding trial was conducted with juvenile red drum to determine the bioavailability of synthetic taurine in diets processed by cold pelleting and extrusion technology.

Experimental diets were formulated without animal ingredients to contain 38% digestible protein and 20% lipid. Diets were supplemented with taurine at either 0, 0.5, 1, 2 or 4% of diet. Diet mash at each taurine level was processed by either cold pelleting or extrusion cooking with a twin-screw extruder at the US Fish and Wildlife Service, Bozeman Fish Technology Center, Bozeman, Montana, resulting in a total of 10 different dietary treatments. Across all diets pre-extrusion taurine concentration was 98.8% of target values with a coefficient of variation of 4.1%. Post-extrusion taurine concentration was 98.9% of target values with a coefficient of variation of 2.4%.

Groups of 12 juvenile red drum (averaging 4.3 g/fish) were sorted by size and stocked into each of 30, 38-L aquaria as part of an indoor brackish water (6-7 ppt) recirculating system. Each diet was fed to three replicate groups of fish twice daily at rates close to apparent satiation for 6 weeks. Fish were sampled weekly to monitor weight gain and adjust rations. At the end of the trial, three fish per aquarium were collected for determination of body condition indices and whole-body proximate composition. Three additional fish per aquarium were bled at 5 hours post-prandial for determination of plasma taurine concentrations, and muscle samples were also collected and analyzed for taurine. Red drum showed a general improvement in weight gain and feed efficiency with the supplementation of taurine up to 1% of diet. Proximate composition and condition indices of representative fish were not affected by dietary taurine concentration as was plasma and muscle taurine. Plasma and muscle taurine concentrations showed that extrusion processing significantly reduced the bioavailability of taurine on average by 40 and 45%, respectively. Therefore, based on results of this study, red drum generally benefited from taurine supplementation of a plant-based diet, and extrusion processing reduced taurine bioavailability.
Three 9-week feeding trials were conducted to investigate the dietary methionine requirements of blunt snout bream, *Megalobrama amblycephala* for different growth stages (initial weight 3 g, 50 g and 100 g respectively) at a constant dietary cystine level. For each feeding trial, six semi-purified diets (340 g kg\(^{-1}\) CP and 60 g kg\(^{-1}\) lipid) were formulated to contain graded dietary methionine levels from 3.9 g kg\(^{-1}\) to 15.4 g kg\(^{-1}\) in about 2.5 g kg\(^{-1}\) increments which was replaced with glycine. After the feeding trials, the survival ratio was not affected by dietary methionine level at different growth stages. However, specific growth rate (SGR) and protein efficiency ratio (PER), and feed conversion ratio (FCR) were significantly affected by dietary methionine concentrations at all the growth stages. Juvenile Fish (initial weight 3 g) fed the grade level of methionine demonstrated a significant improvement in plasma methionine and total essential amino acid contents, while the similar trends were observed in the bigger fish (initial weight 50 g and 100 g), but not in a significant manner. In juvenile fish, the dietary methionine supplementation also affected the taurine contents in the muscle, liver, intestinal, brain and eye. Analysis of dose response with broken-line regression on the basis of SGR, the optimum dietary methionine requirement of juvenile *M. amblycephala* was estimated to be 8.4 g kg\(^{-1}\) of diet (24.7 g kg\(^{-1}\) of protein) for 3-25 g juvenile fish, 6.3 g kg\(^{-1}\) of diet (18.5 g kg\(^{-1}\) of protein) for 50-130 g fish, and 6.4 g kg\(^{-1}\) of diet (18.8 g kg\(^{-1}\) of protein) for 100-250 g fish respectively.
Meeting dietary protein requirements in cultured fish corresponds to the increasingly well-understood requirements for specific essential amino acids (EAA) (NRC, 2011), which has become a priority given the current constrains on fish meal (FM) availability and cost and thus the need to replace it by plant protein (PP) sources limiting in one or more EAA. Considerable progress has been made in finding suitable alternative protein sources to replace FM in aquaculture feeds. Besides, opportunities may also exist to reduce total protein in aqua feeds by supplementing economically feasible limiting EAA. Protein is one of the most expensive 'nutrient entities' in formulated fish diets. High dietary protein levels are also associated with negative environmental impacts due to potential high nitrogenous losses. Therefore, reducing dietary protein levels and maximizing protein utilization by the fish is a priority both from an economical and an environmental perspective. Hereby, we investigated the extent to which supplemental EAA allow for cost-effective reduction of dietary crude protein level in gilthead seabream diets. For this purpose, 20g fish were fed twice a day until apparent visual satiety for 3 months with one of three isoenergetic (21 MJ/kg) diets having a crude protein (CP) level of 45% (45CP), 40% (40CP) or 35% (35CP). The EAA and EAA to non-essential amino acids ratio (EAA/NEAA) of the diets was balanced through supplementation with limiting EAA. Gilthead seabream performed equally well with diet 45CP or diet 40CP but feed conversion rate (FCR) was significantly increased and specific growth rate (SGR) reduced by reducing CP from 45 down to 35%. Nevertheless, protein efficiency ratio (PER) did not differ among diets. Altogether, results show that CP level can be successfully reduced to 40% in diets for juvenile gilthead seabream provided that dietary amino acid profile is balanced but an additional reduction in the CP level requires further optimization of the nutrient profile of the diets.
DEUTERATED WATER (2H2O) AS A TRACER FOR FISH CARBOHYDRATE METABOLISM - "HEAVY" LEARNING WITH EUROPEAN SEABASS DICENTRARCHUS LABRAX

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The underlying principles of blood glucose (BG) enrichment with deuterium (2H) from deuterated water (2H2O - also known as "heavy water") are well described and validated for mammals since the mid 90's. The advent of modern aquaculture and consequent demand for new fish models to study nutrition contributed to the application of various techniques until then restricted to cell culture and mammal studies. Carbohydrate (CHO) metabolism was particularly attractive since substituting costly fishmeal with less expensive plant-derived substrates could bring economic and environmental benefits to the industry, besides being a scientifically challenging topic in the case of carnivorous fish such as the seabass (Dicentrarchus labrax).

Deuterated water is a relatively inexpensive non-radioactive tracer and is ideally suited for fish since it can be incorporated into tank water for an indefinite period. It rapidly equilibrates with the fish body water (in less than 6h), distributes evenly into all tissues and gets incorporated into hepatic metabolites such as glucose-6-phosphate (G6P). Appearance of glucose 2H-enrichment from 2H2O can be determined by mass spectrometry measurements in a sensitive and rapid fashion using a volume as low as a blood drop. Due to poor sensitivity, 2H Nuclear Magnetic Resonance is dependent on more abundant samples, but compensates with refined positional enrichment information (Viegas et al. 2011).

After 72h in 2H2O after a feeding/fasting/refeeding protocol (BG in mM: 10.7±6.3 / 4.8±1.2 / 9.3±1.4, respectively) G6P-derived glucose accounted for 65±7% and 44±10% of BG appearance in fed and refeed fish, respectively, with the remaining (unlabelled) fraction assumed to be derived from dietary CHO. For fasted fish, despite the unavailability of dietary CHO, BG appearance also had significant contributions from unlabelled glucose (52±16%). In all conditions BG appearance from endogenous G6P was mainly derived by gluconeogenesis (Viegas et al. 2013). In the same feeding/fasting/refeeding protocol (hepatic glycogen in g 100g-1 liver: 3.0±0.9 / 0.7±0.4 / 3.5±0.4, respectively) revealed that the rapid repletion of hepatic glycogen after refeeding was completely sustained by de novo gluconeogenesis. Direct pathway conversion of dietary CHO to glycogen was not detected in any of the groups (Viegas et al. 2012).

Exciting results point that high-CHO diets can spare up to ~30% of gluconeogenic substrates on both BG and hepatic glycogen production, being an effective and direct estimation for a diet's protein-sparing effect (in prep). Up until now, CHO metabolism was largely informed by relating measurements of activities and gene expression levels of key enzymes with changes in BG. While this approach has provided important insight it did not directly unravel its metabolic processes. Despite certain technical and methodological limitations, 2H2O has proven to be a reliable tool able to do so.

The objective of this study was to compile database of apparent digestibility coefficients of the nutrients and energy (ADCs) for plant protein ingredients fed by sea bass (marine carnivores) and tilapia (warm water omnivores), and selected linear model procedures as the statistical framework to model the relationships between ADCs and dietary plant protein level. A database containing plant protein sources digestibility coefficients were collected from 31 studies. The incorporation level of the plant protein sources (PPS) were calculated for each treatment. Simple linear regression analysis to evaluate the relation between predicted (y) and observed (x) values of ADCs of the nutrient performed. With linear regression the coefficient of determination \( R^2 \) illustrates how well the regression line represents the data, whereas the root mean square error (RMSE) indicates the magnitude of variation.

Using dietary plant protein sources level as indicators to predict ADCs in tilapia and sea bass diets, most of the error was attributed to random disturbance ( >90%), indicating a lack of either slope or mean bias errors and high accuracy model.

The results obtained indicated that increasing the incorporation level of plant protein ingredient in sea bass diets have negative effect on the ADC of lipid \( R^2=0.67 \). The models yielded to predict the ADCs of nutrients and energy in sea bass diets based on the incorporation level of plant protein sources (PPS) were as follow:

- Apparent protein digestibility coefficient = - 0.2526* (Incorporation level of PPS) + 102.86
- Apparent lipid digestibility coefficient = -0.2*(Incorporation level of PPS) + 100.13
- Apparent energy digestibility coefficient = -0.2542*(Incorporation level of PPS) + 93.723
- Apparent dry matter digestibility coefficient= -0.2294*(Incorporation level of PPS)+ 85.359

The results obtained indicated that increasing the increasing the incorporation level of plant protein ingredient in tilapia diets have negative effect on the ADC of protein \( R^2=0.74 \). The models obtained to predict the ADCs of nutrients and energy based on dietary plant protein sources (PPS) level of the ingredients were as follow:

- Apparent protein digestibility coefficient= -0.233*(Incorporation level of PPS) + 97.778
- Apparent lipid digestibility coefficient = -0.3898*(Incorporation level of PPS) + 100.93
- Apparent energy digestibility coefficient=-0.2965*(Incorporation level of PPS) + 87.865
- Apparent dry matter digestibility coefficient= 0.0972*(Incorporation level of PPS) + 61.031.
As carnivorous aquaculture species, turbots (Psetta maxima) have got an extended need of crude protein (CP). This demand is commonly met by diets containing high amounts of fish meal. Due to the low sustainability, alternative protein sources are increasingly utilised at present, with potentially adverse effects on supply of sulphuric amino acids (Methionine, Met; Cystine, Cys). Whereas high proportions of legume proteins lead to deficiencies, high amounts of canola or gluten proteins cause oversupply of Met+Cys with potentially negative effects. To our knowledge no information is available about the range of sulphuric amino acid supply, within maximum performance can be expected. Therefore, the aim of this study was to define this range of supply, where outside a lower or higher Met-supply leads to adverse performances.

Methods: In threefold repetition nine different isonitrogenous and isoenergetic diets were fed to tanks of 60L once daily until apparent satiation, stocked with 12 juvenile turbots (mean live weight 25.8 ± 3.0 g), respectively. Met+Cys concentration of seven diets ranged between 0.8 % and 2.0 % (on dry matter (DM) basis), realised by 0.2 % increments of L-Met supply at a constant Cys concentration of 0.3 %. Two diets contained 4.0 and 6.0 % Met+Cys to cover the range of oversupply. Growth and feed intake were recorded for 56 days and expressed as % of body weight per day (relative feed intake, RFI; relative growth, RG). Additionally, the intermediate of the Met-metabolism, S-Adenosylhomocysteine (SAH), was measured in a pooled liver sample per tank. A segmental linear regression model was applied using R software 3.0.2 to determine the plateau phase.

Results: According to the model applied, Met+Cys concentration for an optimum RG ranges between 1.25 % (±0.02 S.E.) and 2.68 % (±0.21 S.E.) in diet DM (2.26 – 4.85 % Met+Cys in CP). For RFI the optimum range lies within 1.23 % (±0.03 S.E.) and 2.33 % (±0.43 S.E.) Met+Cys in diet DM (2.23 – 4.19 % in CP). SAH values showed an inversed distribution compared to RG and RFI.

Conclusions: In this study, based on the lower and upper thresholds, amounts of necessary Met supplementation in deficient diets and maximum inclusion rates of protein sources rich in Met+Cys can be derived for turbot. Due to the relative high S.E. of the upper threshold value further studies are required to improve its precision. The levels of SAH, as well as further intermediates, seem to be relevant factors for the toxicity of an oversupply with sulphuric amino acids.
The complete removal of fishmeal and fish oil is required to promote the sustainable development of aquaculture and for that, fast growing high quality fish that are fed without fishmeal and fish oil are necessary. Early nutritional programming may allow the production of fish better adapted to utilize diets with vegetable meals and oils. Marine fish have a very low bio-conversion ability to produce the omega 3 highly unsaturated fatty acids (n-3HUFA) and the lack of these essential fatty acids in plant oils restrain their use as lipid sources. Nevertheless, plant oils are high in the precursors of n-3HUFA.

In previous studies, it has been seen that, thorough the diet and certain environmental factors, it is possible to regulate the expression of genes of enzymes bio-converting these precursors, although not to such extend to cover the essential fatty acid requirements (Izquierdo et al., 2008). In mammals, dietary fats have been recognised as potent epigenetic factors that exerted at a critical developmental stage early in life may have long-term consequences to the physiological functions later in life (Lima et al., 2011). Therefore, nutritional conditioning of fish during early life stages would allow fish genome adaptation for a better utilization of diets without fishmeal or fish oil (Guerden et al., 2007; Vagner et al., 2009).

This presentation will review a series of studies in seabream aimed to define which dietary factors influence the epigenetic profile, where are the most sensitive time-windows along life-span and the length of intervention required to be able to program fish for a better utilization of plant meals and oils. For that purpose we conducted three types of programs by early exposure of embryos, larvae or post-larvae to different diets. After several months of monitoring growth and metabolism, fish were challenged with either plant diets or fishmeal diets. The studies demonstrated for the first time that it is possible the early programming of fish through broodstock feeding for a better use of vegetable meals and oils and demonstrated the very long-term effects resulting from parental epigenetic control of embryogenesis, even along the whole life cycle of the fish.

Vagner M. et al., 2009. Ontogenic effects of early feeding of sea bass (Dicentrarchus labrax) larvae with a range of dietary n-3 highly unsaturated fatty acid levels on the functioning of polyunsaturated fatty acid desaturation pathways. Brit J Nutr 101, 1452-1462.
EFFECTS OF PHOSPHOLIPIDS ON GROWTH, LIPIDS TRANSPORT AND PHOSPHOLIPIDS METABOLISM OF LARGE YELLOW CROAKER (LARMICHTHYS CROACEA)

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The study was conducted to investigate the effects of dietary phospholipids (PLs) on growth, lipids transport and phospholipids metabolism of Larmichthys croacea. Three isoprotric and isolipidic diets were formulated with graded levels of PLs (2.53%, 4.75% and 11.86%) for larvae. The diets and live copepod were randomly allocated to triplicate groups of larvae (initial body weight 3.86±0.24) eight times daily for 30 days. Five isoproteic and isolipidic diets were formulated with graded levels of PLs (1.3%, 3.0%, 4.8%, 7.4% and 9.6%) for the juvenile. The juvenile (initial average weight 7.36±0.33g) were fed twice daily to apparent satiation for 60 days. Results showed dietary PLs significantly affect survival, special growth rate (SGR) and body length of Larmichthys croacea larvae. When dietary PLs content was 2.53%, the survival, SGR and body length were significantly lower than control group, PL-4.75% and PL-11.86%. However, dietary PLs had no significant effects on survival and SGR of Larmichthys croacea juvenile. In larvae stage, the expressions of MTP among treatment groups were significantly lower than control group. During juvenile stage, when dietary PLs content was 4.8%, the expression of MTP was significantly higher than other treatments. The expression of phospholipids metabolism related genes, such as choline phosphate cytidylyltranferase (CCT), choline phosphotransferase (CPT), diacylglycerol Oacyltransferase (DGAT), phosphatidylethanolamineN-methyltransferase (PEMT) and cytosolic phospholipase A₂ (cPLA₂) were not significantly affected by dietary PLs in larvae stage. But the expression of LPCAT and secreted phospholipase A₂ (sPLA₂) in treatment groups were significantly lower than control group. During juvenile stage, the expression of CCT, CPT, DGAT, LPCAT, PEMT, cPLA₂ and sPLA₂ were not significantly affected by dietary PLs. In conclusion, beneficial effects of dietary PLs on growth in Larmichthys croacea were restricted to the start-feeding fry stage. Moderate dietary PLs can up-regulate expression of lipid transport related gene of the juvenile. The effects of dietary PLs on the expression of phospholipids metabolism related genes in the larvae and juvenile are not significant in transcriptional level.
DIETARY IMPACTS OF NEW MARINE P INGREDIENTS FROM HERRING BYPRODUCTS ON GROWTH, MINERALIZATION AND SKELETAL DEVELOPMENT IN ATLANTIC SALMON (SALMO SALAR L.) FRY

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The aim of the study was to develop new marine P ingredients from fish bone fraction of herring by-products (head- and backbone) by hydrolysis in a strong acid (H₂SO₄), and to evaluate the dietary impacts of the ingredient on growth, mineralisation and skeletal development in Atlantic salmon fry. Five experimental diets were produced from fish meal formulated basal diet, only differing in the dietary P source and P level; 0.55% soluble P (Low P control); and 2 graded levels of P (0.70 and 0.85% soluble P) from either fish bone hydrolysate (FBH1, FBH2); or from mono-Na-P salt (Na-P 1, Na-P 2). The diets were fed for 168 days, from start feeding (0.17g fish) until 33g size. Periodic and overall daily growth (SGR) did not show diet dependent differences (p > 0.05) except in the initial 6 weeks feeding period (0.2–0.8g) were fish fed FBH1 showed higher mortality and lower growth (p < 0.05) as compared to fish fed the Na-P diets. Mineralization of fish evaluated by whole body and bone ash and mineral contents showed diet dependent differences that related to dietary P level (p < 0.05), but not to dietary P sources (p > 0.05). Fish fed the low P diet showed clear P deficiency signs with significantly reduced tissue ash and mineral contents, reduced whole body Ca:P ratio and morphological deviation from the normal. No morphological deviation appeared in fish fed any of the P supplemented diets. Histological evaluation of the vertebrae of 15g fish revealed stagnation in cartilage development, with accumulation of mature chondrocytes, in fish fed Low P control diet. Half of the fish fed FBH1 had a phenotype similar to the positive control (Na-P 1) and the other half a phenotype similar to the negative control (Low P diet). In addition, FTIR showed that fish fed FBH1 had lower mineralisation and increased cross binding in the vertebral end plates. Fish fed FBH2 resembled the positive Na-P 1 control. Real time qPCR analyses confirmed the histological results, by showing up-regulating of col10a1 (marker for mature cartilage) in fish fed Low P diet and FBH. Osteocalcin (marker for mineralization) was also activated in fish fed Low P control diet and FBH1, possibly indicating a compensatory regulation in response to inadequate P. The dietary impacts on histology, FTIR and qPCR analyses did not appear at 33g size, indicating that direct and compensatory responses to the diets were stabilized in larger fish. In conclusion, results show that P solubilized from bone fraction of herring can be efficiently utilized for growth, mineralisation and skeletal development in Atlantic salmon fry, but that the P availability in FBH may be slightly lower than for mono-Na-P in the initial stages until the fish reach a size of about 15g.
Background Scenario: Over the years, feeding of nutrient dense high-energy diets (with high fat levels) has improved feed efficiency, growth rate and protein retention but also leads to increased fat deposition in salmonids (Sargent et al. 2002). Increased fat deposition in the body is also often associated with P-deficiency (Lall, 2002). Moreover, the faster growth rates achieved with such diets can affect growth allometry resulting in vertebral deformities especially under dietary P insufficiency (Fjelldal et al. 2012). It is felt that the possible interacting effects between dietary supply of fat and P on somatic and bone growth and on whole body P balance need better understanding.

Research question: Does this change in dietary scenario affect growth allometry and thus influence the onset of possible P deficiency affecting linear growth of vertebrae in fast-growing salmonids?

Hypothesis: High fat salmonid diets lacking higher available P levels will affect growth allometry, P balance and bone growth.

Methodology: Two levels of dietary fat (12 and 22%, LF and HF) and two levels of available P namely adequate and high (0.35 and 0.70%, AP and HP) were tested in a 2x2 Latin square design. Groups (n=30 fish) of rainbow trout juveniles (IBW: 49±1g) were distributed into 12 experimental units and each unit was randomly assigned one of the four dietary treatments (in triplicates). All treatments were fed equal restricted rations, twice a day for 12 weeks at 15ºC. Whole body (wk 6 and 12) and vertebral (only at wk12) samples (n=10 fish per tank) were collected for chemical and mineral composition analysis. Individual weight (W), standard length (SL) and caudal peduncle length (CL) were measured (n=20 fish per tank) at the end of the experiment for analysis of tail ratio (TR = CL/SL) and allometric length-weight relation (LWR) expressed as W=aL^b.

Results: HF treatments showed higher weight gain (WG), growth rate (GR) and SL than LF at both P levels (p<0.01) with no interaction (p>0.7). An insignificant (p=0.09) yet noticeable effect of P was observed on TR in HF treatments (HFHP > HFAP) but not in LF. Length exponent (b) from LWR was affected by P (p=0.006), fat (p=0.08) and their interaction (p=0.03). Within HF treatments, a significantly higher b value was observed in HFAP (3.4) compared to HFHP (2.7), but not within LF groups (2.4 and 2.5). Final WB-fat was affected by fat levels even at 6wk (p<0.01); at 12wk, effect of P levels became significant as well (p<0.01). Final WB-ash and P were influenced by both fat and P levels even at 6wk (p<0.05); and at 12wk, interaction appeared (p<0.05). In summary, higher WG, GR, SL and WB-fat along with a significant effect of P and/or fat-P interaction on TR, b, WB-ash and P were observed in HF treatments, but not in LF.

Conclusion: Higher WG induced by high dietary fat requires increased available P supply to assure proper allometric growth and WB mineralisation. Chemical and mineral analysis of vertebrae, plasma and serum underway will provide further responses into bone mineral metabolism.

PREPARING SALMONID DIETS FOR A CHANGING CLIMATE – A SUMMARY OF HIGH TEMPERATURE EXPERIMENTS ON ATLANTIC SALMON AND RAINBOW TROUT

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Longer and more frequent periods with seawater temperatures above the optimum for growth for salmonids cause unfavourable farming conditions along the Norwegian coast. Although large temperature fluctuations have been observed, the mean seawater temperature has increased and is predicted to continue to increase. Temperature affects appetite, metabolism and growth in fish, and temperatures above the optimum for growth results in a number of physiological and fitness consequences. In a large research project several short-term and long-term experiments with Atlantic salmon (Salmo salar L.) reared in seawater has been carried out to elucidate the effect of elevated temperatures on metabolism and growth regulation. The results indicate that larger fish are more affected by elevated temperatures compared to smaller fish, whereas a higher metabolism at elevated temperature resulted in an increased feed conversion ratio and reduced growth rate. The effect of temperature on growth regulation will be presented with emphasis on anabolic and catabolic actions mediated through the growth hormone (GH) – insulin like growth factor (IGF) axis; plasma IGF-I and IGFBP1b and corresponding tissue gene expression and receptors. A comparative experiment between Atlantic salmon and rainbow trout (Oncorhynchus mykiss) reared in seawater was performed to investigate potential differences in coping with high temperature. Both species had an increased utilisation of antioxidant vitamins at high temperature. A microarray analysis (44K cGRASP oligo array) of liver was done to elucidate the influence of elevated temperatures on a transcriptional level, and will be presented in relation to growth performance, tissue and whole body vitamin and free amino acid composition, in addition to endocrine regulation of growth and occurrence of production related disorders. The results from the present project will contribute to increase the understanding of how salmonids cope with elevated water temperatures, and thus contribute to strengthening the fish through tailored feeds. The present work has been carried out in collaboration with research institutions in Japan, Canada, USA and Norway, with support from the Research council of Norway, project no. 199683/S40 – "Local effects of global warming: Effects of period of higher temperature than the normal range for Atlantic salmon", 207155/E40 & 207184/E40, NIFES and the industrial partner Skretting ARC.
REDUCED DIGESTIBILITY OF ENERGY AND LIPID BY RAINBOW TROUT DUE TO INCOMPLETE GELATINIZATION OF DIETARY STARCH IN EXTRUDED DIETS
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Five extruded diets were produced with hydrolyzed wheat gluten (HWG), replacing graded (0, 12.5, 25, 50, 75%) levels of crude protein from LT fish meal. Due to the highly viscous material, the water addition during processing of the diet with 50% HWG was reduced from 24-25% to 13.5% compared with the other diets to be able to shape pellets. The diet with 75% HWG had no water added in the extruder, but was supplemented with 6.6% plant oil in the feed mesh, to reduce viscosity to obtain functional pellets.

The diet with 50% HWG had notably reduced expansion compared to the diets with lower content of HWP, moderately reduced water stability, and lower recovery (P < 0.05) (when fed into tanks with no fish in them). The diet with 75% HWG had highly reduced breaking force, lower water stability, and lower sinking rate in water than the other diets. The digestibility of energy and lipid were highly reduced in the diets with 50 and 75% HWG, in a second-degree polynomial manner. This is rationalized by low gelatinization and corresponding low digestibility of starch in these two diets. Reduced digestibility of lipid can be explained by the occurrence of indigested starch in the intestine, affecting the viscosity of the chyme in a similar manner as soluble non-starch polysaccharides.

In conclusion, complete gelatinization of starch in extruded diets should be sought when making fish feed, not only for digestibility of starch, and lipid, but also for the availability of other lipid soluble nutrients.
IN VIVO DIETARY SUPPLEMENTATION WITH ARGinine AND IN VITRO EXPOSURE TO POLYAMINE INHIBITORS AFFECTS METABOLIC PROFILING IN ISOLATED ATLANTIC SALMON LIVER CELLS

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Background: Arginine has been demonstrated to enhance glucose and lipid oxidation in mammals through activation of polyamine turnover. Arginine is the only precursor of the polyamines putrescine, spermidine and spermine, and may act through this pathway in fish via ornithine decarboxylase (ODC), the rate-limiting enzyme for polyamine synthesis. We investigated how arginine influences energy utilization and liver cell metabolism in salmon.

Methods: Primary liver cells were isolated from Atlantic salmon which had been fed diets containing low arginine (LA, 25.5 g/kg) or high arginine (HA, 36.1 g/kg) concentrations for 12 weeks, to investigate the effect of long-term arginine supplementation. The cells were cultured in L-15 medium to which was added either DFMO (alpha-difluoromethylornithine) or DENSPM (N\textsuperscript{1},N\textsuperscript{11}-diethylnorspermine), inhibitor of ornithine decarboxylase (ODC) and activator of spermidine-spermine acetyltransferase (SSAT), respectively, for 24 h. Cell culture media was profiled for metabolite content using 500MHz high field NMR (nuclear magnetic resonance) of the water soluble fraction. Metabolite profiling was done using Chenomx metabolic profiling software and statistical analysis using Simca-P software.

Results: In vivo supplementation of salmon diets with arginine did not result in weight change differences. When primary hepatocytes were grown in culture, analysis of the culture medium by NMR revealed significant differences between the two dietary groups (HA and LA, n=10 each) with $R^2$ and $Q^2$ of 0.85 and 0.45 respectively, demonstrating that the in vitro cells retained their in vivo phenotype. A variable importance calculation for the difference of HA and LA ranks the main variables glucose $>$ ornithine $>$ lactate $>$ arginine $>$ fumarate. Treating hepatocytes in vitro with polyamine inhibitors DFMO and DENSPM also resulted in a significant change in culture media metabolites ($R^2$ 0.98, $Q^2$ 0.92). DFMO inhibition of ODC resulted in increased hepatocyte media concentrations of lysine, pyruvate, glucose, fumarate and phenylalanine compared to controls and DENSPM treatment; effects related to arginine metabolism.

Conclusion: Primary salmon hepatocyte cultures in vitro retain their in vivo phenotype when arginine is the only dietary change. Arginine effects on cultured hepatocyte metabolism is not precisely mimicked by polyamine inhibitors, suggesting several pathways mediate arginine effects.
NEW ASPECTS ON ENERGY METABOLISM IN COMMON CARP (CYPRINUS CARPIO)

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In order to gain new fundamental knowledge of energy metabolism in common carp (Cyprinus carpio) two consecutive experiments were conducted. The first experiment focused on determining apparent nutrient, energy and amino acid digestibilities of wheat starch (WS), corn starch (CS), corn gluten meal (CGM), soybean meal (SM), soybean protein concentrate (SPC) and pea protein concentrate (PPC) applying the stripping methodology. Six test diets and a reference diet were fed until apparent satiation to triplicate fish groups two times a day for nine weeks. The reference diet consisted of fish meal, fish oil, wheat starch, a vitamin/mineral mix and titanium oxide as inert marker. Test diets included 70\% reference diet and 30\% test feed ingredients respectively. After analyses of nutrients and marker in feed and feces apparent digestibility of energy and nutrients of the ingredients (ADI) was calculated using the difference method. ADI of crude protein in SM (79\%) and SPC (75\%) were found to be higher than in CGM (59\%). Average ADI of total amino acids (TAA), of essential amino acids (EAA) and of non-essential amino acids (NEAA) were higher in SM (82\%/82\%/81\%) than in all other tested ingredients. Furthermore ADI\textsubscript{EAA} and ADI\textsubscript{TAA} in SPC (72\%/74\%) were higher than in CGM and PPC. ADI\textsubscript{NEAA} in SPC was higher than in CGM. ADI of tested amino acids of SM and SPC always showed higher values than those of CGM and PPC. ADI of N\textsubscript{E} in CS (90\%), WS (88\%) and CGM (82\%) were higher than in SM (26\%) and higher values were found in CS than in CGM. No significant differences between ADI of gross energy (70.3\%-83.17\%) were determined.

Based on the results of experiment 1 in the second study interactions between digestible energy (DE) contributed by dietary protein, fat or carbohydrates were evaluated using ingredients investigated in trial 1. Isoenergetic diets (16 MJ DE/kg) with different protein levels and fat/carbohydrate energy ratios were formulated and arranged according to the central composite design. All diets were formulated to contain minimum levels of EAA according to AMINOCarp\textsuperscript{®}. Carp were fed for 8 weeks 4 times a day at a feeding rate of 2.5\% body weight, which was adjusted bi-weekly. At termination of the trial body weights, liver weights and lengths were recorded and whole body samples were taken to analyze body compositions. All diets resulted in excellent performance with feed conversion ratios (FCR) lower than 1, specific growth rates (SGR) higher than 2.4 and protein efficiency ratios (PER) higher than 3.1. Data analysis revealed that fish fed diets with high lipid in combination with low protein energy or high carbohydrate in combination with high protein energy showed higher SGR compared to the other treatments. Best PER were achieved along with high dietary lipid incorporation.

Presented studies revealed that applying the stripping methodology used for digestibility measurement in carp is a viable method compared to conventional passive feces collecting technique. Moreover, energetic utilization of protein, fat and carbohydrates seems to be mainly influenced by protein supply suggesting a big influence of protein metabolism.
CURRENT NUTRIENT RECOMMENDATIONS ARE INSUFFICIENT FOR JUVENILE ATLANTIC SALMON (SALMO SALAR) FED DIETS WITH HIGH INCLUSION OF PLANT BASED INGREDIENTS

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Knowledge on quantitative requirements is for many nutrients limited to rainbow trout (Oncorhynchus mykiss) and common carp (Cyprinus carpio), and even for these species, the information is often limited to the juvenile stages (NRC, 2011). Consequently, there is a clear lack of knowledge on quantitative nutrient requirements for maximal growth performance and optimal health in Norway’s most important aquaculture species; the Atlantic salmon (Salmo salar). With a shift in dietary protein and lipid sources from fish-based to plant-based ingredients, there is a concomitant change in quantity and availability of a range of dietary micronutrients.

A study was designed where Atlantic salmon parr in the freshwater stage were fed 7 diets with graded levels of a nutrient premix (NP) added to a basic diet with a low content of marine ingredients (10% fish meal+ krill meal of the total recipe; 20% fish oil of the added oil; protein ingredients and oil mix were identical for all diet groups). The inclusion of the NP ranged from no addition to four times the current estimated requirement (NRC, 2011) for each nutrient. Methionine, histidine and taurine were included in the nutrient premix, as well as cholesterol, vitamin A, D, E, C, biotin, folate, niacin, pantothenic acid, vitamin B₆, riboflavine, thiamine, and cobalamine. Furthermore, the minerals selenium, iodine, copper, cobalt, manganese, iron and zinc were added to the nutrient premix.

The trial lasted twelve weeks, and fish grew from an initial weight of 18.3 g (± 2.2) to a range of 78.6 g (± 1.9) to 87.3 g (± 4.5). Both fish growth and protein accretion increased with increasing dietary nutrient premix, while lipid accretion decreased, together with liver index and viscera-somatic index, all indicating nutrient deficiencies in the groups fed diets with low addition of the nutrient premix. Tissue status for the different nutrients added was assessed, in addition to parameters of fish health, antioxidant status and stress resistance. Results for some nutrients gave indications of a revised requirement level within the current experimental design, while for others continuously increasing tissue status did not indicate a requirement level. The results will be discussed for the different nutrients and compared to the current recommendations. Finally, suggestions will be made for revision of nutrient requirements in modern diets for Atlantic salmon parr.

References: The study is part of the EU-project ARRINA (Advanced Research Initiatives for Nutrition and Aquaculture, 7th framework programme).
POSTER PRESENTATIONS

Posters will be located in the main exhibition and catering area of the symposium (Exhibition Hall C & D), Cairns Convention Centre.

Poster presentations are listed in alphabetical order by presenter surname.

POSTER 001
COMPARATIVE EFFECTS OF SUPPLEMENTARY EXOGENOUS ENZYMES ON GROWTH PERFORMANCE AND FEED UTILIZATION OF NILE TILAPIA, OREOCHROMIS NILOTICUS
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Supplementation of aquafeeds with exogenous enzymes is a recent development for fish and thus requires more research for refinement. Therefore, a study was conducted to evaluate comparative effects of exogenous enzymes on Nile tilapia (Oreochromis niloticus) with specific emphasis on growth performance, feed utilisation and health status. Tilapia (38.74 ± 0.51g) were fed one of four plant-based diets (40.8±0.2% protein, 7.8±0.3% lipid); one of which was a control and the remaining three diets were supplemented with exogenous enzymes (Ronozyme Hiphos CT (phytase), Ronozyme ProAct (protease) and Ronozyme G2 G (carbohydrase) at 0.3g kg⁻¹, 0.2g kg⁻¹ and 0.3g kg⁻¹ respectively). The tilapia were reared at 28°C and fed the experimental diets, with triplicate tanks per treatment for six weeks. Fish fed phytase, protease and carbohydrase supplemented diets performed better than those fed a control diet (P < 0.05), in terms of growth performance and feed utilisation. Body proximate composition, condition factor, hepatosomatic and visceromatic indices were not affected by dietary treatment. Also, haematological parameters were not affected by respective dietary treatments (P > 0.05). Thus, the supplementation of diets with exogenous enzymes have the potential to enhance growth performance without apparent impairment of tilapia health (P > 0.05). Histological analysis of gut and liver morphology as well as microbiology analysis of gut microbiota of the tilapia are on-going.
AMINO ACID COMPOSITION OF SOUTH AFRICAN DUSKY KOB (ARGYSOMUS JAPONICUS)

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Introduction
Argyrosomus japonicus is a carnivorous fish found in the southern and northern hemispheres (Griffiths and Hecht, 1995). Argyrosomus japonicus is also known as mulloway in Australia and dusky kob in South Africa. Attributes that makes it suitable for aquaculture in South Africa include their marketability, high fecundity, fast growth, non-territorial or cannibalistic nature (Fielder et al 1999). Dietary protein supply is one of the major factors influencing the productivity of all fish and it is an important constituent of diets. The essential amino acid requirement pattern of fish has been shown to correlate well with the essential amino acid pattern of the whole body tissue of that fish (Wilson and Poe, 1985). There are several reports to confirm that amino acid profile of whole body tissue of a given species of fish resemble those of the dietary requirements of the fish (Wilson & Poe 1985; Mambrini & Kaushik, 1995). The aim of this study was to analyse the whole body profile of dusky kob.

Materials and methods
Juvenile dusky kob Argyrosomus japonicus with average weights of 1.2 g were used for the analysis. Amino acid in whole body tissue of fish was determined using Waters AccQ Tag kits analyser, following hydrolysis in 6 N HCl for 22 hours.

Results and discussion
The amino acid data are expressed as g/100 g amino acids. Seventeen amino acids were identified. EAA identified were histidine, arginine, threonine, lysine, methionine, valine, leucine, isoleucine, phenylalanine and NEAA identified were serine, glycine, aspartic acid, glutamic acid, alanine, proline, cysteine and tyrosine. (Tryptophan was not detected). The most predominant amino acids were glutamic acid, aspartic acid, glycine and lysine (ranging from 8.12 % to 16.08 %). Of these amino acids, glutamic acid was the most predominant. Glutamic acid represented 16.08 % of the amino acid content of the whole body profile of dusky kob. Glutamic acid is essential for cell proliferation (Zhao et al. 2010). The same results in glutamic acid have been reported for rainbow trout, yellowtail flounder, Japanese flounder, Atlantic halibut, Atlantic salmon, Rainbow trout (Kim & Lall, 2000; Wilson & Cowey 1985). Cysteine was the amino acid with the lowest concentration in dusky kob. The ratio (expressed as percentage) of the essential amino acids to the total amino acids is 45.06 %. Lysine and leucine had the highest concentration among essential amino acids in Argyrosomus japonicus. Histidine was the essential amino acid with the lowest concentration in dusky kob. The whole-body AA values determined in the present study for juvenile dusky kob can be utilised to provide guidelines for formulating diets for this species.
Feed is the single largest cost to a prawn farm and therefore feed management strategies that maximise prawn growth but optimise feed utilisation are critical to the cost effectiveness of the farm. The two key components to any feed management strategy are the number of times fed per day (feed frequency) and the amount fed as a percentage of the estimated satiation level (ration). While a number of studies have investigated the effect of either feed frequency or feed ration on the growth of *Penaeus monodon*, there are no know studies on the combined effect. Furthermore, as new diets enter the market there is a need to determine the optimal administration strategies for such diets. The aim of this study was to evaluate juvenile *P. monodon* growth, survival and FCR at feed frequencies of 2 and 6 times per day and when feed ration is reduced from 100% to 80% and 60% satiation. This design was also compared between two diets; and industry standard and the same diet but with the inclusion of the growth enhancing additive Novacq™.

Juvenile *Penaeus monodon* with a mean weight of 3.10 ± 0.02 g were stocked into 100L tanks at a stocking density of 8 per tank with 5 replicates per treatment. Filtered seawater was supplied to each tank at a continuous rate of 0.6 L min⁻¹ and 29°C. The prawns were fed on the 12 different frequency x ration x diet combinations for 6 weeks and then individually weighed. The amount of feed fed was recorded throughout the trial.

Survival was above 90% for all treatments throughout the experiment and not significantly different between the main treatments effects. However, there were significant effects of frequency, ration and diet on prawn growth. When averaged across diet and ration, prawn growth was significantly higher (*P*<0.05) when fed 6 times per day (1.41 ± 0.05 g wk⁻¹) compared to 2 times per day (1.25 ± 0.05 g wk⁻¹). When averaged across diet and frequency, there was a significant increase (*P*<0.05) in prawn growth as ration increased (1.13 ± 0.04 g wk⁻¹ at 60%; 1.40 ± 0.05 g wk⁻¹ at 80% and 1.47 ± 0.06 g wk⁻¹ at 100%). When averaged across frequency and ration, prawn growth was significantly higher when the diet included Novacq™ (1.49 ± 0.05 g wk⁻¹) than without Novacq™ (1.18 ± 0.03 g wk⁻¹). There was also an interaction effect between frequency and ration, which demonstrated that the difference in growth between 2 and 6 feeds increased as ration decreased.

There was a significant frequency and ration effect on FCR but no diet effect. When averaged across diet and ration, FCR was significantly lower (*P*<0.05) when fed 6 times per day (1.22 ± 0.04) compared to 2 times per day (1.42 ± 0.04). When averaged across diet and frequency, there was a significant increase (*P*<0.05) in FCR as ration increased (1.18 ± 0.04 at 60%; 1.26 ± 0.04 at 80% and 1.52 ± 0.06 at 100%). The results demonstrated the growth benefits of feeding more than 2 times per day and the potential feed efficiency benefits of a ration below 100% satiation.
Mitochondrial membrane composition may be a critical factor in the mechanisms of aging processes by influencing the propagation of reactions involved in mitochondrial function during periods of high stress. Changes affecting either lipid class or fatty acid compositions could affect phospholipid properties and alter mitochondrial function and cell viability. Nutrition and feeding regimes are one of the more actively studied factors for their potential impact on metabolic rate, oxidative stress, disease and aging. In the present study, the effect of diet lipid composition on mitochondrial oxidative status, gene expression and membrane phospholipid compositions were analysed in zebrafish of two well differentiated ages.

Materials and Methods
The experiment was performed on zebrafish, *Danio rerio*, of two different ages (10- and 24-months-old) belonging to a resident colony. Fish were kept in 31 L or 13 L tanks (for young and old fish, respectively) at the same biomass density. Tanks were cleaned daily with approx. 1/2 of the water replaced each day. Photoperiod was set at 12L/12D and temperature remained constant throughout the experiment (26 ± 1 ºC). Fish were fed twice a day by hand (ration: 2% of body weight) for 8 weeks. Each age group was split into two experimental groups (per duplicate). One group was fed on a diet with rapeseed oil as the only source of lipid (RO diet) while the other group was fed a diet with a high-DHA oil (DHA diet). Both diets had the same protein and lipid contents (50 and 11%, respectively).

After the experimental period, fish were sacrificed and samples taken for mitochondrial isolation (whole fish) or gene expression (muscle, liver and brain). The purified mitochondrial isolates were analysed to determine lipid composition (phospholipid content and class composition, and phospholipid fatty acid composition) and lipid peroxidation (TBARs) of the organelle while tissues were analysed for mitochondrial gene expression (COXIII, ND3, ND4I, ND4 and ND5).

Results
The fatty acid composition of the diet significantly influenced the mitochondrial membrane lipid composition and lipid peroxidation of whole zebrafish and also affected mitochondrial gene expression in fish tissues. These effects were different in the two age groups. TBARS content was significantly higher in young fish fed the DHA diet. Mitochondria of young fish fed the RO diet showed higher expression levels of mitochondrial genes compared to that of the DHA group while no statistical differences were found in mitochondria from old fish tissues. Regarding mitochondrial lipids, diet composition affected mitochondrial membranes from whole fish, mainly those of young animals. Fish fed the DHA diet had higher levels of polyunsaturated fatty acids in their membranes this being associated with metabolic rate, free radical production and molecular damage.

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The study aimed to evaluate the effect of lysine in productive and reproductive performance, hormonal characteristics, body indices and total amino acid incorporation in oocytes and Rhamdia voulezi’s body confined in cages in the first reproductive cycle, for 185 days. We used 400 fish with an average initial weight of 35.18 ± 0.25 g, identified with tags and distributed in a randomized experimental design with four treatments and four replications in 16 cages with 25 fish each. The treatments consisted of four diets prepared to contain lysine levels of T1 = 1.20, T2 = 1.40, T3 = 1.60 and T4 = 1.80% with 30% crude protein and 3,500 kcal. kg⁻¹ digestible energy. It was randomly selected 18 females per treatment that had rounded abdomen, swollen and reddish urogenital papilla and cannulation intraovarian to verify the migration of the germinal vesicle, at the same time, males were selected for semen collection and subsequent artificial fertilization. The fish were weighed (g), measured (cm) and submitted to hypophysation with carp pituitary extract at a dose of 5.5 mg.kg⁻¹ of females. After 240 accumulated thermal units oocytes were collected (time of spawning) which were divided into parcels: Solution Gilson (oocytes diameter), frozen (amino acid analysis) and the remainder fertilized being arranged in conical incubators (20L) to assess other reproductive parameters. Then, the blood was collected (analysis of estradiol and cortisol) using syringes, the next, sacrificed by cervical column dislocation, dissected, where ovarian, liver, fat and viscera were removed, weighed and their indexes body were estimated. The female body was dried for amino acid analysis. For productive performance it was observed effects (p<0.05) for the mean weight and final length, weight gain, feed conversion and condition factor were observed, whereas treatment with 1.80% lysine showed the highest values of weight gain (84.80±18.88g) and lowest feed conversion ratio (0.74±0.13). The distribution of the diameters of oocytes before the hormonal induction was distinct and polymodal between the diets: T1 = 410, 590, 750 and 820 µm; T2 = 410, 570, 670, 790 and 880 µm; T3 = 410, 550 and 750 and T4 = 550, 750 and 850 µm. At the time of spawning the percent frequency distribution of the diameters was similar to polymodal for the diets with 1.20, 1.40 and 1.60% and bimodal for the diet with 1.80% lysine. The values average rates of fertilization and hatching were lower in T2 differing (p<0.05) of the others. The largest number of released oocytes (13,210±6,520 units) and weight of the remaining ovary (7.60±5.22 g) were recorded for the fish that received 1.80% lysine. Regarding hormonal characteristics was not significant (p>0.05) for cortisol and estradiol. Among the body indices only visceral fat was influenced (p<0.05) with lower value (0.90±0.54%) in the diet with 1.80% lysine. The lysine levels affected (p<0.05) the incorporation of amino acids in the body, however, the oocytes had no effect (p>0.05). Lysine has effect in productive and reproductive performance in visceral fat accumulation and incorporation of amino acids in the body.
POSTER 006
ENTEROCYTES TURNOVER AND INTESTINAL TROPHISM ACCORDING TO FRACTAL DIMENSION ANALYSIS IN COMMON SOLE (SOLEA SOLEA) FED DIETS WITH INCREASING MUSSEL MEAL LEVEL
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The intestine is the most important organ in digestion and absorption of nutrients and therefore monitoring of this organ is considered necessary in formulating new diets for cultured fish. However, the role of dietary ingredients in regulating intestinal homeostasis is poorly investigated, since most of the studies on the gut evaluated the inflammatory effects of certain plant ingredients. In fish, the morphological peculiarities of intestine, as the lack of distinct crypt compartments and the presence of folding of the mucosa require morphometric techniques. The fractal dimension (FD) calculation has been widely used in physics, biology and medical fields and is able to transduce shape complexity in analytical quantitative data. The present study was undertaken to investigate the effects of graded level of mussel meal (MM) dietary inclusion on growth performance and feed utilization in common sole (Solea solea) juveniles, evaluating the influence of this ingredient on the enterocyte turnover and intestinal trophism according to fractal dimension analysis. Four isoproteic (53%) and isolipidic (11%) diets were formulated to contain graded levels of MM (0%, 25%, 50% and 75%: MM0, MM25, MM50 and MM75, respectively) to replace fishmeal. Triplicate groups of 70 individuals each (weight 13 g) were hand-fed the experimental diets, to apparent satiation, over 91 days. Intestine was processed for routine histology at the end of the trial; immunohistochemistry (anti-PCNA antibody and TUNEL method) was performed and cellular kinetics indexes evaluated by image analysis. Fractal dimension was calculated by the Box Counting method on ImageJ 1.46 software. One-way ANOVA, Newman-Keuls’ post hoc test and linear regression analysis were used to analyze data (p ≤ 0.05). For all growth parameters and feed utilization indices, the linear regression produced a good R² (>0.69) and no departure from linearity was noticed. MM-based diets gave significantly higher Specific Growth Rate and Feed Intake and the lower Feed Conversion Rate when compared to the MM0. Protein Efficiency Ratio and Gross Protein Efficiency were significantly improved in fish fed diets containing MM. Histomorphological evaluation did not show signs of enterocytes degeneration or inflammation. Cell proliferation index and FD were significantly reduced with a diet high in MM, while apoptotic index did not show any significant difference for the same comparison. According to the results, MM is an effective ingredient for enhancing growth and feed utilization in sole. A relationship between reduced enterocytes proliferation and a lesser complexity of mucosal folding, expressed by a lower FD, resulted in fish fed MM75 diet. From this study, emerged that FD could be used as a numeric indicator complementary to in situ quantification methods to measure intestinal trophism, in conjunction with functional parameters.
Knowledge about the nutritional requirements of common sole (Solea solea) is limited and no information of optimal dietary lipid level is available yet. Thus, a study was undertaken to assess growth response and feed utilization of common sole juveniles fed diets with increasing lipid levels. Four isonitrogenous (59% protein) pelletized diets with different dietary lipid levels (8, 12, 16 and 20%; L8, L12, L16 and L20, respectively) were fed to triplicate fish groups of 80 individuals to apparent satiation, over 150 days. Fish were weighed at day 35, 70, 105 and 150. At the end of the trial, samples of fish were taken for proximate composition of carcass, somatic parameters and gut histology. One-way ANOVA, and Tukey’s post hoc test and linear regression were used to analyze data (P≤0.05). At the end of the trial, final body weight was significantly higher in fish fed L8 (40.7 ± 1.7 g), followed by those fed L12 (35.1 ± 1.2 g), L16 (27.9 ± 2.5 g) and L20 (22.1 ± 0.3 g). A similar trend was observed for final length. Specific growth rate was higher in fish fed L8 and L12 compared to the other treatments and it was the lowest in L20. Voluntary feed intake decreased with increasing dietary lipid level. Feed conversion rate of the fish fed L20 was the lowest and no significant differences between fish fed L8, L12 and L16 were noticed. Protein efficiency ratio and gross protein efficiency were lower in fish fed L20 while no significant differences were observed between L8, L12 and L16. The trend of gross lipid efficiency values was related to dietary lipid level. Viscerosomatic index was significantly higher in fish fed L16 and L20 than in those fed L8 and L12. No differences were found in the hepatosomatic index. Histology of intestine was evaluated and correlated to the different lipid level of the diets.

In conclusion, the results of this trial evidence a low lipid requirement by common sole juveniles. Increasing dietary lipid level led to a substantial decline in performance. This should be taken into consideration in formulating specific practical diets for this species.
A high dependence on fish meal and oil use in aquaculture feeds has led to the assessment and development of a range of grain protein meals as alternatives. From these initiatives, lupins have emerged as one the grain sources now being used in aquaculture feeds throughout the world. However, as with all raw materials, learning to manage variability is one of the major limitations to improved adoption and maximisation of the value of these raw materials. In a series of experiments, the variability in the digestibility of protein and energy was assessed in rainbow trout from 136 different samples of lupin kernel meal using a diet substitution approach. Lupin lines were obtained from the Australian National Lupin Breeding Program’s germplasm lines, and had been selected on the basis of maximal crude protein variability as assessed by existing crude protein near infrared spectroscopy (NIRS) calibrations. Chemically measured crude protein values varied from 277 to 613 g/kg dry matter (DM) & gross energy values ranged from 187 to 230 MJ/kg DM. The digestible protein and energy values varied from 244 to 595 g/kg & 77 to 205 MJ/kg respectively.

Other compositional parameters assessed included amino acids, total lipids, ash, total carbohydrates, cellulose, hemicellulose and lignin. Two reference diets were also included in each experiment to ensure that a high degree of robustness in the across-experiment evaluations was maintained.

Using this sample and data set, this study assessed the ability of NIRS spectra to predict nutrient composition, energy value and digestibility parameters (digestible protein and digestible energy) of lupin kernel meals when fed to rainbow trout. The same lupin kernel meal samples were also scanned using a diode array near infrared spectrophotometer (DA-NIRS). The spectra were obtained by the DA-NIRS and were chemometrically calibrated against both the chemical composition and the digestible value data using multivariate analysis software. The results in terms of standard error of cross validation (SECV) and correlation coefficient ($R^2$) showed strong relationships ($R^2 > 0.8$) between the predicted and observed parameters for most of the chemical and digestible value parameters assessed. This study therefore demonstrates that within one raw material type, not only does significant variability exist in the digestible value of the raw materials, but that it is possible to use NIRS technology to provide rapid estimates of the digestible value of those raw materials in near real-time prior to incorporation into dietary formulations.
THE RESPONSE OF YELLOWTAIL KINGFISH (SERIOLA LALANDI) DIGESTIVE ENZYME ACTIVITY TO THE REPLACEMENT OF DIETARY FISH OIL
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Yellowtail kingfish (Seriola lalandi; YTK) is a rapidly growing carnivorous, temperate marine species, currently being cultured in several countries. In southern Australia, YTK are commercially cultured in water temperatures fluctuating from 10°C in winter to 25°C in summer. There is limited information published on YTK nutritional requirements associated with fish oil substitution at different water temperatures. In a recent study we fed YTK five diets in which the dietary fish oil component was replaced with 100% of either poultry oil (PO), canola oil (CO), a blend of fish oil and poultry oil (FO/PO; 50:50) or a blend of fish oil and canola oil (FO/CO; 50:50).

Results from this previous study demonstrated that after five weeks, 100% replacement of fish oil with poultry oil was possible in the presence of ~5% residual fish oil. However, 100% substitution with canola oil was not recommended, particularly at the sub-optimal water temperature of 18°C where growth performance was significantly reduced. Further investigation of how the diets affected the digestive enzyme profile is necessary for understanding the nutritional physiology of fish and to synchronise dietary ingredients and feeding practices on a physiological and species-specific level.

Therefore, the aim of the second study was to 1) understand the role of water temperatures in regulating the activities of digestive enzymes (e.g. trypsin, lipase and amylase) in juvenile YTK, and 2) determine whether the partial or total replacement of fish oil with poultry oil or canola oil affected the digestive enzyme activity at optimal (22°C) and sub-optimal (18°C) water temperatures.

The partial and total replacement of fish oil with poultry oil and canola oil in diets for YTK significantly affected the digestive enzyme activities of trypsin and lipase activities ($P < 0.05$), but not amylase activity. Water temperature also had a significant effect, causing reductions in digestive enzyme activity at 18°C compared to 22°C ($P < 0.05$). The digestive enzyme activity results correspond closely to the growth performance and feed efficiency results as previously described.
Among the native Brazilian fish species with the potential for aquaculture, the tambaqui (Colossoma macropomum) stands out. Considering the lack of information involving protein requirements for this species, it is necessary to establish dietary requirements of this nutrient. The purpose of this study was to determine the requirements of digestible protein (DP) for the intensive production of tambaqui juveniles.

1750 juveniles were used in 35 tanks of 450 L with an initial density of 50 fish/m² and an initial weight of 6.53 ± 0.43g. The experimental design was completely randomized with seven treatments (14, 17, 20, 23, 26, 29 and 32 % DP) and five replications. The fish were hand fed during 120 days, three times a day. The statistical analysis was conducted using a software program, SAS version 9.2. Averages for the level of protein were subjected to an analysis of segmented regression - broken line model.

The increase in the levels of digestible protein of the diets up to the 29% level of DP significantly improved the averages of final body weight, weight gain (WG), daily feed intake, specific growth rate and protein crude gain. The segmented regression analysis for WG showed that there was a growing response up to the level of 29.00 % DP (optimal level of DP). Higher values were not proportionally beneficial in the production of juvenile tambaqui. FAPESP fellowship (2011/12963-2 and 2012/09126-4)
The experiment were conducted to study the effect of pectin and arabinoxylan on growth performance and lipid metabolism in yellow catfish (Pelteobagrus fulvidraco). In test groups, 8% pectin (diet P) and 30% wheat bran (Wheat bran was used as the source of arabinoxylan) (diet A) were added into the diets respectively. In control diet, pectin and wheat bran were degraded by pectinase (diet P-D) and xylanase (diet A-D) respectively before utilization. The diets in all groups were isonitrogenous (CP36%) and isolipid (EE6%). Fish with initial body weight of (23.5±1.7) g were fed for 8 weeks before growth performance, body composition and lipid level were determined. Results showed that final body weight, weight growth rates (WGR) and specific growth rates (SGR) in fish fed diet P-D and A-D were significantly higher than in fish fed diet P and A. The feed conversion ratios (FCR) in fish fed diet P and P-D were significantly higher than in fish fed diet A and A-D, and the condition coefficient in fish fed diet A-ED was the highest (P<0.05). The body and muscle lipid content in fish fed diet P was the lowest, and its moisture content was the highest. Meanwhile, the body and muscle lipid content in fish fed diet P and A were significantly lower than in fish fed diet P-D and A-D (P < 0.05). Compared with fish fed diet P-D and A-D, serum content of total TC, HDL and LDL were decreased in fish fed diet P and A, and the fish fed diet P had the lowest TG content, which was significantly lower than that in fish fed diet A-D (P < 0.05). These results suggested pectin and arabinoxylan inhibit the growth of catfish, and the reason might lie in reduced lipid accumulation. Moreover, the growth inhibition of pectin may be greater than arabinoxylan.
Introduction
Soybean and plant based ingredients are increasingly being used as major feed ingredients in fish diets. However, some of these ingredients may affect fish metabolism and intestinal health. In particular, soybean meal (SBM) has been reported to induce damage at different levels of the intestinal mucosa in salmonids, leading to enteritis in some cases. SBM is also able to affect the intestinal microbiota in rainbow trout (*Oncorhynchus mykiss* Walbaum) but a link between the modulation of the intestinal microbiota and the development or prevention of enteritis remains unclear. A considerable amount of research has been published about the positive effect of probiotics such as lactic acid bacteria (LAB) in fish. Thus, this study aimed to assess the potential benefit of using *Pediococcus acidilactici* MA18/5M as a probiotic to ameliorate the adverse effect caused by a shift from a typical fish meal based diet to high plant based diets in rainbow trout.

Materials and methods
The trial was conducted at the recirculation aquarium facilities at Plymouth University, UK. The trial consisted of three groups of juvenile rainbow trout. The fish were acclimatized for 30 day on a standard commercial diet (Sigma® 50, EWOS, UK). At the end of the acclimatization period, fish were fed three different diets in which part of the FM was replaced by plant ingredients: a FM diet (100% protein derived from FM), a SBM diet (39% SBM) and a MPP diet (19% SBM and 8% pea protein concentrate). The diets were iso-lipidic (20%) and iso-nitrogenous (50%). Two sets of each diet were produced, one served as the control, and the other one was supplemented with the probiotic Bactocell® (10⁶ CFU/g *Pediococcus acidilactici* MA18/5M). After five weeks of feeding, samples from the digesta and the intestinal mucosa were taken and the microbial community and probiotic recovery was assessed by plate counts, PCR-DGGE and scanning electron microscopy (SEM). Growth performance was assessed by mean final weight gain, specific growth rate (SGR) and feed conversion ratio (FCR).

Results and Discussion
The groups fed diets with the SBM, PPM and all diets containing Bactocell® showed a significant increase in total LAB counts in digesta in comparison with the group fed the FM diet. This increase was higher in diets supplemented with *P. acidilactici* and PCR-DDGE confirmed the presence of the probiotic. PCR-DGGE analysis evidenced a proportional relation between the content of plant proteins in the diet and the bacterial diversity and probiotic abundance. Thus, the group fed with FM rich diet had less diversity in bacterial microbiota and a relatively lower level of probiotic abundance. Electron microscopy confirmed the presence of *P. acidilactici*-like bacteria populations associated with the epithelial mucosal layer in treatments where Bactocell® was supplied, which suggest that *P. acidilactici* is able to colonize the mucosa under different dietary regimes. These results, together with the numerical improvement in growth performance (SGR and FCR) measured at week four, suggest that the use of *P. acidilactici* MA18/5M may be particularly interesting to limit possible negative effects associated to diet changes (plant ingredients) and feed transition. Future studies are required to validate this hypothesis.
Protein and lipid are the main nutrition demanding for fish. Suitable protein and lipid levels in fish diets are essential for its maintenance growth, reproduction and spawning. This study was conducted to determine the appropriate dietary protein and lipid levels on growth, reproductive and spawning performance of short-bodied mackerel. Six treatments and three replicates were applied to the total of 18 plastic round tanks (500 L) with 10 fish tank each. Three protein levels (40%, 45% and 50%) and two lipid levels (15% and 18%) within each protein levels were tested through formulated practical diets labeled as 40P15L, 40P18L, 45P15L, 45P18L, 50P15L and 50P18L, respectively. Feeding was carried out five times per day on 2-month-short-bodied mackerel for 2 months prior to spawning. Results showed that dietary protein level significantly influenced final weight and specific growth rate, while dietary lipid level did not influence of any these growth parameters. Best growth was observed in short-bodied mackerel fed with diets containing protein 45% and 50% with both lipid levels. Dietary protein and/or lipid levels did not affect to survival rate. Gonadosomatic index (GSI) values were positively influenced by the dietary protein level, while the dietary lipid level did not exert any influence. A 40% protein level treatment with both lipid levels gave good GSI values. For the first spawning time, the treatments of 45% protein level with 15% or 18% lipid levels provided a shorter time for the first spawning than those of 30% and 50% protein levels with both lipid levels. Spawning performances, comprising of egg production (total egg and fertilized egg), quality (buoyancy rate and fertilization rate), larval production (hatching larvae) and quality (hatching rate), obtained from diet 45P18L, were all highest, while diet 40P15L provided the lowest egg and fry production and quality. At all protein levels, increasing dietary lipid from 15 to 18% resulted in a significant increase in egg quality, larval production and quality. This present study indicates that the dietary protein and lipid requirements for short-bodied mackerel for optimized growth and spawning performance will be at 45% protein and 18% lipid, respectively.

Keywords: protein, lipid, growth, spawning performance, Rastrelliger brachysoma, broodstock
POSTER 014
EFFECT OF BATH TREATMENT OF HORMONES ON THE DIGESTIVE ENZYME PROFILE OF CATLA CATLA LARVAE DURING ONTOGENIC DEVELOPMENT
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Digestive enzymes are good indicators of the physiological status of fish larvae. Effect of exogenous hormone bath treatment on the growth and digestive enzyme activities were studied on Indian major carp Catla catla larvae (1.0±0.01 mg). Larvae (4 days old) were given bath treatment in cortisol (Hydrocortisone, 0.2 mg/l), 3,5,3′-triiodothyronine (T3, 2.5 mg/l) and a combination of cortisol and T3 for 30 min. Digestive enzyme profile was recorded on 2 days interval and was continued up to 34 days. Larvae were fed with live food for initial 12 days and then weaned to a mixed feeding of live food and prepared diet. Significantly (P<0.05) higher amylase, total protease, trypsin, chymotrypsin, lipase, chitinase and chitinobiase activities were found in the hormone treated groups compared to the control one during ontogenic development. Among the treated groups, amylase activity was higher in cortisol treated larvae. Total protease, trypsin, chymotrypsin, lipase, chitinase and chitinobiase activities were significantly (P<0.05) higher in mixed treated group compared to the other groups in most of the days. Average weight of hormone treated larvae was higher compared to the control group. The immersion of tilapia larvae in cortisol enhanced the growth at early developmental stage (Mathiyalagan et al., 1996). This study showed that bath treatment increased the digestive enzyme activities in first feeding catla larvae. The combined effect of application of cortisol and triiodothyronine was reported in marine fish larvae (Brown and Kim, 1995).

References: References
As plant products are used in an ever increasing rate to replace fishmeal in the feeds of fishes, even marine fishes, how to ensure the health quality of the fish products has been a challenging issue. Among the health quality criteria of seafood, level of highly unsaturated fatty acids (HUFA) reflects critically the uniqueness of seafood products. Both dietary Vitamin E and tissue oxidative tone have been linked to synthesis and tissue deposition of HUFA. Here we reported the results of a 14-wk growth trial that was aimed to unravel which of Vit. E or oxidative tone is the decisive factor. The 2x2 factorial trial had 4 test diets, 3 replicate tanks for each diet, and 8 juvenile groupers in each tank. Vit. E and Se were supplemented to a basal diet in which fishmeal (containing PUFA) was the major protein source. The basal diet contained 10% of an oil mixture from linseed, sunflower, and coconut oils and with a ALA/LA ratio of 3. The growth performance of the fish was not significantly different among diets. Liver TBARS concentrations in fish fed FE (Vit. E only) and FSE (Vit. E and Se) were significantly lower than fish fed F (no supplement). The fish fed FS (Se only) had the significantly highest liver TBARS. While Vit. E supplementation resulted in significantly higher Vit. E and lower TBARS concentrations in the liver and muscle, PUFA (in terms of EPA and DHA) concentrations did not differ. Calculation of HUFA deposition or accumulation revealed that oxidative tone affected limitedly HUFA deposition in the groupers.
POSTER 016
EFFECT OF OXIDIZED FISH OIL AND A-TOCOPHEROL ON GROWTH, ANTI-OXIDATION STATUS, GENE
EXPRESSION LEVEL OF HEPATOPANCREAS HSP70 AND RESISTANCE TO AEROMONAS HYDROPHILA
CHALLENGE OF CHINESE MITTEN CRAB ERIOCEIR SINENSIS
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The impact of dietary α-tocopherol on juvenile Chinese mitten crab Eriocheir sinensis was experimentally evaluated in a 10-week study. Crab were fed with nine diets including three levels of α-tocopherol (0, 100 and 300 mg kg−1 diet) and three levels of fish oil oxidation: fresh, moderate and high) in triplicates. Fresh and moderate oil oxidation enhanced weight gain, but moderate and high oil oxidation lowered survival and feed efficiency. The 100 mg α-tocopherol kg−1 diet resulted in lower hepatopancreas MDA than other α-tocopherol diets. The mRNA expression of Hsp70 in the hepatopancreas was measured by quantitative realtime PCR assay. The expression level of Hsp70 in hepatopancreas was up-regulated by the oxidized oil. High oil oxidation led to the lowest serum superoxide dismutase (SOD) and glutathione peroxidase (GPH-PX). The serum SOD and GPH-PX activities in crab fed 100 mg α-tocopherol were higher than those fed other α-tocopherol diets. The diet without α-tocopherol addition lowered lysozyme and phenoloxidase (PO) activities compared to other α-tocopherol diets. Fresh fish oil diet increased PO activity compared to oxidized oils. High oil oxidation caused significantly more mortality than fresh or moderate oxidation after 7-d post challenge with Aeromonas hydrophila. Supplementation with α-tocopherol significantly enhanced resistance to bacterial infection. This study indicates that α-tocopherol can protect lipid from peroxidation and enhance disease resistance.
POSTER 017
GROWTH, IMMUNE RESPONSE AND RESISTANCE TO AEROMONAS HYDROPHILA CHALLENGE IN DARKBARBEL CATFISH PELTEOBAGRUS VACHELLI FED DIFFERENT LEVELS OF LINOLENIC ACIDS, VITAMINS C AND E
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The role of dietary linolenic acids (PUFA) vitamin E (E) and vitamin C (C) in regulating fish growth and immune response was tested on juvenile darkbarbel catfish *Pelteobagrus vachelli*. Five dietary combinations were used (C-E-PUFA, C+E-PUFA, C+E+PUFA, C+E+PUFA and –C+E+PUFA) in triplicates. Fish weight gain, specific growth rate and feed efficiency ratio in the C+E+PUFA group were highest. Red blood cells in fish fed the C+E+PUFA diet was highest, but hematocrit and hemoglobin of fish fed C-E-PUFA were lowest. Superoxide dismutase, catalase, glutathione peroxidase, and glucose-6-phosphate dehydrogenase activities in fish fed C-E+PUFA were higher than in fish fed other diets. Malondialdehyde in fish fed –C+E+PUFA was highest. Fish fed C+E+PUFA had higher levels of lysozyme activity, serum protein, complement C3, C4, and immunoglobulin than fish fed other diets. Fish fed C+E+PUFA showed lower mortality and higher antibody titer than fish fed other diets after bacteria challenge for 14 d.

This study suggests that the growth of darkbarbel catfish was improved by increasing dietary linolenic acids. Diets with high linolenic acid, vitamins E and C enhanced immune responses and resistance to *A. hydrophila* challenge in darkbarbel catfish.
An experiment was conducted to determine the combined dietary effects of cholesterol (CHL) and NUTRAFITO Plus (NP), a mix of plant extracts containing mainly saponin, on Pacific white shrimp Litopenaeus vannamei. A two-factor factorial design was used consisting of four cholesterol levels (0.00%, 0.05%, 0.10% and 0.15% of diet) and three NP levels (0.00%, 0.05% and 0.1% of diet), the experiment was carried out for 3 months. The results indicated that diets supplemented with either CHL and/or NP significantly improved shrimp survival (SUR), weight gain (WG), specific growth rate (SGR), average body weight (ABW), and food conversion ratio (FCR). There was a highly significant interaction between CHL and NP on SUR, WG, SGR, ABW and FCR. The dietary cholesterol level to achieve significant shrimp growth was 0.10%, while that for NP was 0.10%. Shrimp fed diets supplemented with CHL had no significant effects on immune capacity of total haemocyte count (THC), superoxide dismutase (SOD), total antioxidant (TAS), glutathione peroxidase (GPx), glutathione reductase (GR), alanine aminotransferase (ALT) and aspartate aminotransferase (AST). However, supplement of 0.10% NP has significantly improved shrimp’s THC, SOD and TAS, but not other parameters. When increasing CHL content in diet, total lipid content of shrimp body (LB) decreased, but no different was found in total cholesterol of shrimp body (CB), and total lipid (LH) or cholesterol (CH) of hepatopancreas was fluctuated. Increased supplementation of NP resulted in increasing in LH, CB and CH but not in LB. The concentration of serum cholesterol (SC) was not affected. NP was beneficial as a feed supplement in the white shrimp, which could replace cholesterol partially or totally in diet as well as reduce the cost of feed.
POSTER 019
APPARENT DIGESTIBILITY OF PROTEIN AND TOTAL CARBOHYDRATES IN SALMONID FEED IMPROVES WHEN SUPPLEMENTED WITH A PROTEASE
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A digestibility trial was conducted with three salmonid species, coho salmon (Oncorhynchus kisutch), Atlantic salmon (Salmo salar), and rainbow trout (Oncorhynchus mykiss) at Universidad Catolica de Temuco, in Chile. Three isonitrogenous diets (~45% CP) were formulated. A control diet (diet 1) with 35% fishmeal (FM) and two other diets with reduced fishmeal (diets 2 & 3, 15% FM) were formulated, where diet 3 was supplemented with a protease obtained from Jefo Nutrition Inc., Canada at 175 g/ton of feed. Chromium dioxide was used as digestibility marker and added at 1% in all three diets.

Apparent digestibility coefficients (ADC) of CP, lipid and energy in coho salmon (88.6-90.1%, 94.9-95.7% and 87.1-88.4%, respectively) and rainbow trout (89.4-91.9%, 96.0-97.5% and 89.1-90.7% respectively) were not significantly different among the dietary treatments. However, in Atlantic salmon fed both the low fishmeal diets, ADCs of CP were significantly higher (89.4-90.3%) than those fed the high fishmeal control diet.

Interestingly, there is a significant increase in ADCs of total carbohydrate in all three species fed the diets containing protease than those fed diets without protease. The increase in total carbohydrate digestibility in coho salmon, Atlantic salmon and rainbow trout fed the protease supplemented diet were 9% and 4%, 8.2% and 3.8%, and 3.4% and 3.9% higher than those fed the high fish meal control diet and low fish meal diet without protease, respectively.

It can be hypothesized that some of the protein bound complex carbohydrates present in oilseed meals were released and available to the animals for digestions. Further investigations are recommended to validate this hypothesis.

It can be concluded from this study that a properly formulated cost effective low fishmeal diet (in this instance, a savings of ~100 US$/ton of feed compared to the control diet) doesn’t affect the quality of the feed when supplemented with a protease.
An 11-month growth trial was conducted at Universidad de Chile with rainbow trout, *Oncorhynchus mykiss*. The experiment consisted of three treatments with three replicates for each treatment, a total of 360 fish were randomly distributed in nine 2-m³ circular tanks with 40 fish in each. Average weight of fish in each treatment was ranged between 390 and 401 g. During the trial, average water temperature was maintained at 10.5°C. At which time, fish were fed an extruded commercial feed (40% CP) supplemented with graded level (0, 175, and 250 ppm) of a proteolytic enzyme.

Fish fed both protease-supplemented diets (175 and 250 ppm) showed significantly higher final body weight (FBW, 971 and 987 g, respectively), improved feed conversion ratio (FCR, 1.35 and 1.33, respectively) and better growth rate (thermal-unit growth coefficient – TGC, 2.94 and 3.03) than those fed the control diet (FBW = 849 g; FCR = 1.43; TGC = 2.52). No difference was observed in performance between the fish fed the two protease-supplemented diets.

A linear increase in intestinal microvilli height was observed with increasing levels of dietary protease ranging from 630-µ in fish fed the control diet to 663-µ and 734-µ in those fed diets supplemented with 175 and 250 ppm of protease, respectively. Insignificant improvement in growth performance and nutrient utilization between the protease-supplemented dietary treatments despite linear increase in villi height suggested that most of the dietary proteins were hydrolyzed by protease at the lower inclusion level of 175 ppm.
POSTER 021
EVALUATING TWO NOVEL IN-VITRO METHODS TO ASSESS EFFECTS OF A DIETARY PROTEASE ON COMMERCIAL PRODUCED AQUACULTURE FEED
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Increasing reliance on more economical feedstuffs, the formulation of feeds to much lower or narrower nutritional specifications and the formulation of diets on a digestible nutrient basis are leading feed manufacturers to explore and use different approaches to improve availability of nutrients or maintain more stable digestible nutrient levels in feeds. The use of exogenous protease enzymes in aquaculture feeds is becoming increasingly popular and the benefits of using such enzymes in certain feed formulations have been demonstrated. In-vivo studies in both poultry and swine have reported improved protein digestibility, true nitrogen digestibility and feed efficiency in animals fed diets containing these enzymes. However, in-vivo trials are both expensive and time consuming, and cannot be implemented as part of regular QA/QC processes at the feed mill. In contrast, in-vitro methods can be cheaper, require less time and they are frequently part of QA/QC process. The objective of this study was to develop and compare two in-vitro techniques (HCl-Pepsin protein digestibility and total soluble amino acid content) and use these techniques to compare both digestibility and total soluble amino acid content of shrimp feed that contained a protease (AG175™, Jefo Nutrition Inc., Canada) at 0, 175 or 1000 ppm inclusion levels.

In the HCl-Pepsin method, finely ground feed samples were incubated at 45°C for 16 hours in a shaking water bath, solids were separated and analyzed for nitrogen (N) content. Crude protein digestibility was determined from the difference in pre- and post-digestion N content. In the second technique, finely ground feeds were incubated for 5 hours at 40°C under alkaline condition (8.5 pH). After 5 hours of incubation, supernatant was separated and analyzed for total amino acid content after hydrolyzing with sulfosalicylic acid (SSA).

The results suggested greater solubility (1 to 3% increase) and higher soluble essential amino acid concentration in the diets containing protease. A linear increase in digestible protein content as determined by the HCl-pepsin method was observed with the increasing level of enzyme (y = 1.45x +64.3; R²=0.96). The digestible protein content increased from 66% in the control feed to 67% and 69% in feed supplemented with 175 and 1000 ppm of protease, respectively. Most essential amino acids were in higher concentration in the soluble fraction of the diets supplemented with the protease except for methionine.

The findings of this study highlighted the potential of the two in-vitro methods for assessing effects of an enzyme or comparing effects of two or more enzymes on protein quality of pelleted or extruded feed. These methods appear more robust and useful than traditional enzyme activity assessment that relies on a particular substrate and measured based on one or more indicator amino acids.
An *in-vitro* digestibility trial was conducted to assess the effects of protease treated fish solubles on protein quality of a 38% CP extruded feed. Six diets of two pellet sizes (6-mm and 8-mm) and three levels of protease (0, 175 and 350 g/ton of, a protease from Jefo Nutrition Inc., Canada) were assessed. The feeds were formulated with fishmeal (10%), soybean meal (4.5-6%), poultry by-product meal (5%), lupin meal (7%), corn gluten meal (4%), soy protein concentrate (4%) and partially dried fish solubles (6%) as major protein sources. The solubles were treated with 6 and 12 g/L of protease at 40°C to attain final concentration of 175 and 350 g/ton of protease in the feeds. Semi-liquid fish solubles were added continuously at 6% during feed manufacturing. The temperatures were ranged from 96-97°C for conditioning, from 110-125°C for extrusion and from 167-185°C for drying.

Feed samples were collected every 10-min for an hour during production and then, pooled together for analyzing physical quality and protein digestibility. Hardness, water stability, leaching of oil, % crumbles and % fines were tested for pellet quality. Protein digestibility was assessed using an *in-vitro* HCl-Pepsin method. Significant effects of pellet size and size-enzyme interactions were observed on hardness, oil leaching, % crumble and % fine, while water stability was affected only by the pellet size. Despite significant size-enzyme interactions, the levels of enzyme in the feed alone did not affect any pellet quality parameters.

Digestible protein content significantly increased from 83% and 78% in 6-mm and 8-mm pellet without enzyme, respectively to 85% and 84% in pellets containing 175 g/ton of enzyme. Despite a slight increase, no significant differences were observed in digestible protein contents between the pellets containing 175 g/ton and those with 350 g/ton of protease. The results from HCl-pepsin digestibility analyses revealed significant increase in digestible protein contents in both 6-mm and 8-mm size feeds when treated with the protease.
Nutritional assessment of alternative ingredients to fish meal and soybean meal for shrimp feeds is necessary due to the high costs and uncertainty in the supply of these ingredients. Proteins of vegetable origin are potential ingredients to be used in aquafeeds, since they have good availability and their cost is generally low. Mexico produced 0.1 MT of coconut (Cocos nucifera) in 2011, and 54.7 MT were produced worldwide (FAO, 2012). Coconut meal (paste) is a byproduct of the process of oil extraction from the flesh, being moderately used in feeds for terrestrial animals (Hertrampf and Piedad-Pacual, 2000). However, its potential use in shrimp feeds has not been sufficiently explored.

The objective of the present work was to evaluate the nutritional value of two coconut meals as ingredients in diets for Pacific white shrimp Litopenaeus vannamei.

Two coconut meals were used: commercial meal (CM) 25.3% crude protein and 13.0% lipids, and a defatted coconut meal (DCM, 28.5% crude protein and 6.80% lipids) obtained in the laboratory by further extracting oil from the coconut paste using petroleum ether. A 45-day growth trial with juvenile shrimp L. vannamei (0.25 g mean initial weight) was performed under intensive culture conditions (27°C, 38.5‰ and 4.9 mg/L dissolved oxygen) using 9 diets: a reference diet (35% protein and 8% lipids) containing a mixture of soybean-wheat (31.4:68.6) which was replaced by increasing dietary levels (25, 50, 75, and 100%) of commercial paste (diets CM25, CM50, CM75 and CM100, respectively) and defatted coconut meal (diets DCM25, DCM50, DCM75 and DCM100, respectively).

Each treatment consisted of 4 replicates (60-L tanks) with 10 shrimp/tank. Survival, growth, apparent feed intake, feed conversion ratio and protein efficiency were determined for each treatment. Survival at end of the experiment was high (>90%) and unaffected by diets. Shrimp fed diets DCM100 and CM100 had the highest final average weights (3.39 and 3.63 g) growth rates (1,274.6 and 1,345.8%), and showed the highest feed intake (0.19 and 0.20 g/shrimp/day). No significant differences (P>0.05) in feed conversion and protein efficiency ratios between any of the dietary treatments was detected.

The results indicate that coconut pastes can be used as ingredients in feed formulation and can partially or totally replace a mixture of soybean-wheat (31.4:68.6) without having adverse effects on shrimp growth or feed utilization.

POSTER 024
LONG TERM FEEDING RAINBOW TROUT WITH FISH MEAL AND FISH OIL FREE DIET: CONSEQUENCES ON GROWTH PERFORMANCE, WHOLE BODY LIPID CONTENT AND FATTY ACID PROFILE
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Although it is possible today to replace a large amount of fish meal (FM) and/or fish oil (FO) by terrestrial plant products in alternative aquaculture feeds for several species, it is recognised that there is still a lack of information regarding the effects of such dietary changes throughout the whole life cycle of fish. This study aimed to determine the long-term effects of FM/FO free feeds from first feeding onwards, with specific focus at key points along development.

Rainbow trout alevins (IBW: 0.14 g) from the same cohort were fed from the first feeding three different diets: a marine diet (M) based on FM and FO, a commercial-like diet (C) with an equal mixture of marine ingredients (FM-FO) and plant products, and a diet containing only plant-products (V). Fish were fed by hand until apparent satiation (gradual decrease of daily meal number: 8 meals a day at first feeding to twice a day from 3 months onwards). Fish were reared at 7°C until 6 months, when they were transferred to 17°C. Growth performance, whole body composition and fatty acid profile were analyzed after 6 months (juveniles) and 11 months feeding the respective diets.

Survival was significantly lower in fish fed the V-diet (70% vs 95%) during the first 6 months after which there was no difference (96 vs 98%). Daily growth index and body weight at 6 months after the first feeding were not significantly different, but after 11 months significantly lower values were observed for the V-fed group. A reduction in feed efficiency (-10 to 13%) was found with the V-diet at both juvenile and ongrowing fish stages. The whole body lipid content in juveniles was significantly higher for the V-fed group (13% vs 9-10%) whereas no significant difference was found in ongrowing fish (15%). The fatty acid profile of body lipids reflected that of the diets. However our results showed that rainbow trout was able to synthesize eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids from dietary 18C precursor. In trout fed diet V although dietary intake of LC-PUFA n-3 was zero, around 0.9% EPA and 2% DHA were recovered in body lipid of fish at both developmental stages.

This study showed that rainbow trout are able to grow when fed a totally plant-based diet, without any use of marine ingredients from first feeding onwards, although finer adjustments of feed formulations are required to optimize growth performance and fatty acid content of the fillet.

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Three years life cycle breeding with a fish meal and fish oil free diet: reproduction performance and potential carry over generation effects in rainbow trout (Onchorhyncus mykiss)

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Terrestrial plant products are increasingly used as substitutes for fish meal (FM) and fish oil (FO) in diets for aquaculture species, however they are completely devoid of long chain polyunsaturated fatty acids (LC-PUFA). The lipid content and fatty acid (FA) composition of broodstock diet have been identified as major dietary factors that determine successful reproduction and survival of offspring. We were wondering if trout deprived of any dietary supply of n-3 LC-PUFA during the whole life cycle would be able to reproduce and to generate progeny with different aptitude to use alternative diets. This study aimed to assess the effects of the maternal diet history on reproductive performance and the response of progeny to 3 different diets (M: FM-FO based, V: plant-based, C: commercial-like).

Two groups of female rainbow trout were fed either a commercial (COM) diet made of a blend of marine and plant ingredients or a 100% plant-based (VEG) diet throughout a 3-years life cycle, from first feeding onwards. Ova samples (400/female) of the second spawn were taken from 10 females of each group. The ova were fertilized with sperm collected from males fed the commercial diet and reproductive performance assessed (ova and swim-up fry weight, survival). Alevins from the two cohorts were respectively split into three groups and each group received one of the first feeding diets M, V or C, for 3 weeks. Lipid content and FA profiles of ova, swim-up fry and 3 weeks feeding alevins were analyzed.

Females from the VEG group produced smaller ova (weight -15%) and swim-up fry with slightly lower body weight (-13%) however no difference in survival rate of fry was observed. Neither total lipid content of ova (5-6%) and fry (11%) nor lipid composition was significantly affected by the maternal diet history. Neutral lipids were around 65-70% and polar lipids 35-30% in both groups. Analysis of fatty acid profile showed that VEG females have synthesized significant amount of eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids from dietary precursor (linolenic acid, 18:3 n-3), and transferred them to ova, not only in polar lipid fraction but also in the neutral lipid fraction. Whatever maternal origin, progeny responded in a similar way to the dietary treatments with reduced growth when fed V diet. Total lipid content of alevins was around 4-5% and fatty acid profile mirrored that of diets. In V fed groups an increase in 18:1, 18:2 n-6 and 18:3 n-6 with a concomitant decrease in LC-PUFA n-3 was observed and changes were more pronounced in neutral than in polar lipid fraction. Meanwhile, the initial differences in weight and FA composition resulting from maternal history (COM or VEG) still remained after 3 weeks feeding whatever the progeny diets.

This study shows for the first time that rainbow trout has a remarkable capacity to produce viable offspring when fed a totally plant-based diet free of FM and FO over the whole life cycle and that maternal dietary history does not significantly affect the response of progeny to diets of different composition.

Study supported by the French national program FUI VegeAqua and the EU project ARRAINIA
Background: Reducing the reliance upon soy protein concentrate (SPC) and fishmeal (FM) in feeds for Atlantic salmon in Europe is likely to further improve sustainability of salmon farming. Bean Protein Concentrate (BPC), made by fine grinding and air classification of faba beans (Vicia faba), contains ~55 to 65% protein and offers a potentially low cost protein concentrate. The purpose of this work is to examine if it can replace part of the protein provided by SPC, FM or their mixtures.

Materials and Methods: Commercially produced BPC (61% crude protein) was purchased from Sotexpro, France. Analogues of FM, SPC and BPC were designed to have the same proximate and amino acid composition and could replace each other on a 1:1 basis. The study used these three analogues as the components in a mixture design so as examine a wide range of possible combinations. BPC itself varied from 0 to 50%, FM itself from 12% to 32% and SPC itself from 0 to 50%; sixteen experimental feeds were made based on a D-optimal design. A high soybean meal (SBM) feed was also included. The study was conducted at EWOS Innovation facilities, Dirdal, Norway using 70 0.4m² square tanks supplied with good quality freshwater (13°C) initial body weight was 1.5g and by the end of the eight week study fish fed most treatments grew to about 12g. Weight gain, body composition and enteritis assessment in distal intestine were the main response variables. Mixture polynomials with increasing complexity (null, linear, quadratic and special cubic models) were fitted to the weight gain and body composition data and the most plausible model was selected on the basis of likelihood ratio tests. Histology scores for enteritis were modelled with multilevel ordinal regressions.

Results: Data analysis showed that the dietary inclusion of moderate amounts (10 – 15%) of BPC increased weight gain and protein deposition and thus can reduce the inclusion of FM or SPC or both. The improvement in growth with a reduced dietary FM was particularly noteworthy. Intestine enteritis was not apparent in fish fed these inclusion levels, although it was apparent in fish fed the high SBM feed. However, there was no measureable benefit in BPC use when inclusion was beyond approximately 20% whilst beyond about 30% inclusion, fish gained less weight than those on a feed in which BPC was not included. Fish fed the treatment with the highest BPC examined for enteritis showed some sign of this condition.

Outcomes: This study suggests that BPC shows good promise as a protein source for salmon and is likely to be beneficial up to inclusion levels of around 20%.

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EVALUATION OF THREE SOY PROTEINS ON GROWTH AND ENTERITIS IN SALMON
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Background: As a candidate to replace fishmeal, soy beans, or their derivatives, offer many advantages. This study examined three alternative sources namely soybean meal (SBM), soy protein concentrate (SPC) and in-house cooked soy beans (COS) to help determine which one to focus efforts upon.

Materials and Methods: SBM meal (47% protein, 2% fat), SPC (60%, 1%) and in-house, wet cooked, mechanically extracted soybeans (57%, 10%) were added to feeds such that the amount of protein provided by each soy was tested at 20, 35, 50, 65 and 80% of the total dietary protein. The rest of the dietary protein was provided by LT fishmeal and fish bone meal. As soy inclusion increased the protein content was allowed to decrease but the ratio of protein to fat was made constant by addition or removal of fish oil. Synthetic sources of methionine, lysine and threonine were added in increasing quantities as soy inclusion increased. A control feed in which fishmeal was the only source of protein was also included in the study. The feeds were fed to Atlantic salmon (initial body weight 1.6g) for 8 weeks in 0.6m tanks, five replicates per treatment at EWOS Innovation facilities, Dirdal, Norway. Cubic B-splines were used to model the weight gain of fish in relation to soy inclusion and credible intervals were calculated by posterior simulation. Three tank replicates for the SBM and COS treatments and all five tank replicates for the SPC treatments (ten fish per tank in all cases) were taken for enteritis assessment using the six parameter ordinal scoring system described in Uran et al., 2009 and modelled using multilevel ordinal logistic regression.

Results: By the end of the study the fish had grown to 4.3g, mortality was low on all treatments. As the amount of protein from each soy source increased, the weight gain of fish was fairly constant until an inflection point after which fish showed lower growth. The inflection point varied with source; for the fish fed the SBM series of feeds it occurred at approximately 30%, for those fed the COS series it occurred at approximately 50% and for those fed SPC series it occurred at 65% of dietary protein from soy. Both SBM and COS feeds generated symptoms consistent with SBM enteritis with little or no difference in scores between the two soy types. The SPC feeds did not generate enteritis and scores were similar to the fish fed the soy-free feed.

Outcomes: The results suggest that the component(s) causing growth reduction may be different to that(those) causing enteritis and that SPC is a good choice on which to focus further efforts.

Saponins and polyphenols are bioactive compounds which are commonly found in many plants. The beneficial effects of supplementing saponins and polyphenolic extracts on growth performance and health of humans and various livestock species are well documented. Two of the most important commercial sources of saponins and polyphenols are Yucca schidigera, which is a plant native to southwestern USA and Mexico, and Quillaja saponaria, a tree that grows in arid areas of Northern Chile. The effects of saponin and polyphenols extracts, from Quillaja saponaria and Yucca schidigera, on growth performance and hepatic antioxidant enzymes activity of rainbow trout juveniles were determined in the present experiment. In total, 840 juvenile rainbow trout (Oncorhynchus mykiss) with an average weight of 17.8±3.4 were used. The fish were divided into seven groups of three tanks (100 L) each: a basal diet without any extract supplementation was offered to the control group (I) while 0.025, 0.05 and 0.1% of two commercial extracts derived from Quillaja saponaria (DK-QL Perm) and a blend of Quillaja saponaria and Yucca schidigera (DK-NutrafitoPlus) were included in the diets of treatment groups II, III, IV, V, VI and VII, respectively. After 60 days of feeding the growth performance was very similar across all dietary treatments. Immediately after the feeding trial, fish were subjected to a stress assay conducted during 10 days; afterwards, the activities of antioxidant enzymes such as glutathione peroxidase, superoxide dismutase and catalase as indicators of lipid peroxidation, were measured in homogenates of the liver. Our findings suggest that the supplementation of the commercial extracts DK-QL Perm (Quillaja saponaria) and DK-NutrafitoPlus (Quillaja saponaria and Yucca schidigera) at the doses studied does not lead to a negative effect on the growth performance and feed utilization. Based on the result the potential use of extracts derived from Quillaja saponaria and Yucca schidigera as antioxidants and health promoters in fish cultured under intensive conditions is discussed.
POSTER 029
FATTY ACID PROFILE OF DUSKY GROUPER LARVAE (EPINEPHELUS MARGINATUS: TELEOSTEI SERRANIDAE) DURING INITIAL DEVELOPMENT IN SOUTHEAST OF BRAZIL
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Lipids and fatty acids are essential energetic substrates during egg and fish larvae development. These substrates are important in all phases of fish life, but specially in larval phase, when fatty acids are important in metabolism, energy production, components of biological membranes, vitamins carriers, eicosanoids precursors, hormones, vitamin D, cofactors and coenzymes. Studies on fatty acid profile in fish larvae are very important to understand the metabolism of these substrates in order to develop future feeding protocols based on these findings. The aim of this study was to understand the fatty acid metabolism of triglycerides and phospholipids in dusky grouper larvae until the 8th day after hatching (DAH). The larvae were reared in 1 m³ tanks in a density of 10 larvae/l and fed with rotifers at a density of 2 rotifers/ml. Total lipids of larvae were extracted using a mixture of chloroform: methanol: water (2:1:0.5) and separated into triglycerides (TG) and phospholipids (PL) by thin layer chromatography (TLC). Fatty acids from TG and PL were analyzed by gas chromatography (GC) using hydrogen as carrier gas. TG class of the oocytes was mainly composed by saturated and monounsaturated fatty acids and the PL class was mainly composed by saturated and polyunsaturated fatty acids, with a predominance of n-3 fatty acids in both classes, mainly docosahexaenoic acid (DHA). Grouper larvae used mainly the saturated fatty acids of PL for hatching and after the mouth opening, fatty acids present many interesting transitions during the development which will be probably important sources to develop future feeding protocols focused on fatty acids requirements.
POSTER 030
GENE EXPRESSION OF SOLEA SENEGALENSIS FED WITH THE SYNBIO TIC COMPOSED BY SODIC ALGINATE AND THE PROBIOTIC SHEWANELLA PUTRE FACIENS PDP11 KAUP, 1858
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The sole, Solea senegalensis, is a common flatfish of Atlantic and Mediterranean waters with a high potential for aquaculture. However, cultivation is hampered by high sensitivity to different stresses and several infectious diseases. The use of antibiotics can avoid this, but has adverse effects such as accumulation in the tissue, immunosuppression, and development of antibiotic resistant bacteria. Probiotic, prebiotic and mix of both, synbiotic, can give protection from pathogens because seem to stimulate the immune system playing an important roles in health of the organism. In this study, we selected genes related to immune system (C3, C7, TF, G-lys, GPx and NADPHox) for relative quantification of transcript by real-time PCR. For this experiment, individuals of S. senegalensis were separated in two groups, one fed with a control diet and the second with the commercial diet supplied with a synbiotic composed by sodic alginate and the probiotic Shewanella putrefaciens Pdp11, which was isolated and studied in our laboratory. Fish were fed each 8 hours during 10 days. The synbiotic was prepared with 10⁹ cfu of Pdp11 per g of feed and 2 g of sodic alginate per Kg of feed. After 10 days, samples of head kidney were removed and RNA was extracted using GeneJET RNA Purification Kit (Thermo Scientific, Spain). cDNA was synthesized from total RNA (1 µg) of each sample using SuperScript III reverse transcriptase kit (Thermo Scientific, Spain). Real-time PCR analysis was carried out using an iCycler (Biorad). Comparisons between groups were made by one-way analysis of variance followed by Fisher analysis. Significant differences were accepted for P < 0.05. After 10 days feeding with the synbiotic, gene expression showed a significant upregulation in comparison to control fishes in all studied genes. These results can demonstrate that the synbiotic can alter the expression of genes related with immune system functions. The immuno-stimulation produced by the synbiotic may provide an additional overprotection against pathogens or stressors processes. However, the permanent application of the synbiotic and the continuous immuno-stimulation could be harmful to the fish. Therefore, more studies are necessary to see how would affect to fishes if we still adding the synbiotic and if the synbiotic could give protection against experimental infections.
GENE EXPRESSION OF TILAPIA OREOCROMIS Niloticus FED WITH THE PROBIOTIC BACILLUS SUBTILIS

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The tilapia Oreochromis niloticus is the main species produced in Brazil representing 39% of aquaculture. Probiotics are dietary supplements composed by live microorganisms that benefit the health of the host by improving its intestinal microbiota balance. The analysis of gene expression can evaluate the response of an organism to nutritional, environmental and reproductive management changes. The objective of this study was to evaluate the influence of a probiotic composed by Bacillus subtilis in female and male tilapia broodstocks on gene expression of five genes related to immunology (hsp 70, C8β, C1NH, ILβ and TNFα). The experiment was conducted at São Paulo Agrubusiness Tecnology Agency - Pirassununga, SP/Brazil during 110 days. One hundred and eight females and 48 males (527.65 ± 185.98g and 30.16 ± 3.57cm) were used to this trial. The three treatments were: T0- control (without probiotic), T1 – continuous intake of probiotic and T2 – alternate intake of probiotic (7 day diet with probiotic and 7 days without probiotic) at a dose of 0.5 g kg⁻¹ of feed (commercial product composed by Bacillus subtilis with 10¹⁰ CFU g⁻¹). To quantify gene expression by Q-PCR, the liver was removed from the experimental fishe (male and female) and immediately conserved in RNA later®. The organs were homogenizated and total RNA was isolated using the Gene JET RNA purification kit (Thermo scientific). Total RNA (1 ug) from each sample was reverse transcribed for converting RNA into DNA molecules using the iScript_ cDNA Synthesis kit (Bio-Rad). Real-time PCR analysis was carried out using an iCycler (Biorad). The amplification protocol used was: initial denaturation and enzyme activation for 7 min at 95 ºC, followed by 40 cycles of 95 ºC for 15 s, and 65 ºC for 30 s. Comparisons between groups were made by one-way analysis of variance followed by Tukey analysis. Significant differences were accepted for P < 0.05. The hsp 70 gene, whose function is to regulate homeostasis and stress, showed higher gene expression in T1♀, while in T2♀, T1♂ and T2♂ this gene was suppressed. The C1NH gene, that is responsible to suppress the inflammation, it was expressed in all treatments with higher detection in T1♀. The C8β gene is responsible of MAC (Membrane Attack Complex) formation that induces the destruction of bacteria and eukaryotic cells. The quantify of this gene showed suppression at T1♀ and expression in T1♂ and T2♀. The ILβ gene, responsible for the inflammatory response, showed suppression in T1♀ and T2♀, while in T1♂ and T2♂ it was more expressed. The TNFα gene, responsible for the apoptosis activity, enhanced neutrophil migration and respiratory burst activity of macrophages. This gene was expressed in the T1♀ and T2♀, but was suppressed in T1♂ and T2♂. The use of Bacillus subtilis in tilapia broodstocks was efficient in enhance immune system of the animals treated.
In gilthead seabream (*Sparus aurata*) vegetable meals diets have been found to successfully replace fish meals to a large extent. However mineral imbalances may interfere in such substitution and adjusted mineral supplements may be required. The use of appropriate delivery vectors for these minerals could improve the bioavailability for these nutrients to achieve a more effective mineral supplementation. In the present study, vegetable meal and oil (VM/VO) diets were formulated including the target minerals: Se, Mn, Fe and Zn, in the form of inorganic, organic or encapsulated minerals. Their performance was compared with two diets without mineral supplementation one with the VM/VO formulation and another with a fish meal/fish oil (FM/FO) based formulation. Two thousand and seventy juvenile gilthead sea bream (*Sparus aurata*) were randomly located into eighteen 500 L circular fiberglass tanks and fed one of these diets for 84 days. Fish performance in terms of growth and feed utilization, vertebra and body biochemical composition, gene expression of antioxidant enzymes genes and biomarkers of bone development and x-ray studies to determine vertebral morphology were conducted.

Low haematocrit values were only found in fish fed the VM/VO diet without target mineral supplementation, denoting the requirement for Fe supplementation in these diets and the good availability of inorganic, organic or encapsulated Fe. Results of the GPX transcript expression levels also suggested the high availability of inorganic, organic and encapsulated Se.

Fish fed FM/FO diets showed a lower vertebral weight and ash content suggesting that this type of diet requires supplementation of Zn or Mn, in relation to the high FM content in Ca and P as previously reported. Fish fed VM/VO diets without the target minerals supplementation showed the lowest mineral content, vertebral weight and vertebral length/vertebral height as well as a down-regulation of both BMP2 and OC, biomarkers of bone differentiation and mineralization, and suggesting a deficiency in Zn or Mn, which play an important role in bone mineralization. However, these minerals were effectively incorporated when they were supplemented in an inorganic form as denoted by the higher vertebral weight and mineral content, as well as the larger vertebral length/vertebral height. On the contrary, encapsulation of inorganic minerals reduced the mineral content in vertebrae and led to a lower final weight, suggesting that Zn or Mn, were not so available as in the free inorganic form.

Finally, feeding the organic Mn and Zn, despite increasing vertebral mineral content, caused a significant reduction in growth, suggesting that a higher availability of organic minerals may require reducing dietary levels to avoid a potential toxic effect of an excess of these minerals. Further dose-response studies are being conducted to further understand the effect of organic or inorganic Mn and Zn in diets containing high vegetable meals inclusion.
POSTER 033
EFFECTS OF SEPARATE AND JOINT SUPPLEMENTATION OF DIETARY COPPER AND ZINC INTERACTION ON JUVENILE ORIENTAL RIVER PRAWN MACROBRACHIUM NIPPONENSE UNDER NORMAL OR STRESS CONDITION
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Semi-purified diets containing seven graded levels of copper (2.8, 12.2, 20.9, 29.8, 43.1, 78.9 and 157.1 mg kg⁻¹ diet) from CuSO₄·5H₂O were fed to juvenile Macrobrachium nipponense (initial weight 0.101±0.002 g) in five replicates for 8 weeks. Prawns fed with 2.8-78.9 mg Cu kg⁻¹ obtained higher weight gain rate and lower feed conversion ratio than 157.1 mg Cu kg⁻¹ group. Activities of hepatopancreas Cu-Zn superoxide dismutase, glutathione peroxidase and total antioxidant competence (T-AOC) were the highest in the 43.1 mg Cu kg⁻¹ group. The hepatopancreas malondialdehyde content was lower (P < 0.05) in the 29.8 mg Cu kg⁻¹ than others. The level of hepatopancreas hemocyanin mRNA expression was significantly higher in the prawns fed 43.1-157.1 mg Cu kg⁻¹ diet than that fed 2.8-29.8 mg Cu kg⁻¹ diet. The content of hemolymph oxyhemocyanin peaked at 43.1 mg Cu kg⁻¹ diet. After the feeding experiment, prawns were challenged with Aeromonas hydrophila by injection, and the cumulative mortality rate of the prawns fed with 20.9-43.1 mg Cu kg⁻¹ was lower than others. The optimum requirement of dietary Cu in juvenile prawn was estimated at 26.9-27.8 mg kg⁻¹ diet based on the cumulative mortality rate and whole-body Cu retention.

In the second study, Macrobrachium nipponense were fed four level of Cu (0, 30, 60 and 180 mg Cu kg⁻¹ diet) for 8 weeks under two level of nitrite (0.01 and 1.90 mg L⁻¹). Growth, antioxidant enzyme activities and hepatopancreas hemocyanin mRNA expression of prawns with low nitrite were higher than those of prawns with high nitrite. Hemolymph oxyhemocyanin content of prawns with high nitrite were higher than prawns with low nitrite. Hemocyanin expression and oxyhemocyanin content elevated with the increase of dietary Cu level, peaked at 60 mg Cu kg⁻¹ diet, then exhibit a decreasing trend regardless of nitrite level. This study indicates that the harmful effects by nitrite could be mitigated when prawn fed 30-60 mg Cu kg⁻¹.

In the second experiment, prawn were fed with nine semi-purified diets including three levels of Cu (0, 30 and 180 mg kg⁻¹ diet) and three levels of Zn (0, 35 and 210 mg kg⁻¹ diet) in five replicates for 8 weeks. Moderate dietary Cu or Zn increased prawn weight gain (WG) and special growth rate (SGR). Cu concentrates in whole-body increased with increased dietary Cu regardless of dietary Zn levels, whereas high dietary Zn decreased hepatopancreas Cu content. Zn concentrates in hepatopancreas and whole-body increased with increased dietary Zn regardless of dietary Cu levels. Serum alkaline phosphatase (AKP) activity elevated with the increase of dietary Zn levels regardless of dietary Cu level. Higher serum lysozyme (LYZ) and phenoloxidase (PO) activities were seen in prawns fed moderate dietary Cu or Zn. After the feeding experiment, prawns were stressed with hypoxia. After stress, hepatopancreas hemocyanin mRNA expression was the highest in prawns fed high dietary Cu. Moderate or high dietary Zn elevated hemocyanin expression. Oxyhemocyanin contents of prawns after hypoxia stress were higher than that of prawns before hypoxia stress except prawns fed low Cu and Zn.
The Chinese mitten crab (Eriocheir sinensis) is an important economic aquatic animal farmed in China, however, the nutrients requirement of this species has not been well established. Selenium is an important component in glutathione metabolism, which plays critical roles in anti-oxidative system. However, the requirement for selenium of the Chinese mitten crab is unknown. In the present study, a 6-week feeding trial was firstly conducted to investigate the effects of dietary Se supplementation on juvenile Chinese mitten crabs. Six diets containing 0, 0.2, 0.4, 0.6, 0.8 and 1.0 mg/kg selenium were fed to quadruplicate juvenile Chinese mitten crabs (0.27±0.01) g, respectively. The results showed that hepatopancreas and muscle selenium contents were positively related to the dietary Se supplementation. The Se supplementation with 0.4 mg/kg brought the highest weight gain, survival rate, whole body crude protein content, and the highest activity of glutathione peroxidase and glutathione reductase in serum and hepatopancreas (P<0.05), whereas, the serum malonaldehyde content was the lowest in this group. Quadratic broken line analysis with serum GPx showed that 0.51 mg/kg selenium supplementation could meet the optimal anti-oxidative status of juvenile Chinese mitten crabs.

The second study was then performed to investigate the effects of Magnesium (Mg), which is an essential cofactor for numerous enzymatic and also plays a key role in antioxidation and immunity mechanism. A 10-week study was conducted to determine the dietary magnesium requirements of juvenile Eriocheir sinensis, by feeding diets containing L-aspartic acid magnesium with 0, 1.5, 3.0, 4.5, 6.0, 7.5g/kg diet, respectively. Quadratic broken line analysis with glutathione shows that a level of 3.76g/kg magnesium supplementation (total magnesium is 4.39g/kg) can meet the optimal growth of juvenile Chinese mitten crab.

In order to know the joint supplemental effects of selenium and magnesium, a 8-week feeding experiment was followed to study the effect of organic magnesium and organic selenium on the growth performance and antioxidation system of Eriocheir sinensis. Four treatments with control, organic Se (0.4mg/kg), organic Mg (0.3%) and the combination of organic Se and Mg were designed. The results showed that in serum, organic Se significantly decreased feed coefficient and reactive oxygen species (ROS), meanwhile, Se improved the total antioxidant capability (T-AOC) and the content of glutathione (GSH) significantly(P<0.05). The content of GSH and T-AOC in the serum and hepatopancreas for the crabs fed organic Mg also increased significantly, which might cause significant decrease of the ROS and MDA(P<0.05). There was a significant interaction (P<0.05) between organic Mg and organic Se in promoting growth, feed efficiency and anti-oxidation ability.
This study aimed at demonstrating the effects of total fish oil replacement by plant oils on the oxidative stress response and several immune system parameters in Nile tilapia (*Oreochromis niloticus* L.). Five iso-nitrogenous and iso-lipidic diets (34 % CP and 14 % CL on a dry matter basis) were formulated to totally replace Fish Oil (FO100) by sunflower seed oil (SF100), Canola Oil (CO100), Linseed Oil (LO100) and equal combination (FO25/SF25/CO25/LO25) of these vegetable oils. A total of 375 fish (32.5±0.5 AMBW) were randomly assigned to 15 190 l (130 l rearing volume) rectangular fiberglass tanks and fed 3 % BW/d two times in equal amounts both for morning (09:00-10:00 h) and afternoon feeding (17:00-18:00 h). Glutathione peroxidase (GSH-Px), Catalase (CAT), Malondialdehyde (MDA) and Super Oxide Dismutase (SOD) activity levels in liver were analysed to demonstrate the effects of plant oil replacement on oxidative stress response in fish. Myeloperoxidase (MPO), Lysozyme and Nitric Oxide (NO) activities were also measured to understand the immune functions of Nile tilapia fed dietary plant oils. Fish tripled its initial live weight after 90 day of growth period and there were no significant differences (*P*>0.05) between dietary groups. There were also no significant differences (*P*>0.05) in terms of GSH-Px, Lysozyme, MPO and SOD levels of fish fed different dietary treatment in this experiment. However, fish fed LO100 diet had significantly higher (*P*<0.001) CAT and MDA levels than that of fish fed the other dietary treatments indicating that high dietary α-Linolenic acid is prone to peroxidation leading to accumulation of MDA. Nitric oxide levels in fish fed diets SF100, CO100, LO100 and FO25/SF25/CO25/LO25 had also significantly higher (*P*<0.001) Nitric oxide levels than that of fish fed FO100 diet indicating that plant oils either replacing fish oil in diets individually or in combination with fish oil might behave as immuno-stimulant in Nile tilapia.

**Keywords:** Nile tilapia, growth, plant oil replacement, oxidative stress response, immune system
The demand for fish meal is increasing due to the high dietary protein requirement for carnivorous marine finfish diets, and the continuous growth of aquaculture production over the last few decades. The use of alternative protein ingredients would help to reduce the dependency on fish meal. Hence, partial replacement of fishmeal by plant protein and other ingredients has been accomplished in many carnivorous cultured fish. As alternative protein sources are not well balanced in indispensable amino acids (IAA) according to the requirement of fish, supplementation with crystalline amino acids (AA) is often necessary. Therefore, knowledge of the ideal dietary AA profile is paramount in order to allow an optimal protein growth. This ideal AA profile depends on the AA profile of the proteins being synthesized and also on the relative use of the different AA for energy production or for other metabolic purposes.

This work aims to study how the bioavailability of different IAA is affected by two different DP/DE ratios in gilthead seabream (Sparus aurata) juveniles using 14C-labelled IAA. Bioavailability will be assessed by quantifying gut absorption, oxidation and retention of tracer IAA. Gilthead seabream were reared following standard procedures and the normal feeding schedule used in grow-out production systems, and feed the two different DP/DE ratios for one month. The experiments were done by tube-feeding gilthead seabream juveniles with the test feed marked with 14C-labelled IAA as tracers. A set-up to determine the handling of 14C-labelled IAA was used, in order to quantify the absorption, catabolism and retention of the tracers fed to gilthead seabream juveniles.

The experiments demonstrated significant different IAA absorption, retention and catabolism among the IAA tested. Results point to diverse digestion/absorption capacity for different IAA in gilthead seabream juveniles, as well as their relative utilization for energy production. Differences in IAA bioavailability in relation to the dietary DP/DE ratios will be discussed.
EFFECT OF SQUID WASTE SILAGE ON THE GROWTH AND FEED EFFICIENCY OF SUTCHI CATFISH, PANGASIUS HYPOPHTHALMUS

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The effects of squid waste silage (SWS) on the growth and feed utilization of juvenile Pangasius hypophthalmus (Sauvage, 1878) was investigated. Squid waste silage was produced by the addition of 3% formic acid. There was significant decrease in protein and increase in lipid and ash (P<0.05) in the squid waste silage than that of squid waste. Seven isonitrogenous (30% protein) and isolipidic (12% lipid) experimental feeds were formulated incorporating squid waste (SW) and squid waste silage (SWS) to replace fish meal at 33.33, 66.67 and 100% of dietary protein (SW 33.33, SW 66.67, SW 100, SWS 33.33, SWS 66.67 and SWS 100) respectively. Each diet was fed to three replicate groups of fish with an initial weight 5.43 – 5.70 g for 60 days. The mean weight gain, percentage weight gain, SGR and ADG of fish fed SW 33.33 diet were significantly higher than those fed other diets (P<0.05). However there was no significant differences in the mean weight gain, percentage weight gain, SGR and ADG between the fish fed on control, SW 66.67, SW100, SWS 33.33, SWS 66.67 and SWS 100 diets. Feed conversion ratio and feed conversion efficiency of fish fed SW33.33 was significantly better than those fed other diets (P<0.05). The FCR and FCE of fishes fed SW66.67 and SW100 were significantly lower. Protein efficiency ratio (PER) of fish fed SW 33.33 was significantly better than those fed other diets (P<0.05). There was significant difference (P<0.05) in mean feed intake and it was highest in SW 33.33 diet. HSI was significantly higher in control diet than those fed other diets (P<0.05). The fishes fed all diets showed 100% survival. The whole body composition of sutchi catfish fed the squid waste and squid waste silage diets did not show any variations in moisture, protein, lipid and ash.

The present study indicated that fish meal can be replaced up to 100% by squid waste and squid waste silage based diets without any adverse effect on the growth, feed utilization and body composition.
VEGETABLE OIL REPLACEMENT IN GROWTH PERFORMANCE AND THE EFFECT ON BLOOD PARAMETERS IN WHITE SEABASS

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The primary lipid source in formulated feeds is fish oil (FO), rich in highly unsaturated fatty acids (HUFA). The annual global production of FO in aquafeeds has increased and its demand from capture fisheries has reached its limits of sustainability. Vegetable oils (VO) production has been increasing consistently; some VO contain higher levels of linolenic and linoleic fatty acids than those in FO. Partial replacement of FO by VO have been tested across a variety of cultured fish species, with good and controversial results on growth parameters. Replacing FO with VO can decrease the important fatty acids in fish tissues with a negative impact on fish health. White seabass (WSB) is an important commercial species and its production for stock enhancement is now a well-controlled process although more information regarding the nutritional profile of this carnivorous marine fish is needed to improve growth and ensure economical and environmental sustainability. The aim of this study was to identify the impact of feed supplemented with linseed and maize oil on growth performance, proximate body composition, digestibility and the outcome on hematological and biochemical blood parameters of juvenile white seabass.

Five experimental diets were formulated with 18% lipid from 100% FO, 3:1 blends of linseed oil and maize oil: 37.5%/12.5% (FVO1); 12.5%/37.5% (FVO3) respectively; 1:1 blend of 25%/25% (FVO2), 50% maize oil (FMO); diets with blends of VO contained 50% FO. Fish were fed 13 weeks in a semi-closed recirculating system at 20°C. Growth performance parameters were measured; fish muscle, liver and whole-body composition analysis were taken; apparent digestibility coefficients were measured, lipids were extracted with chloroform and blood samples collected for hematological and biochemistry parameters. Data was analyzed with one-way ANOVA (P=0.5).

Fish overall growth performance increased from 45.0 to 51.9 g. Although proximate composition of whole fish, muscle and digestibility had slight differences, values were within those found in recent studies for marine fish. HUFAS augmented in muscle in fish fed oil blends. The contrary was observed in muscle PUFAS (polyunsaturated fatty acids). HUFAS and PUFAS were lower in liver in fish fed oil blends. Dietary VO inclusion for WSB had no effect on red bloodline. Plasma glucose and triglycerides were higher in fish fed with blends of linseed and maize oils.

VO as a replacement of FO did not affect the overall growth parameters, proximate composition and digestibility of WSB. Based on the limited data available and the contrasting results in the still scarce studies related to FO replacement by VO and the effect in marine carnivorous fish blood biochemistry, results suggested that though there are yet many questions to elucidate regarding lipid and glucose metabolism in fish fed with VO, the inclusion of blood biochemistry in response to fish diet formulation indicated that though fish fed the experimental diets had similar growth performance in this short term study, blood biochemistry results clearly pointed toward fish health alterations. Further discussion and fatty acid analysis will be presented.

The Sciaenidae shi drum (*Umbrina cirrosa*) is considered to be a new candidate in Mediterranean aquaculture. It is a common wild species in the Mediterranean Sea showing a very fast growth. Although shi drum has a great potential for aquaculture currently there is a limited number of studies on its nutritional requirements. The aim of the present study was to evaluate the nutritional needs of shi drum juveniles by feeding different protein/lipid diets.

Six diets were formulated containing 47% and 52% protein in combination with three different lipids level 10%, 15% and 20% (2X6), containing the same raw materials at different percentages. Diets were tested using shi drum of 7.2g initial body weight, held in 18 cylindroconial tanks of 180lt capacity, for two months. Each diet was tested in triplicate and 30 fish were placed in each tank. Fish were fed by hand at libitum twice a day and water temperature ranged from 20 to 22°C during the whole duration of the experiment. The growth of fish was followed by weighing all the population at the begging in the middle and at the end of the trial. Samples of the fish from each tank were collected at the end of the trial for whole body and liver composition.

At the end of the experimental trial fish duplicate their weight. The best growth parameters were achieved in fish fed on 52% dietary protein level both at 10 and 15% dietary lipid although differences were not significant from diet containing 47/10. Fish fed on diets with the highest lipid content 20% gave the worst performance results irrespective of the dietary protein content. Feed efficiency was the best at the high dietary protein level both at 10 and 15% lipid content. Protein utilization by fish was the best in diet 47/10 significantly different from the rest of the diets except from diets 52/10 and 52/15.

Hepatosomatic index was the highest in diets 47/10 and 47/15 and the lowest in diets 52/15 and 52/20 and differences were significant between them. Fat and glycogen content were inversely correlated. Body composition didn’t reveal differences among experimental diets. The increase in dietary lipid from 10 to 20% didn’t result in increase in lipid deposition.

The results of the present study suggest that the optimum dietary protein requirement for shi drum juveniles is around 52% in combination with 10 or 15% lipid. High fat diets were not recommended for shi drum since they resulted in poor growth and feed utilization.

Key words: shi drum, *Umbrina cirrosa*, protein/lipid, growth, feed efficiency
PRELIMINARY RESULTS ON GROWTH, FEED UTILIZATION, BODY AND FILLET COMPOSITION OF MEAGRE (Argyrosomus regius) REARED UNDER DIFFERENT PROTEIN/LIPID REGIMES TO COMMERCIAL SIZED FISH

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Mediterranean aquaculture is based mainly on gilthead sea bream (Sparus aurata) and European sea bass (Dicentrarchus labrax). Since there is an increase demand for new products the effort has been concentrated on diversification of the aquaculture industry by the introduction of new species. The sciaenid meagre (Argyrosomus regius) is a relatively new and promising species in aquaculture, as it can be adapted easily to captivity, exhibiting high growth rates and tolerating a wide range of salinities and temperatures. In pertinent literature there is little information about its nutritional requirements on dietary protein and lipid over a long rearing period to commercial size fish. The aim of the present study was to evaluate the nutritional needs of a new species for aquaculture by feeding different protein/lipid diets over a long rearing period in commercial like conditions.

Four diets containing 43% and 47% protein in combination with two different lipid level 15% and 20% (2X2), were formulated containing the same raw materials at different percentages. 35% fish meal was included to all diets and diets were extruded by a commercial extruder. Diets were tested using meagre of 350g initial weight, held in 12 (3x3x6 m³) cages, in the experimental unit of HCMR, reared from June 2012 to April 2013. Fish density was 5kg/m³ and fish were fed by automatic feeders. Water temperature ranged from 26 to 16°C during the whole experimental period. The growth of fish was followed by weighing 20% of the population each month and the whole population at the end of the experimental trial. Samples were collected at the end of the trial for whole body and fillet composition.

The highest growth parameters (SGR, DGI, TGC) were achieved at 43% dietary protein both at 15 and 20% dietary lipid although differences were not significant from diet 47/15. Diet 47/20 showed the lowest growth performance parameters. Feed efficiency and feed conversion ratio was the best in diets 43/15 and 43/20. Hepatosomatic index was high in diets with 20% dietary lipid both at 43 and 47% protein. Body and fillet composition didn’t reveal differences among treatments. The increase in dietary lipid didn’t result in increase in lipid deposition neither in the body nor in the fillet of the fish.

The results of the present study suggest that the optimum dietary protein requirement for meagre from an initial body weight of 350g to a final body weight of 1000gr is around 43% in combination either with 15 or 20% dietary lipid.

Key words: meagre Argyrosomus regius, protein, lipids, growth, fillet composition
Cachara, *Pseudoplatystoma reticulatum*, is a carnivorous freshwater catfish with high commercial value for Brazilian aquaculture, whose dietary protein and energy requirements are still unknown. To determine the protein requirement for the initial growout phase, groups of 15 juveniles (16.08 ± 1.13 g) were fed isocaloric diets (19.26 MJ/kg gross energy) with increasing levels of crude protein (30, 35, 40, 45, 50, and 55%) for 60 days, in triplicate. A polynomial regression analysis revealed a quadratic effect (P < 0.05) of increasing the concentration of crude protein on weight gain, feed efficiency, feed consumption and protein retention. The proteases activities, measured in the stomach and intestines, were highest (P < 0.05) in fish fed the 50% protein diet. Protein, dry matter, and energy digestibilities did not vary significantly among diets containing 40, 45, and 50% crude protein. Therefore, the estimated protein requirement for juvenile cachara was 49% crude protein for the maximum daily weight gain of 1.4 g. This equates to approximately 45% of digestible protein and to a dietary digestible protein/gross energy ratio of 23.3 g/MJ. On a second study, to estimate cachara’s energy requirement, isonitrogenous diets (49% crude protein) were formulated with increasing levels of gross energy (15.5, 17.0, 18.2, 20.2, and 21.9 MJ/kg) and fed to groups of nine juveniles (84.43 ± 13.59 g) for 90 days, in triplicate. Similarly to the previous study, a polynomial regression analysis revealed a quadratic effect (P < 0.05) of increasing the concentration of dietary gross energy on weight gain, feed efficiency, feed consumption, and protein retention. However, body fat increased linearly (P < 0.05) as dietary gross energy increased, unlike body moisture. Protein, dry matter, and energy digestibilities did not vary significantly among diets containing 17.0, 18.2, and 20.2 MJ/kg gross energy. Therefore, the dietary energy requirement estimated for juvenile cachara was 18.8 MJ/kg gross energy for the maximum daily weight gain of 1.7 g. This equates to 14.6 MJ/kg digestible energy.
Gnotobiotic models help understanding the mechanisms involved in the interactions between intestinal microbiota and host by excluding microbial communities belonging to non-axenic animals (reared in non-sterile conditions) and by including the microorganisms to be tested. In this preliminary study, jundiá (Rhamdia quelen) catfish eggs from induced spawning were previously rinsed three times in autoclaved natural water and subsequently disinfected with hydrogen peroxide for 3 min at different concentrations (0%, 0.3%, and 3%) in quadruplicate. All eggs were kept in erlenmeyer flasks, in their respective solutions, which were prepared with autoclaved natural water. The flasks were sealed, kept under constant light and sterile aeration. Before and after disinfection, samples of 30 eggs, in triplicate, were transferred to sterile stomacher® bags and homogenized for 120 s, at low intensity, and for 120 s, at high intensity. Samples were plated (pour-plate) over Luria Bertani agar and incubated for 48 h at 31 ± 2 °C. After 24 h from hatching, larvae were transferred to beakers containing the respective solutions. The flasks were sealed and kept without aeration or illumination and larvae were kept without feeding. Larvae survival was assessed within 72 h after transfer. Non-disinfected egg samples presented countings higher than 300 colony forming units (CFU) per plate whereas hydrogen peroxide treated eggs reached the desired disinfection (less than 30 CFU). No significant differences were found in hatching rate among larvae kept as control or submitted to 0.3% hydrogen peroxide concentration (28.8% and 30.0%, respectively). Similar results were registered for larval survival (87.2% and 90.5%, respectively). However, hydrogen peroxide at 3% impaired egg hatching. An identical experiment was subsequently conducted, where jundiá catfish eggs were disinfected with 0.3% hydrogen peroxide, followed by the addition of an amylolytic potential probiotic strain of Aeromonas sobria, just after hatching. A. sobria was added at 10^9 CFU/ml and the triplicate flasks incubated for 120 h. Control larvae were submitted to the same procedures except for the bacteria addition. Larvae were histologically examined and showed no organ damage, demonstrating the potential of this strain for future use as a probiotic to improve starch digestibility. The concentration of 0.3% hydrogen peroxide was effective in the disinfection of eggs, being suitable for use in gnotobiotic models of jundiá catfish larvae.
Poultry by-product meal is a suitable protein replacement for the carnivores cobia (Rachycentron canadum) and the freshwater catfish cachara (Pseuodonatystoma reticulatum)

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Poultry by-product meal is highly available in Brazil, since the country is the world’s largest exporter of poultry. However, using poultry by-product meal as a substitute for fishmeal in aquafeeds shows conflicting results. Two studies were designed to evaluate a good quality poultry by-product meal (68% crude protein, 14% ether extract) as a salmon by-product meal (72% crude protein, 11% ether extract) protein replacement for two carnivorous species of interest to Brazilian aquaculture. The protein replacement levels tested were 0, 20, 40, 60, 80, and 100%. In the first study, groups of five juveniles (46.09 ± 1.49 g) of cobia (Rachycentron canadum) were fed six isonitrogenous, and isocaloric diets (47% crude protein, and 4,100 kcal/kg gross energy) for 56 days, in quadruplicate. Diets were supplemented with DL-methionine, as follows: 0.00, 0.03, 0.09, 0.15, 0.20, 0.26%, respectively. For the second study, other six isonitrogenous and isocaloric diets (50% crude protein and 5,000 kcal/kg gross energy) were fed to groups of nine cachara catfish (Pseuodonatystoma reticulatum) juveniles (87.75 ± 15.91 g) for 60 days, in triplicate. There was no dietary amino acid supplementation in this study. Weight gain (3.1 g/fish/day), feed efficiency and protein retention of cobia were not significantly different for all replacement levels but viscerossomatic and hepatosomatic indices increased as the protein replacement increased. Whereas for cachara catfish, best weight gain (3.0 g/fish/day), feed efficiency and protein retention were achieved when fish was fed 80% protein replacement (P<0.05). Histology of liver (both species), stomach and intestines (cachara only) revealed no morphological differences with increasing protein replacement. However, significant differences in body composition (protein, fat, ash, and dry matter) were registered in cachara catfish fed 100% protein replacement diet. Additionally, protein (90.32 ± 0.8%), energy (80.39 ± 1.7%) and dry matter (73.39 ± 3.4%) digestibility coefficients did not vary significantly among cachara fed diets with 0, 40, 60, and 100% protein replacement. Our findings showed that high levels of dietary fish meal protein replacement are possible in diets for cobia (100% with methionine supplementation) and cachara catfish (80%), without hindering growth, when a good quality poultry by-product meal is used in the formula.
POSTPRANDIAL METABOLIC RESPONSE AND BIOENERGETICS OF GENETICALLY DIVERSE POPULATIONS OF BARRAMUNDI, *LATES CALCARIFER*

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Australian barramundi are found across 16 degrees of latitude from the Noosa and Mary Rivers on the east coast to the Ashburton River in Western Australia. Local thermal adaptation has been shown to result in genetically diverse populations of barramundi over time. Currently, there are no data describing the nutritional physiology and bioenergetics of genetically discrete barramundi stock. Identifying the differential effects of increasing temperature as a result of climate change on the nutritional physiology of discrete populations will provide important information towards optimizing feed formulations and stock selection for the implementation of commercial breeding programmes. The increase in oxygen consumption (\(M_O^2\)) that occurs after feeding, specific dynamic action (SDA), represents the energetic cost of ingestion, digestion, absorption and assimilation of a meal. The SDA response is strongly influenced by characteristics of the meal e.g. composition, ration size and feeding frequency. \(M_O^2\) can also be used to indirectly determine an organism’s energy demand during the SDA period by using an oxyenergetic conversion coefficient. This study investigated the effects of SDA response, duration and peak, in relation to various substrates and the relationship between temperature and aerobic metabolism; mass-specific routine metabolic rate (RMR) and aerobic scope (AS) across genetically diverse populations of barramundi.

The RMR and AS of barramundi stocks from Jungle Creek, Karrumba, Gladstone and Broome, mean body weight of 262.5 ± 3.854 g, was determined across broad temperature ranges (18 to 38 °C) in open top static respirometers. There was no significant difference in RMR and AS between genetically different stocks at any temperature (\(p>0.05\)). RMR and AS increased linearly with increasing temperature; the relationship between: RMR and temperature (\(T; 18 \text{ to } 38 \, ^\circ\text{C}\)) can be described as 3.464\( T - 27.605 \, \text{mg} \, \text{O}_2 \, \text{kg}^{-0.8} \, \text{h}^{-1}\) and AS and temperature (\(T; 18 \text{ to } 38 \, ^\circ\text{C}\)) can be described as 4.212\( T + 27.684 \, \text{mg} \, \text{O}_2 \, \text{kg}^{-0.8} \, \text{h}^{-1}\). SDA is discussed further in relation to various substrates.
POSTER 045

THE EFFECT OF DIFFERENT DIETARY ENERGY SOURCES (STARCH, FAT) ON METHIONINE REQUIREMENT AND UTILIZATION EFFICIENCY FOR NILE TILAPIA (OREOCHROMIS NILOTICUS)

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An 9-week feeding trial was conducted to determine the relationship between dietary energy resources and methionine requirement in Nile tilapia, Oreochromis niloticus. Eight practical diets (four contain 17% starch and four contain 7% fat) were formulated to contain graded levels (0, 0.1, 0.3, 0.6%) of methionine. Each diet was randomly assigned to triplicate groups of 30 tilapia (approximately 5.23g) and tilapia were fed 3 times a day. The results indicated that the weight gain and specific growth rate increased with the supplementation of methionine in the diet with starch. Diet with fat shows the same trend except the 0 dietary methionine. Hepatosomatic index were increased significantly with the supplementation of methionine in both starch and fat diets. Both viscerosomatic index and intraperitoneal fat ratio have no significant differences in starch diet which is the same with fat diet. But HSI and IPF in the fat diet were significantly higher than that of starch diet. There were no significant differences observed in survival and condition factor among all treatments. These results indicated the growth and protein efficiency ratio of tilapia significantly increased as the supplementation of dietary methionine in both starch and fat diets. But the growth and protein efficiency ratio of tilapia in starch groups were much higher than those in fat groups in each dietary methionine level. It indicated that tilapia could use supplemented methionine much better with starch than with fat.
The present study was conducted to evaluate the effect of various feeding patterns of emodin on growth, non-specific immune responses, and disease resistance to Aeromonas hydrophila in Wuchang bream. Healthy Wuchang bream (initial weight: 3.47±0.032) were grown in a circulating water system for 8 weeks. Five groups were studied: one control group was fed with a basal diet for eight weeks (P1), and four treatment groups were initially fed with a trail diet of 30 mg/kg emodin then switched to a basal diet at intervals of one week (P2), two weeks (P3), four weeks (P4) and none, keeping the fish on a trail diet for the entire eight week study duration (P5). Results indicated that different feeding patterns of emodin significantly influenced the weight gain rate of Wuchang bream (P<0.05). Fish in the P4 treatment group had significantly higher rates of weight gain (WG) than those in other treatment groups. There were no significant difference in survival rates or feed conversion ratios (FCR) between treatment groups and the control group. White blood cell count (WBC), respiratory burst activity, superoxide dismutase (SOD), myeloperoxidase (MPO) and tumor necrosis factor-α (TNF-α) activities were shown to increase under P3 conditions, and decrease under P5 conditions. Fish under P4 treatment had a significant improvement of all tested parameters compared to control. Significantly higher levels (P < 0.05) of plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity were observed in P2 and P4 treatment groups compared with the control group, while no significant differences were observed in the AST and ALT activity of fish under P2, P3, P4 and P5 treatments. In a bacterial challenge experiment with A. hydrophila, fish under P4 and P5 treatment showed lower cumulative mortality than the control group. The results of this study suggest that an initial 4-week feeding interval is recommended for the economic and practical culture of Wuchang bream.
Availability of sustainable raw materials is a prerequisite for sustained increases in global aquaculture production. This study examined the utilization of locally available lipid sources for sustainable aquaculture fish production in Nigeria. Imported fish oil (FO) was replaced with locally available vegetable oils (groundnut oil, GO; soybean oil, SO; Shea butter, SH and palm oil, PO) representing treatments 1-5 (T₁-T₅) respectively as dietary lipid sources for *Clarias gariepinus* fingerlings in an iso-nitrogenous (40% crude protein) and iso-caloric (4816 kcal/kg) diet to evaluate the impacts of dietary lipid source on feed utilization (Feed intake, FI, Protein Efficiency Ratio, PER and Feed Conversion Ratio, FCR), growth (weight gain and specific growth rate), hematology (packed cell volume, PCV; hematocrit, Hb; erythrocytes, RBC; leucocytes, WBC; mean corpuscular volume, MCV; mean corpuscular hemoglobin concentration, MCHC) and serum lipid levels (total cholesterol, TOC; free cholesterol, FCH; triacylglycerols, TAG; high density lipoprotein, HDL; low density lipoprotein, LDL and very low density lipoprotein, VLDLP). 25 fish with an initial weight of 6.71 ± 0.69g were stocked in individual net fish happa (1m x 1m x 1m) suspended by bamboo poles in an earthen pond (36m x 30m x 1.5m) and fed experimental diets at 5% body in two equal portions offered at 09.00 and 16.00hrs daily for 56 days. Data obtained were analyzed using Analysis of variance (ANOVA) and correlation analysis. Although feed utilization and growth were similar in treatments, with survival in the order T₁>T₅>T₃>T₄>T₂; weight gain was in the order T₅>T₄>T₃>T₂>T₁, indicating that all the locally available dietary oil sources supported growth in *C. gariepinus* better than the imported source. There were significant differences (P≤ 0.05) in all the hematological parameters evaluated except in MCH and MCHC. The highest packed cell volume (38±0.84%) was observed in T₃, while T₅ had the least (33±0.82%). T₃ had the highest hemoglobin (12.8±0.06 g/dl) and red blood cell contents (44±0.07 mil/mm³), while T2 had the least values of 10.8±0.06 g/dl and 3.6±0.06 mil/mm³ respectively. White blood cell content was similar in the control diet (T₁) and T₃, T₄ and T₅ (5200 ±10⁴/mil³), but was significantly lower (P ≤ 0.05) in T₂ (5000 ±10⁴/mil³) suggesting that T₂ reduced stress in experimental fish compared to other diets. Serum lipids of *C. gariepinus* varied significantly (P ≤ 0.05) with dietary lipid source in the order T₃>T₁>T₄>T₅>T₂. A negative but statistically insignificant correlation was observed between serum lipids and growth; thus confirming that locally available groundnut oil, soybean oil, Shea butter and palm oil can serve as suitable alternatives to fish oil as dietary lipid source for the African catfish, *Clarias gariepinus*.

**Key words:** fish oil, vegetable oils, *Clarias gariepinus*, growth, hematology, serum lipids.
BIOCHEMICAL COMPOSITION OF MARINE SEAWEEDS AND THEIR USE IN ECOFRIENDLY SHRIMP FEEDS

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The Northwestern Bay of Bengal supports the mangrove dominated deltaic complex known as Sundarbans at the confluence of the Ganga-Bhagirathi-Hooghly River system and the Bay of Bengal. Three abundantly found seaweeds (Enteromorpha intestinalis, Ulva lactuca and Catenella repens) were collected from three different sites of Indian Sundarbans that are significantly different with respect to salinity and compared for their biochemical composition. Carbohydrate, total protein, total lipid and astaxanthin were estimated in these species and their seasonal variations were critically analyzed. The study revealed that Enteromorpha intestinalis is the most nutritionally rich species in terms of biochemical composition compared to Ulva lactuca and Catenella repens.

Traditional method of tiger prawn culture is a common practice in this area, which is done without any proper scientific back-up, formulated feed and water quality management. The shrimp farmers use traditional feed of animal origin that often results in fouling of water quality and disease outbreak in culture species. The present paper highlights the effect of total replacement of animal ingredients in shrimp feed with floral ingredients using high protein seaweeds collecting from Indian Sundarbans on the water quality and shrimp health. Weight gains, condition index, Feed Conversion Ratio (FCR), survival, body pigmentation (astaxanthin level) were analyzed in shrimps along with pond water quality. Higher condition index values, survival rate and gain in shrimp weight were observed in experimental pond (E) compared to control pond (C). Low FCR values were observed in the experimental pond than the control pond. Astaxanthin level in shrimps of the experimental pond were also higher than the control pond which points towards Catenella repens as the source of carotenoid in the shrimp tissue. Cost-benefit analysis also proved better yield and profit in case of experimental pond indicating the sustainability for the present venture.
A series of experiments were conducted with black tiger shrimp (*Penaeus monodon*) juveniles to firstly determine the threshold to fishmeal inclusion in a diet and then to evaluate the potential for a bioactive growth stimulant to support complete replacement of both fish meal and oil in feeds when fed under both clear-water and green-water conditions. The isoproteic and isoenergetic replacement of fishmeal resulted in a consistent decline in growth performance indicating that at every decrease in fishmeal below a basal level of 50% there was a decline in performance. In a subsequent trial undertaken in a clear-water tank system diets devoid of both fishmeal and oil fed to shrimp were demonstrated to produce poorer performance than a standard fishmeal and fish oil reference diet. However the addition of a microbial bioactive to the diet resulted in not only a compensation for the replacement of these ingredients but additional growth. Replication of the clear-water trial in a green-water tank system produced similar results, but also showed that the green-water system largely compensated for the performance lost through replacement of fishmeal and oil. However it was also shown that the use of the microbial bioactive in the diets still resulted improved growth performance of shrimp over than achieved using a standard diet based on fishmeal and fishoil. This study has effectively demonstrated a viable strategy for complete replacement of all fishery products in shrimp diets and delivers not only a replacement strategy, but also improved performance.
POSTER 050
EFFECT OF DIETARY COMPOSITION ON THE FEASIBILITY OF TILAPIA (OREOCHROMIS NILOTICUS)
PRODUCTION IN AN AQUAPONIC SYSTEM
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A pilot scale aquaponic system was designed to produce 200 tons of tilapia (whole cycle production) and vegetables. In order to assess the technical and economic feasibility of the production system, 3 commercially available on growing tilapia diets were selected. The diets contained similar dietary energy levels (Diet A: 14.8 KJ/Kg DE; Diet B: 13.5 KJ/Kg DE and Diet C: 14.8 KJ/Kg DE) but different protein (40%, 37% and 33%) and lipid (5%, 7% and 13%) dietary levels and ratios (7.8, 5.4, & 2.5). Two groups of fish with an initial body weight of 30 g were fed 3-5 times by automatic feeders during 174 days with Diet A and Diet C. A third group of fish with an initial body weight of 100 g was fed on Diet B 3 times a day during 174 days. The three groups of fish were fed on Diet A before both on growing trials started. At the end of the feeding trials, fish fed on Diet A showed the best growth and feed efficiency followed by those fed on Diet C. Fish fed on Diet B showed lower final weights than the rest of groups indicating a poor growth and feed efficiency. Regarding the digestive utilization of the diets: alkaline phosphatase (AP), trypsin, amylase, neutral lipase (BAL) and phospholipase A2 (PLA2) activities were determined in digestive contents of fish. Higher AP activities were observed in fish fed Diet A, and both higher BAL and PLA2 activities in fish fed diets with higher dietary protein and lipid levels, thus Diet A and C, respectively. However, higher dietary EPA and DHA levels did not increase BAL and PLA2 activities in fish fed diet B. Although, trypsin and amylase activities were slightly higher in fish fed diet B. Results indicated that the quality and an adequate mixture of the raw materials used in Diet A, together with a higher protein to lipid ratio of the diets enhance growth performance of on growing tilapia in a recirculating system, allowing better production feasibility.
In recent years, due to the increasing demand in the marine ornamental fishes globally, but still we depend on wild sources which may soon affect their diversity. So, their hatchery production has been practiced. The captive breeding of marine ornamentals has been developed in many countries, but the mass mortality during first feeding is prevalent. This could be due to inadequacy in meeting nutritional requirements of the larvae. In the present study, the effect of the live prey enrichment with n-3 polyunsaturated fatty acids rich marine pelagic fish oil on the growth and survival of the marine ornamental fish larvae were studied. The rotifers (Brachionus plicatilis) has been used as a candidate species and the enrichment study duration for 24 h have been analyzed at 3 h time intervals to understand the suitable enrichment period. It shows that enrichment for a period of 3 h is best. The enriched rotifers have been used for the experiment. The newly hatched larvae were separated from the broodstock tank and transferred with care to three different larval rearing tanks with triplicates, enriched with marine microalgae (A), DHA protein selco (B) and polyunsaturated fatty acids rich sardine oil (C). The trend was used as different experimental combinations to understand their individual and synergistic effects. The growth and survival in treatment C was confirmed to be superior among other experimental combinations.

Keywords: PUFAs; pelagic fish oil; marine ornamental fish larvae; rotifer; enrichment
POSTER 052
NUTRIGENOMIC DIFFERENTIATION OF GENETICALLY DISCRETE BARRAMUNDI (LATES CALCARIFER) POPULATIONS
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The increasing trend of replacing fishmeal in aquafeed for carnivorous species with plant-based ingredients has a great potential to introduce genotype and diet (G x E) interaction. Today, there is no available information of how genetically discrete Australian barramundi population may exhibit different capacity in utilizing fishmeal-free diet. This research examined the existence of differential phenotypic and transcriptomic performances of two genotypically discrete barramundi (Lates calcarifer) stocks from Bowen and Northern Territory when fed with fishmeal or fishmeal-free diet under two different temperatures. A split-plot designed experiment was conducted in temperature-controlled recirculation systems, 28 °C and 18 °C at Marine and Aquarium Facility Unit (MARFU), James Cook University (JCU) for 6 weeks. Two relatively iso-proteic (44%), iso-lipidic (21%) and iso-energitic (23%) diets (fishmeal-based and fishmeal-free) were manufactured and used in the experiment. Important phenotypic data such as Thermal Growth Coefficient (TGC), Feed Conversion Ratio (FCR), and Voluntary Feeding Intake (VFI), plasma metabolites activities and carcass body composition were collected during the study. In addition, transcriptomic data will be generated from the liver tissue using high-throughput direct-RNA sequencing method to understand the underlying process of differential phenotypic performance. Using three way ANOVA analysis, from the phenotypic parameter recorded (TGC, FCR, and VFI), significant genotypes, diet and temperature effect was detected (p<0.05). Therefore, we concluded that the different capacity in utilizing fishmeal or fishmeal-free diet in these two studied Australian barramundi population is greatly under G x E control.

Keywords: Barramundi, Nutrigenomic, Genetic-environment interaction, fishmeal-free diet
A twelve week investigation into the effects of feeding substituted palm oil (PMO) with coconut oil (CNO) as an alternative source of energy, on the growth performance and nutrient utilization of *Clarias gariepinus* juveniles was conducted. Six (6) isonitrogenous 40% crude protein diets were formulated where CNO replaced PMO at 100%, 80%, 60%, 40%, 20% and 0% inclusions. Juveniles of *C. gariepinus* (40.39g± 2.98g) were stocked at 20 fish/50 L bowls in triplicate, and were fed diets twice daily to satiation with weight changes recorded weekly. Samples of experimental diets and fish carcass were analyzed for proximate composition, while growth performance and quality of diets were evaluated. Fish fed diet 4 (containing 60% palm oil, 40% coconut oil) inclusion had the highest mean weight 203.36g ±2.27 after the twelve week study. Fish fed diet 2 (containing 20% palm oil, 80% coconut oil) inclusion gave the highest gross food conversion efficiency of 125.0g ±3.18. There was a significant difference (P< 0.05) in the percentage weight gain between fish fed diet 1 (70.05 ±3.80g) and fish fed diet 3 (4.18 ±0.55g). For better growth therefore, a 40% partial replacement of PMO with CNO as dietary lipid in diet of African mud catfish (*Clarias gariepinus*) juveniles as alternative source of energy is recommended.

**Key words:** *Clarias gariepinus*, diet, coconut oil, palm oil.
Growing interest for reducing the fish meal inclusion in aquaculture feeds has encouraged the evaluation of new alternative protein sources. Nowadays, the use of plant protein ingredients is common for aquaculture feeds and high protein plant sources, like soybean, are well known. In this context, lupin seeds are gaining interest as fish feed ingredients due to their rich protein content. Lupinus mutabilis seeds possess the highest levels of oil and protein of all domesticated lupin species. A feeding trial was conducted to assess the suitability of a low-alkaloid (<0.05%) variety of Andean lupin (Lupinus mutabilis) as partial fish meal replacement in diets for Yellowtail amberjack (Seriola lalandi) juveniles on the basis of feed acceptability, growth, feed conversion, survival and the occurrence of deformities. A fish meal based diet was used as control. Experimental diets were formulated to contain different inclusion levels of lupin (15, 30 and 40%) and identified as L15, L30 and L40 respectively. Since soybean is considered a standard plant protein source in aquafeeds a diet formulated to contain 30% of this ingredient was included in the essay (S30). All diets were isonitrogenous (43% crude protein), isolipidic (19% crude lipid) and isoeenergetic (22.5 MJ/kg). Triplicate groups of fish (4.69±0.06 g) were assigned to each experimental diet and fed over a 33 day period. The mean consumption did not vary significantly between groups fed all diets with the exception of the group fed diet containing 40% lupin. The results indicate that weight gain and specific growth rate at the end of the experiment were not different (P>0.05) among the groups of fish fed diets L15, L30 and S30. In general growth performance and feed utilization were better for diets L15, L30 and S30 in comparison with the control group fed the fishmeal based diet. However, the best results in terms of final body weights were obtained with diets L15 and L30. Malformation and mortality were significantly higher in the group of fish fed diet L40 in contrast with the rest of the groups. In conclusion, Andean lupin meal can be included up to 30% in extruded diets for juveniles of Seriola lalandi without adverse effect on growth performance and feed utilization. Based on these results, sweet varieties of Andean lupin represent a potential alternative for fish meal and soybean in diets for Yellowtail amberjack juveniles.
The salmonid fish *O. kisutch* is an important species in Chilean aquaculture industry, so that a detailed knowledge of its digestive physiology is advisable to improve production results. In the present work, a group of fish (119±23 g) was reared in freshwater conditions, fasted for three days and then fed an artificial moist diet (14% w/w fishmeal (73% prot:7% lipid), 20% v/w squid extract as attractant, 0.75% chromium oxide, 63.25% w/w water, gelatinized with 1% w/w gelatin and 1% w/w agarose). Diet moisture, 80.8±0.7%, and titration curve resembled that of muscle in prey fish: diet pH in water (1:5 w/v) equaled 6.1±0.0 and its buffering capacity to pH 3.0 resulted in 396.0±2.8 μEq OH⁻/g.

Animals were sequentially euthanized under anesthesia at 0, 2, 4, 6, 8 and 24 h. post-feeding and sampled (n=8-10) in order to characterize gastric contents: wet and dry weight (WW, DW), moisture (M), osmolality (O), pH, and acid protease activity at pH 2.5 (PA) were measured. Water temperature during the feeding trial was approx. 14.5±0.5°C. Stomachs were dissected and stored at -20°C until further analysis.

Mean DW followed a nearly linear decreasing pattern during the first 8 hours after feeding, reaching 0.66, 0.48 and 0.29 g at times 0, 4 and 8 h. respectively. In spite of the high moisture of the diet, M continuously increased to ~90% at 24 h. after food ingestion. The mean osmolality of the liquid phase of gastric contents showed no significant differences in the interval 2-8 h post-feeding (306±8 mOsm/kg). The temporal evolution of the mean gastric pH, measured at pyloric stomach just after dissection, fitted a decreasing quadratic pattern for the whole experimental period 0-24 h., reaching ~3.9 and ~3.5 at times 4 h. and 8 h. respectively. The mean activity of total acid proteases per mL of the liquid phase of the gastric content increased during the first two hours of digestion, and it kept within the range 600-800 µg Tyr/(min mL) until the eighth hour after feeding without a clear decreasing or increasing pattern.

Data here presented suggest that the process of diet moisturization begins in the stomach short after the ingestion of food, probably due to gastric secretory activity, whereas both the amount of secreted protons and proteases, increase within the first 2 hours of digestion. The relatively low amount of acid protease secreted before the 2nd hour, together with the fact that mean pH is clearly suboptimal for protease activity before the 4th hour, allows to hypothesize that dietary proteins evacuated to the intestine before this temporal point could not have been optimally hydrolyzed in the stomach.
A feeding trial was performed to determine the effect of the partial substitution of fish oil with soybean and linseed oils in the growth and lipid content of juvenile rainbow trout (Oncorhynchus mykiss). The basal diet (crude protein 40%, crude lipid 10%) was formulated with fishmeal (200 g/kg of diet) and soybean meal (400 g/kg) as protein sources. Fish oil was substituted with a mixture 1:1 of soybean and linseed oils at 25 (d25), 50 (d50) and 75% (d75) and as a control (dC), 100% of fish oil was used. Each diet were fed to triplicate groups of 15 juveniles of an initial weight of 0.75 ± 0.05 g (mean ± standard error) for a period of 70 days. At the end of the feeding period, growth performance was evaluated and samples of muscle and liver were took to determine the lipid content. The data obtained were evaluated with a one-way ANOVA and when significant differences were found, a Fisher’s LSD test was performed. Weight gain (%) was higher in the group fed with d50 (872 ± 26%), but no significant differences were observed when compared with the other groups (diet 25, 813 ± 4%; diet 75, 781 ± 38%; diet C, 734 ± 64%). A similar trend of higher values of the fish fed with the experimental diets was observed in the final weight (d25, 6.7 ± 0.1 g; d50, 6.9 ± 0.1; d75, 6.3 ± 0.2;) and the specific growth rate (d25, 3.1 ± 0.05 %/day; d50, 3.25 ± 0.03; d75, 3.1 ± 0.06), than the fish fed with 100% of fish oil (6.2± 0.7 g and 3.0 ± 0.1 %/day, respectively) but no statistical differences were found. Regarding the lipid content, all groups show that muscle deposit higher amount of lipids than the liver. The highest content of lipid deposition was observed in muscle of fish fed with the dC (8.0 ± 1.1 %), while the fish fed the experimental diets have: d25, 7.6 ± 0.8%; d50, 6.6 ± 0.6 and d75, 7.0 ± 0.5. No significant differences were observed. In liver, lipid content range from 4 to 5% in all groups and no significant differences were detected either. The data of the present research show the possibility to use the mixture of soybean and linseed oil up to a level of 50%, without affecting the growth performance and lipid deposition in young rainbow trout, when using a diet with high content of soybean meal as a protein source. With the fish meal and oil prices increasing, their substitution is necessary for a sustainable production and plant-origin ingredients used in this work are good candidates. The authors kindly acknowledge the funding from programs PAPIIT (project TB200112 and RR290112) of the National University of Mexico and SAGARPA-CONACYT (project 164673).

Keywords: Soybean oil, linseed oil, growth, rainbow trout, lipid content
INCLUSION OF TAURINE AND METHIONINE TO ALL-PLANT PROTEIN BASED DIETS IN JUVENILE RAINBOW TROUT (ONCORHYNCHUS MYKISS): EFFECTS ON GROWTH PERFORMANCE
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Plant-origin proteins have been suggested as candidates to substitute the fish meal in feeds for aquatic animals. However, levels of substitution are still low because plant proteins are deficient in some amino acids, particularly the sulphur-containing ones. Supplementation of these compounds may help to use more proteins of plant origin and for this reason, the effects of graded of taurine (Tau) and DL-methionine (Met) were assessed in the growth performance of juvenile rainbow trout (Oncorhynchus mykiss) fed diets based on all-plant protein sources. A basal diet was formulated (crude protein 45%; crude lipid, 10%; digestible energy, 17.8 MJ) with soy protein isolated (392 g/kg of diet) and Spirulina powder (22 g/kg of diet). To the basal diet, 1% of the sulphur amino acids were added as follows: D1 (Met 1, Tau 0); D2 (Met 0.75, Tau 0.25); D3 (Met 0.50, Tau 0.50); D4 (Met 0.25; Tau 0.75); D5 (Met 0, Tau 1). As well, a control group (DC) was set up without additional amino acids. Each diet was fed to triplicate groups of 15 juveniles of an initial weight of 9.8 ± 2 g (mean ± standard error) for a period of 55 days. At the end of the feeding period, growth performance was determined. The data obtained were evaluated with a one-way ANOVA and when significant differences were found, a Fisher’s LSD test was performed (P < 0.05). Survival at the end of the feeding trial was higher than 98% in all groups. Weight gain (%) was the highest in the fish fed with D3 (412 ± 32), but significant differences were observed only when compared with group fed diet D1 (270 ± 32). Rest of weight gain (%) values were: D2, 374 ± 34; D4, 408 ± 43; D5, 395 ± 46 and DC, 345 ± 64. Regarding the specific growth rate (%/day), value of fish fed D1 was significantly lower than the rest of groups (D2, 2.3 ± 0.3; D3, 2.8 ± 0.1; D4, 2.9 ± 0.1; D5, 2.8 ± 0.2; DC, 2.6 ± 0.2). Finally, the hepasomatic index ranged from 1.2 to 1.5 among the groups and not significant differences were observed. The data of the present research show that inclusion of 0.5% of Tau and 0.5% of Met to all plant protein diet improve the growth performance of rainbow trout juveniles. As has been reported for other species of fish (Takagi et al. 2010), the growth performance observed on the juveniles fed with more that 0.5% of Tau, suggests that rainbow trout might have a dietary requirement for this amino acid. The authors kindly acknowledge the funding from programs PAPIIT (project TB200112 and RR290112) of the National University of Mexico and SAGARPA-CONACYT (project 164673).

One of the main problems for complete substitution of live preys by microdiets is the water stability of formulated diets, which is greatly affected by the type of binder used. Most microdiet types have a high leaching rate of water-soluble nutrients, particularly amino acids, risking the ability to meet larval nutritional requirements. In turn, amino acid leaching enhances microdiet attraction and ingestion by the larvae, and therefore a good microdiet needs to have a good balance in leaching of attractants and digestibility of its ingredients. Accordingly, the aim of this work was to test the effect of different binders in Sparus aurata experimental microdiets to determine their effect on larval behaviour, development, growth, survival and enzyme activity. For that purpose, four isolipidic microdiets, Diet 0 GEL (control): 5% gelatin, Diet 1 ARA: 2% arabic gum, Diet 2 DEX: 2.5%, dextrin, Diet 3 SOD: 10% sodium alginate, were fed from 20 to 35 dph (days post hatching) to seabream larvae. At the end of experiment fish fed diets containing Arabic gum as a binder showed the highest survival rate (55%), particularly in comparison to fish fed dextrin (20%) or alginate (22%). As a result, total length of fish fed the diet containing dextrin was significantly higher. Survival after activity test was also significantly higher in fish fed diets with dextrin. Nevertheless, inclusion of Arabic gum produced the higher biomass. Results are discussed in relation to the water stability properties of the different diets.

Saleh, R., M. Betancor, J. Roo, C. M. Hernández-Cruz1, F. Moyano & Marisol Izquierdo. (2013)
POSTER 059
DIETARY ZINC REQUIREMENT OF HYBRID TILAPIA, OREOCHROMIS NILOTICUS × O. AUREUS, FED A SOYBEAN MEAL BASED DIET
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A feeding experiment was conducted to evaluate the dietary Zn requirement of hybrid tilapia fed a soybean meal based diet containing 15.5 g/kg endogenous phytic acid. Juvenile hybrid tilapia were fed diets containing 31 to 252 mg Zn/kg in triplicate groups for 6 weeks. Weight gain (WG), feed conversion ratio, and protein efficiency ratio of fish fed diets containing 127 or 166 mg Zn/kg were better (p<0.05) than those fed diets containing either 31 to 101 mg Zn/kg. WG, plasma Zn level, and superoxide dismutase (SOD) activity of fish increased when dietary Zn increased up to a dietary Zn level around 130 mg/kg. Beyond this level, the values of these parameters were relatively constant. Analyzed by using a broken-line regression model, the dietary Zn requirement of hybrid tilapia fed the experimental diet containing 15.5 g/kg endogenous phytic acid was 120 to 140 mg/kg using WG, plasma Zn level, body Zn retention, and SOD activities as the parameters.
The present study was conducted for 60 days to evaluate the efficacy of phytase supplementation on growth performance and mineral digestibility for Labeo rohita fingerlings. The test diet was consisted of 30% test ingredient (cottonseed meal) and 70% reference diet. Seven cottonseed meal based test diets were prepared by spraying graded levels (0, 250, 500, 750, 1000, 1250 and 1500 FTU kg^-1) of phytase to cottonseed meal-based test diet to assess the optimal dose required for higher fish growth and mineral digestibility coefficients. Chromic oxide was included as indigestible marker in the fish feed. The results of this study showed that phytase supplementation effectively enhanced the growth performance and mineral digestibility of Labeo rohita fingerlings at 750 FTU kg^-1 level followed by 1000 FTU kg^-1 level as compared to reference diet. Relationships between phytase doses and various growth and digestibility indicators were also established through quadratic regression equations. Our results suggest that phytase supplementation to cottonseed meal-based diet at 750 FTU kg^-1 level is sufficient to release adequate chelated minerals for optimal growth performance of L. rohita fingerlings.

Keywords: Phytase; Cottonseed meal; Labeo rohita; Growth performance; Mineral digestibility

ROLE OF PHYTASE SUPPLEMENTATION IN IMPROVING NUTRIENT DIGESTIBILITY FOR LABEO ROHITA FINGERLINGS FED ON PLANT INGREDIENT BASED DIETS

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The present research work (10 weeks feeding trial) was conducted to assess the optimal dose of phytase supplementation required for increasing nutrient digestibility coefficients of *Labeo rohita* fingerlings fed on canola meal-based test diets. A diet containing 30.2% protein and 4.26 Kcalg⁻¹ caloric values was used as reference diet. The experimental diet having approximately similar caloric and protein values was formulated by mixing 70% reference diet and 30% canola meal. This experimental diet was further divided into seven test diets and were sprayed by graded levels (0, 250, 500, 750, 1000, 1250 and 1500 FTU kg⁻¹) of phytase enzyme. Chromic oxide was used as inert marker in reference and test diets. Phytase supplementation at 750 FTU kg⁻¹ followed by 1000 FTU kg⁻¹ effectively enhanced apparent nutrient digestibility coefficients (crude protein 64%, crude fat 76% and gross energy 68%) as compared to reference diet and other phytase supplementation levels based diets. It was concluded that 750 FTU kg⁻¹ level of phytase supplementation to canola meal-based diets is sufficient for increasing nutrient digestibility of *Labeo rohita* fingerlings.

Key Words: *Labeo rohita*, phytase, nutrient digestibility, plant ingredient, canola meal

References


Cage culture of tropical spiny lobster (Panulirus homarus) is a developing industry in Lombok, Indonesia. Wild harvested seed lobsters (0.3 to 2g) are fed solely on mixed trash fish diet until reaching a market size of 250g. Preliminary feed studies with seed lobsters suggest the inclusion of trash fish and/or high moisture levels in formulated feeds is critical for growth and survival during the nursery stage. These findings are hypothesised to be attributed to the attractant and textural properties provided by the use of the fresh ingredients and high moisture which results in an increase in feed intake by lobsters. A series of experiments were conducted with sand lobster (Panulirus homarus) to firstly determine the essentiality of fresh ingredients and then to evaluate the effect that diet dry matter content has on growth and survival. The first study compared four isoproteic and isoenergetic diets which varied only in fresh ingredient inclusion, by incremental reduction from 36% to 0%. The second study compared four isoproteic isoenergetic diets each containing 36% fresh ingredients and which varied only in the level of dry matter, with incremental increases from 65% to 92%. The complete replacement of either fresh ingredients or moisture content resulted in no significant difference in survival performance. The replacement of fresh ingredients down to an inclusion of 13% resulted in a slight decline in growth performance. However, complete replacement resulted in a significant reduction in growth performance. An increase of diet dry matter up to level of 84% resulted in no difference in growth performance. However, a significant reduction in growth performance was observed when dry matter content was increased to 92%. This study supports that there are optimal inclusion levels of fresh ingredient and diet dry matter for feeds for seed lobsters.
Docosahexaenoic acid (DHA) and taurine are very important for early growth and development of marine fish larvae. It was reported that DHA and taurine enrichment improved early growth and swimming behavior of Japanese flounder. DHA and taurine synthesis is achieved by a series of enzymatic reaction. It was reported that fatty acid elongase (elovl) and desaturase (fads) are responsible for DHA synthesis in fish. Taurine is a final product of a sulfur amino acid metabolic cascade and several enzymes are involved in taurine synthesis. It was suggested that cysteine sulfenic acid decarboxylase (CSD) and cysteine deoxygenase (CDO) play a key role in taurine synthesis in fish. However, regulatory mechanism of DHA and taurine synthesis in marine fish are not well understood. Therefore, the present study aimed to determine effect of dietary DHA and taurine intake on gene expression of DHA and taurine synthesizing enzymes in Japanese flounder larvae.

Japanese flounder larvae at 10 days post hatching (dph) (total length 5.5 mm) were divided into four tanks. They were subjected for two treatments with replication. For control, fish was fed rotifers and Artemia nauplii without enrichment. In another group, fish were fed live food enriched with DHA in the morning and with taurine in the afternoon (DHA + taurine group). Fish were sampled daily for measurement of total length and for gene expression analyses of elovl, Δ6fads, CSD, and CDO by RT-qPCR. All fish were sampled at 36 dph for determination of survival, fatty acid and total amino acid composition.

Fish survival was significantly higher in fish of the DHA + taurine group than the control at 36 dph. Significantly higher DHA + EPA content was detected in fish of DHA + taurine group than the control at 36 dph. In the control, fish started to show DHA + taurine deficiency symptom such as slower swimming upon feeding after 20 dph. Significantly better growth started to be detected in the DHA + taurine group than the control after 28 dph onward. Expression of Δ6fads, elovl5, CSD and CDO were detected in flounder larvae as early as 10 dph. It seemed that expression of Δ6fads tended to increase with age of the fish. There was no significant difference in expression level of Δ6fads between the groups. Higher expression level of elovl5 was detected in fish in DHA + taurine group than the control. No significant difference was detected in expression level of CSD and CDO between the groups probably because similar taurine content in fish in these groups. These results suggest that DHA and taurine are important for early stage and elovl5 responds different dietary DHA intake of Japanese flounder.
Barramundi (Lates calcarifer) culture is prominent in Northern Australia, although the freshwater culture is marred by poor flesh flavour and aroma induced by cyanobacterial metabolites. The use of finishing diets for flesh quality enhancement in aquaculture is common, and through the manipulation of many flesh attributes, marketability of the products increases. The common marine algae, Ulva sp, is hardy and easily cultured, and is of interest as an aquafeed additive due to its high concentration of dimethylsulphoniopropionate (DMSP). DMSP, found at a concentration of 2000 ppm, breaks down to form flavour altering compounds including dimethylsulphide (DMS). A fish meal based reference diet was made, with the treatment diet having 30% Ulva ohnoi dry matter (DM) added by substitution. Digestibility of U. ohnoi and DMSP as fed to barramundi (632.8 ± 9.8g (mean ± SE)) over a 45 day trial period was determined. Feeding was to daily to satiation, with manual faecal stripping taking place 3 times a week under anaesthetisation. Initial feeding indicated a preference for the reference diet, however this was overcome during experimental acclimation, following which no preference was detected. Faecal analysis revealed that the 30% Ulva diet DM was 63.7% digestible, DMSP compound within this diet was 83.9% digestable, and DMSP was overall 36% digestible. The results indicate that U. ohnoi is a suitable dietary inclusion for barramundi, providing potential flesh flavour enhancement. This study documents for the first time the digestibility of a flavour contributing compound, providing a sound basis on which to further investigate the properties and benefits attributed.
To further investigate the lipid storage characterization in fish, a preadipocyte primary cell culture system of grass carp was established, and the cellular lipid accumulation and mitochondria development processes were studied. After reaching confluence around day 7 after seeding, the cells were induced by an adipogenic medium (as A0) and the differentiation characteristics were evaluated at A0, A1, A3, A5, A7 days.

Oil red O staining extraction assay and electron microscopy observation showed that the cytoplasm was gradually filled with lipid-rich droplets and the cellular lipid content increased (P<0.05). Meanwhile, mitochondria fluorescent staining was applied and it was found that the mitochondrial number increased markedly (P<0.05), while the mitochondrial protein content elevated (P<0.05) according to the protein concentration analysis. Gene transcript levels measured using quantitative real-time PCR showed that the expression of lipogenesis related genes such as peroxisome proliferator-activator receptors γ (PPAR γ ) and lipoprotein lipase (LPL) increased, that of PPAR α and CCAAT/enhancer-binding proteins α (C/EBP α ) presented a transient increasing after induced, while that of fatty acid synthetase (FAS) and stearoyl CoA desaturase1 (SCD1) increased at the late stage of cell differentiation. On the other hand, the expression of mitochondrial development relevant gene peroxisome proliferator activated receptor coactivator-1α (PGC-1 α) significantly increased, while that of PGC-1 β and nuclear respiratory factor-1 (NRF-1) showed a transient increasing, and the expression of mitochondrial functionally related gene carnitine palmityltransferase-1α (CPT-1 α) was decreased at the initial stage, but elevated at the last stage of the cell differentiation (P<0.05). In summary, the present results indicate that the differentiation characterization of grass carp preadipocytes, such as lipid accumulation and mitochondria development process, is similar to these in other land animals, but the molecular mechanisms are not exactly the same. Therefore, this study highlighted the variation of molecular events, as well as the mitochondria status during fish adipocyte differentiation and provided new information for the fish lipid nutrition study.

**Key words:** grass carp; adipocytes; differentiation; mitochondria
Redclaw (Cherax quadricarinatus) are a crayfish species whose characteristics make them ideal for aquaculture. Considered to be functional omnivores, freshwater crayfish consume a broad range of both plant and animal material. Despite being a native species aquaculture of redclaw in Australia is limited to a small number of farms in North Queensland where production has continued to decline over the past decade. Limited information on the nutritional requirements of redclaw, particularly in aquaculture pond systems has restricted the ability of Australian farmers to increase production and compete on a global scale. Currently the culture of redclaw is conducted in semi-intensive pond systems which attempt to utilise the varied natural diet of crayfish by stimulating and maintaining the natural pond productivity with the addition of fertilisers and supplemental feed. Commercial pellets or food scraps are often used as supplemental feed however this varies from farm-to-farm with the assumption that if a good crop is produced then that particular feeding strategy must be adequate. However, this ad-hoc approach coupled with a lack of an industry standard has resulted in varied production yields and overall uncertainty in relation to feeding practices. Developing a successful feeding regime and appropriate diet requires an understanding of the numerous food sources within the pond system and to what extent they contribute to crayfish nutrition. Stable isotope analysis is a useful way to examine what food sources present are being consumed as plants and animals are distinct in terms of their $\delta^{13}C$, $\delta^{12}C$ and $\delta^{15}N$, $\delta^{14}N$ ratios. By collecting samples of potential food items and comparing the C and N isotope ratios with those found in the crayfish a greater understanding of their diet in pond systems can be established. Four commercial redclaw farms located in the Atherton Tablelands, North Queensland, were sampled. Naturally occurring pond organisms including zooplankton, phytoplankton and macro-invertebrates were collected, pond sediment samples, along with supplemental feed such as lupin, corn and chicken pellets, forage material such as hay and pond macrophytes were sampled. C. quadricarinatus were also sampled from each pond. All collected samples including tail muscle and whole body samples of the crayfish were prepared for C and N stable isotope analysis to determine the food sources assimilated by the crayfish with results and implications discussed herein.
FUNCTIONAL CHARACTERIZATION OF Δ6 DESATURASE FROM NIBE CROAKER
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Most of marine fish require long-chain polyunsaturated fatty acids (LC-PUFA), especially eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) in their diets as they are deficient in the key fatty acid-metabolizing enzymes in the biosynthetic pathway of LC-PUFA. It is therefore necessary to supplement with fish oil to diets for cultured marine fish species. However, this system often faces obstacles related to insufficient and unstable supply of fish oil. In order to improve the LC-PUFA biosynthetic pathway of marine fish, we produced transgenic nibe croaker (Nibea mitsukurii) carrying a gene encoding elongation of very long chain fatty acids protein 2 (elovl2) isolated from masu salmon. As a result of fatty acid analysis of the liver of this strain, the elovl2 metabolites (22:5n-3, 24:5n-3 and 22:4n-6) increased compared to non-transgenic counterparts. However, the EPA and arachidonic acid (ARA; 20:4n-6) level decreased compared to non-transgenic and the DHA level was not altered between transgenic and non-transgenic. These results suggest that the nibe croaker still lack several enzymes in their LC-PUFA biosynthetic pathway. Therefore it is important to understand the function of the endogenous fatty acid-metabolizing enzymes of nibe croaker. However, nothing is known about its functions of desaturase-like gene in this species. Thus, we determine conversion abilities of the nibe croaker desaturase in this study.

The desaturase of nibe croaker was functionally characterized by expressing its coding sequence in yeast (Saccharomyces cerevisiae) which were grown in medium supplemented with one of the following substrates: 18:3n-3, 20:3n-3, 20:4n-3, 22:5n-3, 18:2n-6, 20:2n-6, 20:3n-6 and 22:4n-6. After 48 h culture, the yeast was harvested and then fatty acid methyl esters were prepared for fatty acid analysis. The fatty acid compositions of the resulting yeast were analyzed using gas chromatography. The desaturation conversion efficiencies from exogenously added substrates were calculated by the proportion of substrate fatty acid converted to its desaturated products as [product area / (product area + substrate area)] × 100.

The substrate specificities of the desaturase of nibe croaker indicated Δ6-desaturase activity towards 18:3n-3 and 18:2n-6 substrates. Conversion efficiencies are 24.2% and 16.3%, respectively. In addition, weaker activity towards Δ8-desaturase substrates (20:3n-3 and 20:2n-6) was also exhibited (4.2% and 1.6%, respectively). On the other hand, the desaturase of nibe croaker did not exhibit activity towards Δ5- and Δ4-desaturase substrates. These results suggest that the decrease of EPA and ARA in the transgenic is due to the lack of Δ5 desaturase activity of the endogenous desaturase. Furthermore, no alteration of DHA level in spite of the increase of 22:5n-3 in the transgenic is due to the lack of Δ4 desaturase activity. Since there is still the possibility that the desaturase of nibe croaker has another Δ6-desaturase activity towards C24 fatty acid substrates (24:5n-3), this analysis is ongoing in our laboratory.
POSTER 068

EFFECT OF LOWERING FISHMEAL WITH COMPOSITE MIXTURES OF SOYBEAN MEAL AND SEA FOOD PROCESSING BY–PRODUCTS ON GROWTH PERFORMANCES AND ELEMENT COMPOSITIONS IN RED SEA BREAM PAGRUS MAJOR

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Sea food processing by-products are the potential protein sources in aquafeed. However, lack of processing technology, high moisture content and heavy metal contaminations etc are the growing concern for the producer and consumer. Very little information is currently available in this regards, specially on the heavy metal contaminants in fish diets and its effect on fish. The present study was a continuation of our previous studies (Kader et al., 2010; 2011; 2012). The aim of this experiment was to utilize sea food processing by-products and soybean meal in replacement of fishmeal (FM) for red sea bream (Pagrus major) and evaluate its effects on growth performances and heavy metal composition of fish.

In the present experiment, skipjack by-product (fish soluble; FS), squid by-product (SQ) and scallop by-product (SC) were used as protein sources. Soybean meal was mixed with SQ and SC in the ratio of 7:3 and 6:4 respectively; and then fermented to facilitate low-cost drying.

Four isocaloric diets were formulated where D1 was the basal diet (0% FM replacement); and 50% FM protein was replaced with composite mixtures of by-products (FS:SQ:SC) in D2 (1:2:2), D3 (1:1:1) and D4 (2:1:1). Triplicate groups (15 fish per group and 45 fish per treatment) of juvenile red sea bream (initial body weight of 2.8±0.01g) were stocked in 100l polycarbonated circular tank. Animals were fed twice a day to apparent satiation for 45 days.

Results showed that there was no significant (P>0.05) difference in survival among treatments. Weight gain (%) and specific growth rate (SGR %/day) of fish were not significantly different between D1 and D4. However, growth performances were significantly (P<0.05) depressed in D2 and D3. Dry matter feed intake (g dry diet/fish/45days) was significantly increased with the increasing levels of FS in diets. Concentration of copper (Cu), zinc (Zn), cadmium (Cd) and lead (Pb) were determined in diets, fish whole body, muscle and liver in different treatments. It was found that dietary Cu, Zn and Cd were significantly increased in all the replacement groups. The analysis of whole body, muscle and liver showed that heavy metal accumulation occurred mainly in liver and may be the other parts of the body rather than muscle. The values were not significant in muscle.

Therefore, it is concluded that skipjack, squid and scallop by-products are the effective protein sources. Based on the present experimental conditions, fishmeal can be easily lowed to 50% with a mixture of FS, SQ and SC (2:1:1) in the diets of red sea bream.

Key words: fishmeal, fish soluble, squid by-products, scallop by-products, growth, heavy metal, red sea bream.

References:
Kader et al., 2010. Aquaculture 308, 136-144.
Kader et al., 2012. Aquaculture Research 43, 1427–1438.
Wheat gluten as a highly digestible vegetable protein has a great potential to be used as an ingredient in rainbow trout feed. This study was to examine the effects of replacement of fishmeal by wheat gluten on energy and protein efficiency in rainbow trout. A total of eighteen groups of fish were assigned in a 2 × 3 factorial design: two diets ('fishmeal' v. 'wheat gluten') and three feeding levels (maintenance, medium and satiation levels). The results of this study indicated that replacement of fish meal with wheat gluten improved growth parameters of rainbow trout (P<0.05). Growth parameters were also affected by feeding levels (P<0.05). Rainbow trout fed higher feeding levels showed better growth than the fish fed lower feeding level. An interaction effect between diet type and feeding level indicated that an increase in feeding level at both diets (fishmeal and wheat gluten) did not improve equally rainbow trout growth (P<0.05). Measurement of metabolism parameters showed that energy requirement of rainbow trout fed fishmeal diet for maintenance was larger than those fed wheat gluten diet (23 versus 32 kJ/day). Similar to energy, maintenance protein requirement of rainbow trout fed fishmeal diet was higher compared to wheat gluten diet (0.63 versus 0.76 g/day). A comparison of regression slope between two protein sources indicated a larger efficiency of wheat gluten for protein retention than that of fishmeal. In conclusion, energy and protein utilisation for growth and maintenance are influenced by protein sources.
POSTER 070
THE EFFECTS OF DIETARY SUPPLEMENT OF ALPHAMUNE ON GROWTH PERFORMANCE, NUTRIENT DIGESTIBILITY AND ENZYME ACTIVITY OF JUVENILE RAINBOW TROUT, ONCORHYNCHUS MYKISS (WALBAUM 1792)
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This research was conducted to evaluate the effect of different levels of alphamune prebiotic (a mixture of mannan oligosaccharides and β-glucan) on growth performance, efficiency of nutrient digestibility and digestive enzyme activity (trypsin, α-amylase and lipase) of juvenile rainbow trout. A basal diet was formulated using common feed ingredients supplemented with alphamun at 0, 0.5, 1, 2, and 4 g kg⁻¹, leading to five experimental diets. Each experimental diet was randomly assigned to triplicate 1,500 L tanks. Juvenile rainbow trout were randomly distributed in the experimental tanks and the experiment lasted for eight weeks. Results showed that inclusion of dietary alphamune significantly affected growth related parameters (p<0.01) of rainbow trout compared to the control. Maximum growth and feed efficiency were observed at diets containing 0.5 g/kg alphamune (P<0.05). Other growth related parameters such as final weight and specific growth rate were higher at 0.5 g/kg alphamune (P<0.05). However, increasing the alphamune supplementation level did not change the growth factors (P>0.05). Rainbow trout survival rate was not affected by prebiotic during eight weeks experiment. Digestibility measurement showed that inclusion of alphamune did not influence nutrients digestibility of rainbow trout significantly (P>0.05). The assessment of the activity of digestive enzymes showed that prebiotic supplementation is associated with an increase in trypsin enzyme activity and this activity increment was significant at 4 g/kg alphamune inclusion (P<0.05). However, the activity of α-amylase and lipase was not affected by the prebiotic inclusion. In conclusion, it seems that the positive impact of alphamune prebiotic on growth related parameters is not as a result of increasing nutrient digestibility and digestive enzyme activity in rainbow trout.
In vitro and in vivo protein digestibility was conducted to investigate the fishmeal, suckermouth catfish protein hydrolysate and substitution fishmeal with suckermouth catfish protein hydrolysate at 0, 25, 50, 75 and 100% diet which were demonstrated in 18.31 g juvenile Nile tilapia. The results showed that the in vitro protein digestibility by intestinal enzymes (protease) of suckermouth catfish protein hydrolysate were higher than fishmeal and highest in the replacement fishmeal at 25% with suckermouth catfish protein hydrolysate (p<0.05) diet. On the other hand, the in vivo protein digestibility was highest in fish fed with replacement fish meal at 50% with suckermouth catfish protein hydrolysate diet. The results demonstrate that suckermouth catfish protein hydrolysate can replace up to 25 – 50% fishmeal protein in diets for Nile tilapia.

A hundred male Nile tilapia (Oreochromis niloticus, L.) were used in this experiment. Initial weight of fish were 18.31 g which were acclimated for one week by feeding with 30% protein feed. Intestine was collected and weighed at 16 hours after feeding. Samples were stored at -80 C until use. The crude enzyme extract was prepared by the method of Gimenez et al. (1999). The enzyme activity of protease was measured in triplicate by the methods of Bezerra et al. (2005) with slight modification. All experimental feeds were formulated to contain approximately 30% crude protein and 3000 Kcal/kg digestibility energy. Diets 100/0 – 0/100 were formulated to produce diets in which 0% (Diet100/0), 25(75/25), 50(50/50), 75(72/25) and 100 % (0/100) of protein from fish meal was replaced by that from suckermouth catfish protein hydrolysate. Two raw materials were fish meal and suckermouth catfish protein hydrolysate and feed trial were measured in triplicate by the cleavage peptides method of Rungruangsak (2002) with slight modifications. The in vivo protein apparent digestibility using feeds with 0.1% of chromic oxide. The chromic oxide levels in diets and feces were analyzed using a modified colorimetric method (Fenucci, 1981). A one-way analysis of variance and Duncan’s multiple range test were used to determine statistical differences of data among dietary treatments.

In conclusion, the suckermouth catfish protein hydrolysate can replace up to 25 – 50% fishmeal protein in diets for Nile tilapia.
In order to satisfy the growth in global salmon farming the inclusion of fishmeal and fish oil in salmon feeds will continue to decrease since, especially for fish oil the availability is limited. Accordingly, in the last 30 years commercial feeds in Norway for salmon have changed dramatically in their composition with an increase in the use of vegetable proteins and oils. The aim of the following fish trial was to evaluate the performance and quality of Atlantic salmon fed commercially relevant feeds compared to two alternative feeds for the future - containing different combinations of plant proteins and reduced levels of fish oil.

Atlantic salmon (mean initial weight 850g) were grown in seawater pens (15 x 15m) at the EWOS Innovation trial site, Western Norway, from April to December 2013. Trial fish feeds were manufactured at the commercial factory (EWOS Florø) and were formulated using raw materials in commercial use today (crude protein 35%, crude fat 35%, digestible energy 21.5 MJ/kg). Each feed had 4 pen replicates (14000 fish/pen). The feed treatments were as follows (% of feed formulation given):

1. Control: fish meal 10%, fish oil 15%.
2. MxdPlants: fish meal 3.4%, fish oil 10%, mixed plant protein sources and concentrates, rapeseed oil.
3. HiSPC: fish meal 3.4%, fish oil 10%, soy protein concentrate (SPC), rapeseed oil.

Fish performance (weight gain, feed conversion rate (FCR), mortality and other parameters (flesh quality: pigment, fatty acid profiles) were monitored. By the end of the study the average whole body weight of fish was 4.3kg, feed conversion ratio was 1.1 and mortality was 3.4-3.7% across the different treatments. Final fish weights (corrected to the same sample date) for the control group was 4.3 kg, for the MxdPlants 4.4kg and for the HiSPC 4.3 kg. The fatty acid profiles of fish flesh varied with the type of feed given and are discussed in relation to the final eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) delivery in the fillet portion. Data evaluation for other parameters (pigment, dress-out percentage, liver index) is in progress.

In summary, the fish trial described demonstrates that Atlantic salmon perform just as well on a low fish meal and fish oil feed when high quality plant protein concentrates and lipids are used as replacements. This data supports a potential future feed for salmon that has larger flexibility in the choice of raw materials and reduced dependence on marine sources.
This study was conducted to improve the quality of copra cake meal through fermentation and to evaluate the effect of the fermented product (fermented copra cake meal – FCCM) on growth and feed utilization of rabbitfish, Siganus javus, reared in floating net cages in Awerange Bay, South Sulawesi, Indonesia. Copra cake meal was fermented with Rhizopus sp. at rate of 0.1 % (w/w) before using it as a dietary test ingredient. Four iso-nitrogenous diets (34% crude protein) were formulated to contain different levels of FCCM at 0, 15, 30 and 45%. Rabbitfish with average initial body weight of 88 g were stocked into 12 cages (1x1x1.5 m³) at a density of 20 fish per cage and fed with the test diets at 2.5-3% of biomass twice a day for 12 weeks. After fermentation, the crude protein content of copra cake increased significantly from 21.8%±1.6 (mean±SD) to 29.3%±0.4, while lipid and fibre decreased from around 13.0%±0.4 to 6.0%±1.1 and from around 20%±1.6 to 16.0%±0.8, respectively. Total amino acid content of copra cake meal increased after fermentation from 17.6 to 20.3%. Survival rate of rabbitfish was high in all treatments (98-100%). SGRs of fish fed FCCM at level of 0–30% were not significantly different, but increase of FCCM level in diet at 45% significantly lower the SGR. There was no clear trend of the dietary FCCM inclusion levels on feed intake, but FCR was not significantly different among rabbitfish fed diets containing FCCM at levels of 0-30% inclusion. However, rabbitfish fed the test diet with 45% FCCM demonstrated significantly higher FCR. Whole body protein content of the rabbitfish tended to decrease with increasing FCCM in the diet at 30 and 45% inclusion. Based on growth and FCR, FCCM could be included in diet for rabbitfish up to 30%, but gives negative effects at the highest inclusion level of 45%.

Keywords: copra cake, fermentation, practical diet, rabbitfish
Considerable progress has been made towards the development of a manufactured feed for Spiny lobster, *Panulirus ornatus*. However, there is little data on the nutritional value of common feed ingredients suitable for use in spiny lobster pelleted feeds. The evaluation of the digestible protein and energy of feed ingredients is critical to the cost-effective formulation of such modern pelleted aquaculture diets. Therefore in this study we aimed to assess the digestible protein and energy value of a range of feed ingredients widely available in the central region of Vietnam for use in feed formulations for spiny lobster.

A reference diet of 548 g/kg DM protein, 119 g/kg DM fat (21 MJ/kg DM gross energy) was formulated and also used as the basis of the other test diets in the experiment. Each test ingredient was combined with a reference diet component in a 30:70 ratio. A total of ten test ingredients were studied, these included; Indian soybean meal, Peruvian fishmeal, KienGiang fishmeal, wheat gluten, shrimp (Acetes) meal, meatbone meal, poultry by-product meal, DaNang fishmeal, squid meal and krill meal. Feeds were fed for seven days to tanks of 10 lobsters per tank before faecal collection commenced. Faeces were collected by the cloacal balloon method. Faeces were collected over a six-week period and pooled within tanks and faecal samples were kept frozen prior to analysis. For analysis the faeces were dried and both faecal and feed samples ground and analysed for chromium, nitrogen, lipid, ash and dry matter. Digestibilities of each of the diets were calculated and the digestibility coefficients of each diet and the composition of the diets and ingredients used to calculate the digestibly of each test ingredient. The results of this study show that spiny lobster can digest protein and energy from a wide range of feed ingredients. These data could be used to better define nutrient and energy requirements and to formulate nutritionally-efficient, cost-effective feeds for this species.

### Table 1. Composition and digestibility of key feed ingredients for marine fish

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>DM (g/kg)</th>
<th>Protein</th>
<th>Lipid</th>
<th>Ash</th>
<th>CHO</th>
<th>Energy (MJ/kg)</th>
<th>Protein ADC</th>
<th>Energy ADC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peruvian fishmeal</td>
<td>900</td>
<td>657</td>
<td>100</td>
<td>201</td>
<td>43</td>
<td>20</td>
<td>85.4^a</td>
<td>81.5^1</td>
</tr>
<tr>
<td>KienGiang fishmeal</td>
<td>888</td>
<td>612</td>
<td>75</td>
<td>224</td>
<td>88</td>
<td>19</td>
<td>83.5^a</td>
<td>80.1^a</td>
</tr>
<tr>
<td>DaNang fishmeal</td>
<td>896</td>
<td>625</td>
<td>80</td>
<td>221</td>
<td>74</td>
<td>19</td>
<td>84.3^f</td>
<td>80.6^e</td>
</tr>
<tr>
<td>Shrimp (Acetes) meal</td>
<td>872</td>
<td>632</td>
<td>70</td>
<td>153</td>
<td>145</td>
<td>20</td>
<td>84.0^f</td>
<td>79.8^a</td>
</tr>
<tr>
<td>Antarctic Krill meal</td>
<td>929</td>
<td>580</td>
<td>140</td>
<td>130</td>
<td>150</td>
<td>22</td>
<td>88.4^h</td>
<td>75.0^cd</td>
</tr>
<tr>
<td>Vietnamese Squid meal</td>
<td>927</td>
<td>600</td>
<td>119</td>
<td>85</td>
<td>196</td>
<td>22</td>
<td>75.5^b</td>
<td>68.8^a</td>
</tr>
<tr>
<td>Meatbone meal</td>
<td>948</td>
<td>507</td>
<td>25</td>
<td>322</td>
<td>146</td>
<td>15</td>
<td>80.0^d</td>
<td>75.4^d</td>
</tr>
<tr>
<td>Poultry by-product meal</td>
<td>900</td>
<td>640</td>
<td>66</td>
<td>80</td>
<td>214</td>
<td>21</td>
<td>79.0^c</td>
<td>73.7^b</td>
</tr>
<tr>
<td>Indian soybean meal</td>
<td>900</td>
<td>434</td>
<td>40</td>
<td>62</td>
<td>464</td>
<td>20</td>
<td>80.0^d</td>
<td>74.0^b</td>
</tr>
<tr>
<td>Wheat gluten</td>
<td>943</td>
<td>750</td>
<td>80</td>
<td>8</td>
<td>162</td>
<td>23</td>
<td>74.7^a</td>
<td>74.4^bc</td>
</tr>
</tbody>
</table>

DM: Dry matter, ADC: Apparent Digestibility Coefficient, CHO: Carbohydrate
A 9-week feeding trial was conducted for the determination of the dietary arginine requirement of red seabream (Pagrus major). Six isonitrogenous (50% crude protein) and isocaloric (4.27 kcal g$^{-1}$ gross energy) experimental diets were formulated to contain 1.2, 1.6, 2.0, 2.4, 2.8 and 3.2% arginine. Triplicate groups of fish (13.3 ± 0.2 g) were fed one of the test diets to apparent satiation twice daily. At the end of the feeding trial, significantly ($P < 0.05$) higher growth was found in fish fed ≥2.0% arginine. Fish survival varied from 86 to 95% without significant differences among treatments. Plasma total protein level was significantly increased in fish fed 2.4-2.8% arginine compared to those fed the basal level of arginine. Plasma alanine aminotransferase activity and glucose level were significantly decreased in fish fed 2.0-2.4% and ≥1.6% arginine compared to those of fish fed the basal diet, respectively. Significant improvements in lysozyme, respiratory burst and myeloperoxidase activities and total immunoglobulin level were achieved by dietary arginine increment. Significantly lower plasma ammonia concentrations were detected in fish fed ≥2.0% arginine compared to fish fed the basal diet. A second-order polynomial regression analysis based on weight gain showed that the optimum dietary arginine level is 2.43% in diet. The findings in this study showed that arginine is an essential amino acid for optimal growth, immune function and health status of juvenile red seabream.
The primary purpose of application of exogenous dietary enzymes is to improve digestion (increasing efficiency and reducing cost) and potential barriers that may wrap around nutrient reserves, being applied as dietary additive and/or as pretreatment of feed ingredients. Literature references on experimental application of dietary exogenous enzymes in aquafeeds (n = 179) register the predominance of phytase (102), NSPases (α–galactosidase 3, β–glucanase 1, cellulase 5 and xylanase 10), protease (19), amylase (9), lipase (2) and combined enzyme applications (28). The specific origin of assessed enzymes is omitted in 69% of studies. Enzymes produced from *Aspergillus* sp. (11% of total references), *E. coli* (4%) and *Bacillus* sp. (2%) comprised the majority of specifically identified enzymes, especially for phytase and amylase. Most studied species were salmonids with 27% of references (the majority for rainbow trout), followed by tilapia (*Oreochromis* sp., 12%), shrimps (8%) and carps (7%). Accordingly, salmonids have been the main assessed group within phytase (35%), NSPases (42%) and proteases (37%), whilst tilapia, carps and shrimps showed particularly relevant for NSPases (37%), amylase (30%) and proteases (32%), respectively.

Combined enzyme applications referred mostly to NSPases complexes with or without protease, amylase and phytase (61%). The majority of enzyme research has been conducted with fingerlings and juveniles (94%) rather than larval stages. Dietary enzyme supplementation for fish meal replacement with plant sources predominated among nutritional objectives (25%), followed by supplementation in varied formulations (19%) and ingredient enzyme pretreatment (9%).

Enzyme inclusion to reduce inorganic P supplementation was also an important objective in phytase studies (9%). Dietary inclusion levels of exogenous enzymes varied widely, averages for either diet supplementation or ingredient pretreatment were 3.0, 0.10, 0.07, 0.03, 2.0, 1.0 and 1.0 g/kg for α–galactosidase, amylase, β–glucanase, cellulase, protease, xylanase and combined enzymes, and 1569 U/kg for phytase. Main nutritional benefits registered for phytase were digestibility (P, other minerals, protein and energy: 45% of publications), nutrient retention (37%), weight gain (32%) and feed efficiency (22%). Combined enzymes and NSPases mainly showed improved weight gain (33%) and digestibility (30%), as proteases that registered weight gain (32%), feed efficiency and digestibility (22%) benefits. Enzyme technology may be useful in bridging the nutritional gap between feed grade ingredients and evolution-driven specificities of food digestion in aquatic species. Wider use of dietary exogenous enzyme should develop according to commercial feed manufacturing conditions and also considering specificities of digestive system of each farmed animal group.

A 56-day feeding trial was conducted to evaluate the effect of graded levels of cottonseed meal (CM) with or without supplementation of a commercially available dietary protease (Jefo Nutrition Inc., Canada) on growth performance of black sea bream (*Sparus macrocephalus*). The control diet contained 36% fishmeal, and 0% (control), 5%, 10%, 15% of cottonseed meal replacing the same protein levels from fishmeal. Another two diets were formulated by supplementing with the protease at 175 ppm in 10% and 15% CM diets (10% CM+P and 15% CM+P, respectively). The six diets, 0%CM, 5%CM, 10%CM, 15%CM, 10%CM+P and 15%CM+P, were fed to black sea bream (initial body weight – 7.0 g) for 8 weeks. During this time, the fish were fed daily at 6%–8% of their body weight. The feeding rate and feed amount were adjusted after weekly sampling.

The weight gains (WG) and feed conversion ratio (FCR) were 300%, 283%, 242%, 201%, 285%, 227% and 1.22, 1.30, 1.52, 1.84, 1.62 of fish fed 0%CM, 5%CM, 10%CM, 15%CM, 10%CM+P and 15%CM+P diets, respectively. The growth performance was linearly decreased with increasing dietary inclusion of CM. While the 5% CM in the diet did not affect the growth (*P* > 0.05) compared with those fed the control diet, 10% and 15% CM in feed significantly affected the growth and FCR (*P* < 0.05) of black sea bream. In all the performance indices, the trend was reversed in fish fed protease-supplemented diets.

The inclusion of protease in 10% and 15% CM diets improved growth rate by 17.6% (*P* < 0.05) and 13.4% (*P* > 0.05), respectively and significantly decreased the FCR (*P* < 0.05), when compared with the fish fed the respective CM level diets without protease.

There was no difference in carcass dry matter, moisture, lipid and ash contents among the dietary treatments except for those fed the 15% CM diets, which was significantly lower than those of other dietary treatments. Free gossypol content in the liver of black sea bream also showed a linear increase with the increasing dietary CM that tended to significantly reduce with the addition of protease.

The activities of protease in stomach and intestine, and the villous height of foregut were significantly decreased by the inclusion of 10% and 15% CM, when compared to the control group (*P* < 0.05). The inclusion of protease in 10%, 15% cottonseed meal diet reversed the trend when compared with 10%, 15% CM group. These results indicated that cottonseed meal could be used as a fishmeal substitute in black sea bream diets up to 5%, or 10% with the addition of the protease. Higher dietary inclusion would impair growth, structure and function of the digestive tract of the fishes.
EFFECTS OF TEMPERATURE, PH AND FEED PROCESSING ON ACTIVITY OF A PROTEASE AND ITS DIETARY INCLUSION ON GROWTH OF WHITE SHRIMP, LITOPENAEUS VANNAMEI

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³Jefo Nutrition Inc., Saint-Hyacinthe, Canada

Effects of temperature, pH and feed processing on stability of a commercially available protease were assessed in an in-vitro trial followed by a growth trial with Pacific white shrimp, Litopenaeus vannamei. Protease activity at different temperatures, pH and pelleting conditions was assessed from the total free amino acid contents in the supernatant after 2 hours, 30 min and 30 min of hydrolysis, respectively, at 40°C using the ninhydrin method. In the second trial, shrimp with an initial body weight of 3.3 g were fed three experimental diets: (1) high fish meal (FM) diet - HFD with 25% FM and 40.0% crude protein (CP); (2) low FM diet – LFD with 22.5% FM and 38.0% CP; and (3) LFD with 175 mg/kg of Jefo Protease (LFD+P) for 6 weeks to assess effects of the protease on growth performance.

The results from the in-vitro trial suggested stability of the protease to wide range of pH and temperature. The ability to withstand high temperature during feed manufacturing processes is manifested in the higher free amino acid contents in feed with protease than those without.

Weight gain (WG) and feed conversion (FCR) were significantly better (+11% and -0.13, respectively; P<0.05) in shrimp fed diet LFD+P compared to the LFD group. However, there was no difference in performance of the shrimp fed the HFD diets (P>0.05). In addition, protease activity in hepatopancreas and proximal intestine was also higher in shrimp fed protease-supplemented diets than those fed the other diets (P<0.05).

It was concluded that the protease maintained good stability during high temperature and acidic pH conditions. Further, use of protease in shrimp feed allowed a reduction of FM and CP by 10% and 5%, respectively compared to those fed the control diets without affecting the performance of the animals.
POSTER 079
EXOGENOUS PROTEASE IMPROVES GROWTH AND DIGESTIVE PROTEASE ACTIVITY OF COMMON CARP (CYPRINUS CARPIO L)
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Two trials were conducted to investigate the effects of a protease obtained from Jefo Nutrition Inc., Canada on growth and digestive protease activity of common carp fingerlings. In the first trial, six iso-nitrogenous diets of three fishmeal (FM) levels (10%, 15% and 20%) and two levels of protease (0 and 175 ppm) were fed to common carp fingerlings (avg. initial body wt., IBW - 11.7 g) for 60 days. In the second trial, a basal low fishmeal (6% FM, Control) diet was supplemented with 175 ppm of the protease (FM+P). The two diets were fed to common carp juveniles (IBW - 48.7 g) for 30 days.

Fish fed a 10% FM diet had significantly lower weight gain (WG) than those fed the 20% FM+P diets. Growth was improved significantly in fish fed the protease-supplemented 10% FM diet. However, no significant improvements were observed in fish fed protease-supplemented 15% or 20% FM diets. In the second trial, the WG was improved by 6.4% ($P<0.05$) and FCR was decreased by 5.4% ($P<0.05$) when the protease was added to the diet.

There were no differences in carcass composition among the dietary groups in both trials. The digestive protease activities of foregut and chyme in 10% FM+P group (trial 1) and 6% FM+P group (trial 2) were significantly increased ($P<0.05$), compared to those fed the control.

It can be concluded that protease supplementation in common carp feed can improve growth performance and digestive protease activities of the fishes and juveniles and may help significantly reducing the overall feed cost without sacrificing the performance.
Mud crabs *S. paramamosain* are found in tropical, subtropical and warm temperate areas where they inhabit brackish and saltwater estuaries or mangrove forest. Because of their large size, high meat yield and delicate flavor, mud crabs are sea-food delicacy and they are in great demand in many markets. However, conventional diets of *S. paramamosain* are some subtropical marine shellfish, such as winkles and *Potamocorbula rubromuscula* in southern China. Therefore, development of a nutritionally optimized diet is considered critically important. With the aim to provide information assisting formulation of more appropriate diets for *S. paramamosain* culture, we conduct a trial to get information on the effects of utilization and metabolism of lipid or fatty acids in pelleted diets for *S. paramamosain*. Five isonitrogenous and isolipidic diets containing 8% level of fish oil (FO), lard (LD), safflower oil (SO), perilla seed oil (PO) and mixture oil (MO, V<sub>FO</sub>: V<sub>SO</sub>: V<sub>PO</sub> = 1:1:1), respectively, and a live food, *Potamocorbula rubromuscula* as the control diet (CF) were fed to groups of 25 juvenile crabs (average initial weight of 7.4 g) in triplicate 400-L tanks twice a day for 6 weeks. The results showed that crabs fed FO and MO had highest survival rate (*P* < 0.05). The tissue fatty acid composition was consistent with those in the diets. The whole body composition and hematology parameters were significantly affected by the lipid sources (*P* < 0.05). The FO and MO diets had the same depression effect like the CF in the fatty acid synthase activity and mRNA expression in hepatopancreas. The results of this study indicated that fish oil and mixed oil could be successfully incorporated in the pelleted diets with better effects for juvenile *S. paramamosain* compared with the live food, and the ratio of n-6/n-3 fatty acids in pelleted diets must be controlled within less than 1.

**Keywords:** *Scylla paramamosain*, dietary lipid, fatty acid, hematology, fatty acid synthase
The intensive aquaculture generates a fodder resource shortage and a potentially stressful environment to the cultured fish. In response, it is crucial to look for new feed ingredients which could produce the physiological merits for the fish. Marine seaweeds are rich in nutrients, some of them produce or contain secondary metabolites, polysaccharides, and glycoproteins with anti-tumor, antiviral, or immuno-stimulatory activity. *Gracilaria lemaneiformis* (GL) have been demonstrated possessing various bioactive functions such as antimutagenic, antitumor, antiviral, antioxidant, anticoagulant and immunomodulation effects, but few studies have paid attention to GL use in feed. In this study, diets containing 3, 5, 10, and 15% *Gracilaria lemaneiformis* (GL) (D1-D4 diet) were prepared to investigate the effects of GL on serum profile, immune and antioxidant status in juvenile yellowfin seabream *Acanthopagrus latus*.

The lysozyme activity in the kidney and the catalase (CAT) activity in the liver of D2 group were greatly higher than that of control group. Total antioxidant capacity (T-AOC) activity in the anterior intestine of D3 group was significantly higher than that of control group. Serum total protein (TP) of D4 group was significantly lower than that of control group ($P<0.05$). The acid phosphatase (ACP) activities in the liver of D3 group and in the anterior intestines of D2 group were significantly higher than that of control group ($P<0.05$). Serum total cholesterol (TC) of D2 group was greatly higher than that of control group. Serum low density lipoprotein cholesterol (LDL-C) of D3 and D4 group was significantly lower than that of control group ($P<0.05$). The results indicated that the best immune and antioxidant status was happened in the fish fed with 5 % GL diet and the lipid metabolism of the fish was affected when the GL inclusion level above 5 %.

Keywords: *Gracilaria lemaneiformis*  *Acanthopagrus latus*  Antioxidant enzymes activities  Immune enzymes activities  Serum profile
IDENTIFICATION OF BACILLUS STRAINS FROM THE INTESTINE OF MUD CRAB (SCYLLA PARAMAMOSAIN) WITH INHIBITORY ACTIVITY AGAINST VIBRIOSIS

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In our previous study, the bacterial communities of the intestine in three populations of crabs (wild crabs, pond-raised healthy crabs and diseased crabs) were scanned by culture-independent methods. In this study, we showed the intestinal communities of these populations of crabs by bacteria cultivation with a variety of mediums and identified the potential probiotics from the intestinal communities. A total of 135 bacterial strains were isolated from three populations of mud crabs (42 strains from milky diseased crab, 60 from healthy crabs and 33 from wild crabs, respectively). These strains were screened for enzyme activity test and antagonistic activity against \(V.\)parahaemolyticus using an agar spot assay. Antagonistic strains were then identified by 16S rRNA gene sequence analysis. The results revealed that the most strongly antagonistic strains belonged to the genera Bacillus.

Further probiotic characteristics of 3 strains (\(Bacillus\) subtilis DCU, \(Bacillus\) flexus D\(_2\)B, \(Bacillus\) cereus HL7) with the most antagonistic activity were also studied. The results showed that 2 (BP and D\(_2\)B) of them were able to survive low pH and high bile concentrations, showed good adherence characteristics and a broad spectrum of antibiotic resistance, which would be potential probiotics. The data showed that the \(Bacillus\) D\(_2\)B and \(Bacillus\) BP are most likely to become probiotics. Based on these results, these strains should be further studied to explore their probiotic effects in mud crab aquaculture. The study demonstrated the capacity of \(Bacillus\) subtilis BP and \(Bacillus\) flexus D\(_2\)B might contribute towards better choice of probiotic strains for mud crab.

The probiotic effect of \(Bacillus\) flexus D\(_2\)B was further tested by feeding juvenile mud crab (\(S.\)paramamosain) food supplemented with D\(_2\)B (\(10^5\) CFU/g) for 28 days before an immersion challenge with \(V.\) parahaemolyticus and \(V.\) alginolyticus at \(10^5\) CFU/mL for 24 h. The mud crabs treated with D\(_2\)B has a higher expression of immune factors such as SOD (superoxide dismutase), CAT (catalase), PO (phenoloxidase), GPx (glutathione peroxidase) than those in controlled mud crabs. The treatment with D\(_2\)B decreased final mortality to 20.25%, compared with 50.75% in the control group. \(Bacillus\) flexus D\(_2\)B has potential applications for controlling pathogenic Vibriosis in mud crab aquaculture.

This study showed the clear evidences that the indigenous mud-associated microbiota might be of probiotics and provided a defensive barrier against Vibriosis.

**Keywords:** Mud crab; Bacillus; probiotics; Vibriosis
The increasing demand for fish meal from the expanding global aquaculture industry and other terrestrial seafood and meat producers, coupled with unstable supply, has inflated the price of the fish meal, making it crucial to look for alternative sustainable aquacultural feed ingredients that are locally available with equivalent nutritional value. The way of solving fodder resource problem is developing new fodder resource promptly and making full use of the existing resource mainly for increasing income and decreasing expenditure. The protein and polysaccharide-rich macro-algae are potential resources and could be use by fish. In this study, diets containing 5, 10, 15, and 20% *Gracilaria lemaneiformis* (GL) (D1–D4 diet), *Ulva lactuca* (UL) (D5–D8 diet) and *Sargassum horneri* (SH) (D9–D12 diet) were prepared to investigate the effects of macro-algae on growth performance, carcass composition, activities of digestive enzymes, transaminases activities of star snapper (*Lutjanus stellatus*) juvenile. In containing GL diets, Weight Gain (WG) and Feed Efficiency Ratio (FER) of the fish fed D3 diet were significantly higher than those of other groups. In containing UL diets, Weight Gain (WG) and Feed Efficiency Ratio (FER) of the fish fed D5 diet were significantly higher than those of other groups. In containing SH diets, Weight Gain (WG) and Feed Efficiency Ratio (FER) of the fish fed D9 diet were highest among those of other groups. Fish fed with the D4, D8, D12 diet showed significantly lower crude lipid of both body and liver and Hepato-Somatic Index (HSI) than those fed with the control diet. Activities of pepsin in the stomach, lipase and amylase in the anterior intestine were significantly suppressed in fish which were fed with the D4, D8, D12 ($P<0.05$). On the other hand, the activities of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were significantly higher in fish feeded with the D4, D8 and D12 diet ($P<0.05$) than those of the control groups. These results indicate that incorporation of the GL in the diet at up to 15%, UL and SH in the diet at up to 5% level for juvenile star snapper is feasible in terms of the growth performance and the physiological state.

**Keywords:** *Gracilaria lemaneiformis*  *Ulva lactuca*  *Sargassum horneri*  *Lutjanus stellatus*  Antioxidant enzymes activities  Immune enzymes activities
Two growth trials were conducted to investigate the growth and lipid metabolism of the Pacific white shrimp, Litopenaeus vannamei (1.98±0.28g), at low salinity.

In trial one, shrimp was grown at salinity of 3‰, 17‰ and 30‰, respectively, for 8 weeks. Shrimp weight gain and survival at 3‰ were significantly lower than at 17‰ and 30‰. The linolenic acid (LNA, C18:3n-3) and n-3 long-chain unsaturated fatty acids (n-3 LC-PUFA) in hepatopancreas and n-3 LC-PUFA, especially eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3), in muscle at salinity of 3‰ were significantly higher than other groups. The activities of highly unsaturated fatty acid (HUFA) enzymes, elongation of long-chain fatty acids family member 6 (ELOVL6), Δ5 and Δ6 fatty acid desaturases (Δ5FAD and Δ6FAD) tended negatively with the salinity decreasing from 30‰ to 17‰ and to 3‰, but no significant differences were found among the groups.

In trial two, shrimp was fed diets containing soybean oil (SO), beef tallow (BT), fish oil (FO), linseed oil (LO), and equal combinations of SO+BT+FO (SBF) or SO+BT+LO (SBL), respectively, as lipid sources at salinity of 3‰ for 8 weeks. Weight gain and survival of SBL were higher than other groups, and the whole body lipid content of SBL was significantly higher than SO and LO groups. EPA and DHA were detected in muscle of all groups. The LNA content of SBL group was significantly higher than SO, BT, FO and SBF. Shrimp of BT group had the highest ELOVL6, Δ5FAD and Δ6FAD activities.

All the results indicate that low salinity of 3‰ will decrease the growth of L. vannamei. Fish oil is unnecessary for achieving optimal growth performance in the diet of L. vannamei. But lack of n-3 PUFA will decrease the growth performance. Though L. vannamei as a marine species, it might have the ability to synthesize both DHA and EPA from LNA.
POSTER 085
PROTEIN SPARING EFFECT OF CARBOHYDRATE IN THE DIET OF WHITE SHRIMP LITOPENAEUS VANNAMEI AT LOW SALINITY
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Juvenile L. vannamei were fed for 8 weeks with diets containing with four ratios of protein to CBH at P26:C30, P30:C25, P34:C19 and P38:C14, respectively at 3.0 g L⁻¹ salinity. Shrimp weight gain of P34:C19 group was the highest and differed from the shrimp fed the P26:C30 or P30:C25 diet. Shrimp fed the P26:C30 diet obtained higher survival than those fed other diets. Shrimp fed the P34:C19 diet contained the highest body protein and lipid, which were significantly higher than those fed the P38:C14 diet. Shrimp fed the P30:C25 diet had the highest hemolymph glucose content, which was significantly higher than those fed the P26:C30 or P38:C14 diet. Shrimp muscle glycogen of the P26:C30 group was the highest. Hepatopancreas B cell number of shrimp fed the P26:C30 diet was lower than those fed other diets and the R cell number was the highest in the shrimp fed the P30:C25 diet. This study indicates that the protein sparing effect by CBH occurred in the P30:C25 and P34:C19 groups because these protein to CBH ratios can support normal growth. Within the range of basic energy demand, the high dietary CBH to protein ratio can improve L. vannamei survival at low salinity.
FISH PROTEIN HYDROLYSATE IMPROVES GROWTH AND DIGESTIBILITY IN JUVENILE TURBOT (SCOPHTHALMUS MAXIMUS L.) GIVEN HIGH PLANT PROTEIN DIETS
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The intention of the study is to investigate the effect of different ultrafiltered fish protein hydrolysate (UF) inclusion levels and molecular weight protein hydrolysates on growth, feed utilization and apparent digestibility coefficient (ADC) for juvenile turbot (Scophthalmus maximus L.) fed high plant-protein diets. Two experiments were conducted at the same time. Fish protein hydrolysate (FPH) was obtained by enzymatic treatment from Pollock (Theragra chalcogramma) frame. Permeate fraction was obtained as UF by ultrafiltered step and retentate fraction was retained as retentate FPH (RF). For experiment (a), experimental dietary protein contained 10% different molecular size soluble protein mixes (54 g kg⁻¹ diet UF, 45 g kg⁻¹ diet crystalline amino acid mixture (CAA), 55 g kg⁻¹ diet FPH and 55 g kg⁻¹ diet RF), about 22% of fish meal protein (180 g kg⁻¹ diet fish meal) and about 68% of mix plant protein. A control diet (FM) contained fish meal as only protein source. For experiment (b), experimental diets were prepared containing 68% of plant protein and fish meal protein was respectively replaced by 0%, 5%, 10%, 15% and 20% UF of dietary protein. A positive control diet (FM) contained fish meal as the single protein source, while a negative control diet (PP) contained about 22% of fish meal protein and about 78% of plant protein. All diets were formulated to be isolipidic, isonitrogenous and isoenergetic and fed to 10 triplicate groups of turbot (initial body weight 16.05 g) for 68 days. High growth rates were obtained in UF-10 group in two experiments. Feed efficiency (FE), protein efficiency ratio (PER) and protein productive value (PPV) was significantly higher in FM group than that in CAA-10, RF-10 and FPH-10 groups (P<0.05), while no significant difference was found between FM and UF-10 groups (P>0.05). FE, PER and PPV in UF-0, UF-5, UF-10 and UF-15 groups were significantly higher than that in UF-20 and PP groups and lower than that in FM group (P<0.05). ADC’s of dietary protein and most amino acids were higher in FM and UF-10 groups than other groups in two experiments. In conclusion, 10% UF could improve the quality of dietary protein (the proportion of amino acid, peptide and intact protein) when experimental diets contained high levels of plant protein. As a result, dietary protein and amino acids were also well digested for fish fed diet UF-10.
EFFECT OF DIETARY CHOLESTEROL AND TAURINE ON EXPRESSION OF GENES INVOLVED IN STEROL METABOLISM IN JAPANESE FLOUNDER PARALICHTHYS OLIVACEUS

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The increasing use of plant protein and lipid sources in aquafeeds is necessary to maintain sustainable aquaculture. However, most plant sources are deficient not only essential amino acids and n-3 highly unsaturated fatty acids (HUFA) but also in phospholipids, cholesterol and taurine. Understanding the molecular basis of sterol metabolism and regulation in fish will help facilitate the use of plant-derived alternatives in aquafeeds. Therefore, we investigated the effect of dietary cholesterol and taurine on expression of LXRα, FXR, SREBP2 and their target genes (HMGCR, CYP7A1, and ABCA1) in the liver and extrahepatic tissues to examine the mechanisms involved in the regulation of sterol metabolism pathways in Japanese flounder. Fish were force-fed either of the seven different treatments once a day, for a 7-day period: (1) agar gel (0.5%), as a vehicle and control group; (2) taurine 1.5 mg/g fish (T₁); (3) taurine 3 mg/g fish (T₂); (4) cholesterol 1 mg/g fish (CH); (5) combination of taurine 1.5 mg/g fish and cholesterol 1 mg/g fish (TC). Increased expression of SREBP-2 and HMGCR in liver of fish fed cholesterol. Increased expression of SREBP-2 in intestine was also observed in fish fed taurine. When fish were fed taurine and cholesterol, expression of SREBP-2, HMGCR, and CYP7A1 in intestine significantly increased. Cholesterol and taurine control hepatic cholesterol and bile acid production via interactive regulation of CYP7A1, HMGCR and SREBP2 in Japanese flounder.
The inclusion of FM is steadily decreasing in compound aquafeeds for salmon from around 300 g kg\(^{-1}\) of the diet in 2006 to 150 g kg\(^{-1}\) in 2012. This amount is projected to decrease further and reach 8% in 2020 (Tacon & Metian 2008). Supplies of fish oil and fish meal are finite and prices have increased dramatically in the past decade. With world fish catches more likely to decline than increase, availability of FM declines and prices are expected to raise further and alternative feed ingredients are required to sustain a healthy industry and allow for future growth. In the past years intensive efforts have been made to use organic waste streams for large scale production of black soldier fly prepupae (Hermetia illucens) in The Netherlands. In this study we tested the replacement of FM with two IM products in diets for Atlantic salmon post-smolt. The IM products were obtained using different nutrient isolation and processing techniques. The modern control diet contained 200 g kg\(^{-1}\) fish meal (FM100), which was stepwise replaced by insect meal A (IM A) as a 25% (A25), 50% (A50) or 100% (A100) FM replacement or insect meal B (IM B) as a 25% (B25) or 100% (B100) FM replacement. Fish oil and rapeseed oil content of the diets high in IM was reduced 17% compared to FM100 due to high lipid content of IM. Selected nutrient parameters were measured in the diets as well as a range of contaminants. After 15 weeks, fish length and weight was recorded and visceral and hepatic indexes calculated. Whole body analysis was done for fatty acids and amino acids and their digestibilities calculated. Morphology of the liver, kidney, mid and hind intestine was evaluated. Finally a sensory testing of the fillets was conducted to test for odour, flavour and texture. A25, A50 and A100 performed equally well as the FM100 diet. The feed intake decreased moderately with increasing IM A inclusion however the FCR decreased resulting in an equal net growth of the fish fed the IM A containing diets compared to the FM100 diet. Product IM B did not perform equally well as IM A. The most remarked difference was the reduced feed intake of the B25 and B100 diet groups compared to both FM100 and all IM A based diets. We speculate that palatability of IM B based diets played a decisive role in the effects seen. Histology did not show any differences between any of the dietary groups and sensory testing of fillets from FM100, A100 and B25 did not reveal any significant differences in odour, flavour/taste or texture between groups. IM has a favourable AA profile for use in diets for Atlantic salmon. In addition, the tested IM also contained a considerable amount of highly digestible lipid.

REPLACEMENT OF FISH MEAL WITH RENDERED ANIMAL PROTEIN AND PLANT PROTEIN SOURCES ON GROWTH RESPONSE, BIOLOGICAL INDICES AND AMINO ACID AVAILABILITY OF RAINBOW TROUT (ONCORHYNCHUS MYKISS)

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Rendered animal protein ingredients, poultry by-products meal (PBM), hydrolyzed feather meal (FEM), spray-dried blood meal (BM) are potential fish meal substitutes for formulating fish feed. Compared to fish meal, methionine and lysine and methionine, lysine and histidine is limited in poultry by-products and feather meal, respectively. Blood meal is rich in lysine, and can be used to balance dietary lysine content when poultry by-products and feather meal are used alone or in combination as fish meal substitutes. In this study, two feeding trials were conducted (i) to examine the growth performance of rainbow trout fed rendered animal protein and plant protein diets, (ii) to investigate apparent digestibility coefficients of nutrients and amino acid availability in diets, and (iii) to determine the amino acid availability and crude protein digestibility of rendered animal protein sources for rainbow trout.

A 12-week feeding trial (trial#1) was carried out to investigate partial and full replacement of fish meal with PBM, FEM, BM, defatted soybean meal (DSM) and corn gluten meal (CGM) in practical type diets for rainbow trout. Duplicate treatments of rainbow trout (mean initial weight; 16.7±0.1 g) were fed six isonitrogenous (43.7% crude protein) diets. Fish meal based diet (56% anchovy meal) was designated as control and reference diets. In the other five diets, fish meal was replaced by rendered animal protein and/or plant protein sources at the levels of 75% and 100%. For trial#2, the apparent digestibility coefficients of amino acid in PBM, FEM, BM, DSM and CGM were determined for juvenile rainbow trout. A reference diet (RF) and test diets (consisting of 70% RF and 30% of the test ingredients) were used. Fish were randomly selected and reared in each of two tanks at the density of 15 fish per tank for 2 weeks. Each diet was hand-fed to apparent satiation twice a day to fish at 14.1±1.0 °C.

Fish fed the diets replacing fish meal with the combination of rendered animal protein at levels of 75% and 100% showed comparable growth performance with fish fed the control diet except the protein efficiency ratio and feed conversion ratio. Apparent crude protein digestibility coefficients were significantly higher for 75% fish meal replaced by the combination of DSM and CGM treatment than in the 75% fish meal replaced by the combination of rendered animal protein treatment. It was found that FEM showed the lower crude protein digestibility than the plant protein sources. In this study, PBM and FEM showed the lower amino acid availability than the DSM and CGM, but might increase the feed intake for rainbow trout. These results showed that the combination of PBM, FEM and BM might replace most of the fish meal in practical feed for rainbow trout.
The effects of weaning of *Catla catla* (catla) larvae on growth, survival and digestive enzyme activities were investigated. *Catla catla* larvae (0.9 ± 0.2 mg) were cultured for 30 days under six different feeding regimes (1) live zooplankton from day one to day 30 (LF), (2) artificial food from day one to day 30 (AF), (3) mixed food from day one to day 30 (zooplankton and artificial food each 50%, MF), (4) live zooplankton for 12 days then fed artificial food from day 13 onwards to day 30 (LF-AF-13), (5) live zooplankton for 17 days then fed artificial food from day 18 onwards (LF-AF-18) and (6) live zooplankton for 12 days then fed mixed food from day 13 onward up to day 30 (LF-MF-13). Highest survival rate (86%) was recorded in the catla fed with MF however highest average weight (70.5 ± 8.4 mg) of catla was recorded in fish fed with LF-MF-13. Significantly higher activity of different digestive enzyme were recorded for different feeding regimes amylase activity (LF-AF-18), chitinase and chitinobiase activities (LF), protease, trypsin, chymotrypsin, lipase and elastase activities were recorded in LF-MF-13. Carboxypeptidases A and B activities were significantly lower in LF. SDS-PAGE and substrate SDS-PAGE showed the same banding pattern in the digestive tissue extract of fish regardless of feeding regime. Inhibition study with SBTI, PMSF, TLCK and TPCK showed the presence of serine proteases, trypsin and chymotrypsin-like enzymes. In the present study catla wean with LF-MF-13 showed higher activities of digestive enzymes and also a better growth. This study can be a useful in understanding the proper weaning time for this economically important species for producing better fingerlings.

**Keywords:** Catla larvae. Digestive enzyme. Feeding regime. Live food
POSTER 091
THE IMPACT OF DIETARY SUPPLEMENTATION WITH DIFFERENT DHA / EPA ON SPAWNING PERFORMANCE, EGG AND OFFSPRING QUALITY IN SIBERIAN STURGEON (ACIPENSER BAERI)

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The effect of different DHA/EPA on the reproductive performance and offspring quality of the Acipenser baerii was evaluated in a 6-month trial. Two diets were isonitrogenous and isolipidic diets with different DHA /EPA of 1:1.9 and 1:9:1, named after high EPA diet (HE) diet and high DHA diet (HD). Stocking density was 12kg/n², and 136 female fish were randomly divided into two groups of 68 fish in per group. After a 6-month feeding period, the hybridization had been conducted among maturing female Acipenser baerii and male A. schrencki. The broodstock performance had been recorded and 6000 larvae had been cultured to evaluate the growth performance. Compared with the HE females, the egg mass weight, fecundity and fertilization rate in HD groups increased 40.8%, 22.3% and 35.6% respectively. Serum estradiol(E2), 11-keto-Testosterone and luteinizing hormone ( LH ) in HD females was significantly higher than those in HE females (P<0.05). Eggs of females fed HD diet had almost double EPA, DHA and total PUFA to HE eggs. 35-day-old larvae from females fed HD diet tended to present a better growth performance. their length, weight, weight gain and survival rates were significantly higher than larvae from females fed HD diet. Results demonstrate that feeding Acipenser baerii female broodstock with high DHA would improve fecundity, egg hatchability, and the overall quality of the larvae.

Information about diet and the amount of carbon and nitrogen assimilates the animal in their tissues through analysis of stable isotopes has been traditionally used in marine fish, but for the genus Centropomus there are few studies about it. Thus this study aimed to identify the source of food for wild animals in captivity, and check the different tissues (muscle, bone and skin), liver and stomach contents, which is more efficient at incorporation by stable isotope 13C/12C and 15N/14N compared with muscle, bone, liver and stomach contents, not statistically different (P>0.05) between fish in captivity and nature, with similar means.

The liver and stomach contents did not differ (P>0.05) for the isotopes of δ13C for both local collections. The muscle of Centropomus parallelus collected in captivity has proved more rich δ13C (~21.02 %) compared to wild animals (~24.97 %) and these were more rich in δ15N (12.42 %). Based on these results, we can suppose that through analysis of stable isotopes, analyzing muscle, bone, skin, liver and stomach contents, one can differentiate between fish collected from the wild animals in captivity and collected the muscle tissue was more effective in identifying these animals, the variation between the isotopes of δ13C and δ15N.

References:
POSTER 093
PRELIMINARY GROWTH RESPONSE OF CAPTIVE-REARED COMMON SNOOK (CENTROPOMUS UNDECIMALIS) FED WITH A BALANCED DIET IN A FRESH WATER RECIRCULATING SYSTEM
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Common snook (Centropomus undecimalis) is a protandrous hermaphrodite fish species, which is distributed from the north of Florida in the US to the south of Brazil along the coast. Recent efforts towards developing its culture technology (including captive breeding) have been made due to its high demand and aquaculture potential. However, no commercial ad hoc diet has been currently designed for this species and a lack of growth studies in freshwater exists despite its euryhaline capacity.

In the present work we set out to firstly test a purposely-developed diet and secondly describe the growth of adult snook in freshwater in order to gain further knowledge into its aquaculture potential under controlled conditions. For this 15 immature (presumptive male) fish (524±140g) of approximately three years of age were individually PIT-tagged and placed in an 9m³ indoor round tank attached to a closed water recirculating system. Temperature (27±0.7°C) was controlled with ambient heaters and a simulated natural photoperiod was set with a programmable automatic dimmer system (Lutron Electronics, Inc.) during the 6-month period that the study lasted. Water quality was checked and kept within safe levels at all times by flushing the system and doing partial water changes. Fish were fed to apparent satiation twice a day with an experimental diet based on fish and shrimp meal, containing 45% protein and 12% lipid, manufactured in our nutrition laboratory. Individual fish were sampled at the beginning and end of the trial in order to exclude intermediate handling stress events that could affect feeding and growth. Our results show a mean weight gain of 621±157.3g (118.5%) by the end of the trial with no mortalities. Mean Feed Conversion Ratio (FCR) was 2.8 while the Specific Growth Rate (SGR) was 0.41%. In comparison with previous studies and reports, our results show a higher growth performance of C. undecimalis under the previously described culture conditions including against other studies where higher temperatures and protein content were offered. This suggests that apart from the difference in experimental designs, systems used and other variables that could account for the differences observed with previous studies, the diet offered in this study is better than previously tested diets at least at this life stage. Moreover, this work shows for the first time the potential for on growing common snook in freshwater and recirculating systems fed on balanced diets. Such information is relevant for future commercial applications of this species in freshwater bodies either for aquaculture or restocking purposes for fishery management and/or the sport fishing industry.
Six reactors were prepared using PVC tanks of 100 L in capacity. Influent consisted of seawater with 25 mg/L of N, 3.6 mg/L P and some trace elements from plant fertilizer (0.1 mL/L influent). $^{15}$N stable isotope was used to trace the nutrient uptake by the shrimp and was added to the influent as 3% of the total N concentration. A peristaltic pump distributed the influent continuously. The hydraulic retention time was 1 day. The feeding experiment was composed of 5 different treatments with 3 replications. Artificial feed with 40% DW crude protein was used as a control feed. Biofloc treatments were consisted of 50% artificial feed and 50% biofloc with different carbon sourced. The green tiger shrimp (*Penaeus semisulcatus*) was used as the test organism. At the start of the experiment, the shrimp had an average weight of 0.5 ± 0.1 g and were stocked at an initial density of 35 individuals per tank. Feed was given at a weight of 5% of the wet shrimp biomass with a feeding frequency of two times per day. In order to obtain isonitrogenous diets for all treatments, the amount of biofloc given was determined based on the crude protein content of biofloc, which was measured every 5 days. The biofloc was added together with the water. The amount of water with biofloc was determined based on the crude protein content and TSS measurement. Sampling was carried out every 5 days, to measure the average weight of the shrimp and the nutritional quality of the biofloc. Three individuals were taken on every sampling point for whole body fatty acids analysis and three individuals were taken on every sampling for $^{15}$N stable isotope measurement. Survival and growth of shrimp were observed. There was no significant difference in survival and growth of shrimps between treatments in this experiment. The crude protein content of the biofloc grown with wheat bran showed the highest level and was significantly different from the other treatments except from the molasses treatment. The highest total n-3 PUFAs was revealed by the biofloc with wheat bran, which was 0.9 mg/g DW. The biofloc with molasses revealed the highest total n-6 PUFAs. Abnormalities were also observed on the shrimp fed with biofloc with wheat bran as carbon source. There was no significant difference in TVC in water in culture systems of treatments. The highest TVC occurred in the control treatment, i.e. 6.9 x 10⁵ CFU/mL in average. There were significant differences in TAN, nitrite and nitrate concentration between treatments. The TAN concentration in the wheat bran treatment was the highest among treatments. The nitrite concentration was highest in the control treatment. The nitrate concentration of wheat bran was significantly lower than that in other treatments. This indicates that as much as 50% of the artificial feed can be replaced by biofloc in shrimp growing cultures.

**Keywords:** Biofloc, substitution, alternative, protein, health, growth, *Penaeus semisulcatus*
POSTER 095
THE EFFECT OF BIOFLOC FEEDING ON THE SURVIVAL AND GROWTH OF GREEN TIGER SHRIMP (PENAEUS SEMISULCATUS)
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This study investigated the possibility to use microbial floc as a potential novel alternative feed in shrimp feed over 45 day feeding trial. One control diet (with 35% DW crude protein) was compared against two microbial floc treatments (MF1 and MF2). For MF1, Rice bran was used as a carbon source for microbial floc production. For MF2, wheat bran was used as a carbon source for microbial floc production. Four reactors were prepared using fiberglass tanks of 100 L in capacity. Influent consisted of seawater with 25 mg/L of N, 3.6 mg/L P and some trace elements from plant fertilizer (0.1 mL/L influent). A peristaltic pump distributed the influent continuously. The hydraulic retention time was 1 day. The carbon source, Rice bran and wheat bran was added daily to stimulate floc growth.

The green tiger shrimp (Penaeus semisulcatus) was used as the test organism. At the start of the experiment, the shrimp had an average weight of 1.6 ± 0.2 g. The rearing units consisted of fiberglass tanks of 100 L in capacity and were stocked at an initial density of 30 individuals per tank. Feed was given at a weight of 3% of the wet shrimp biomass. Feed was given twice a day in the morning and in the evening. The bioflocs were collected from the reactor by using a net. In order to obtain the same level of protein input in each treatment (isonitrogenous diet), the amount of bioflocs given as feed was determined based on the crude protein content and the dry weight of bioflocs, which was measured every 3 days. When the bioflocs were too small to be collected, the bioflocs were added to the shrimp tank together with the water. The amount of water with bioflocs was determined based on the crude protein content and the total suspended solid (TSS) measurement. The results of the study showed that the shrimps with bioflocs treatment are actually able to consume the flocs. This was also shown by the color of their digestive tract. The rice bran treatment showed the lowest survival and was significantly different from all other treatments. The mortality of the shrimp in this treatment started after the second week of culture, which also occurred to the wheat bran treatment. The highest survival was obtained by the control, which was 96%. There was no significant difference in shrimp growth between treatments, which is shown by the average body weight and growth rate. However, it can be seen that the control revealed a continuously increasing average body weight during the culture period and thus having the highest growth rate. The lowest growth rate occurred in the wheat bran treatment. The average body weights of the shrimps in this treatment tended to decrease in time. This resulted in a negative growth rate.

Keywords: Biofloc, shrimp, alternative feed, growth, survival
POSTER 096
EFFECT OF VARYING LEVELS OF DIETARY CRUDE PROTEIN OF BLACK CRICKETS MEAL ON GROWTH PERFORMANCE AND BODY COMPOSITION IN FINGERLINGS AFRICAN CATFISH, CLARIAS GARIEPINUS (BURCHELL, 1822)

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Black field crickets, Gryllus bimaculatus have been commonly used as a live feed supplement in the ornamental fish industry especially for the Arowana, Scleropages formosus fish. They can be found in abundance in the tropics including Malaysia. Currently, very limited information is available on crickets as alternative protein source for fishmeal in fish formulated diets. The sustainability and growth of the aquaculture industry is being threatened by the continual decrease in supply and increase in cost of fishmeal. It is therefore necessary to find cheaper and sustainable alternatives to fishmeal and insects were proposed as an alternative as it has recently gained in popularity and demand. Therefore, this study is aimed at evaluating the effect of growth performance of African catfish fed with varying levels of crude protein with 100% inclusion level of black field crickets meal. Three levels of crickets meal (30%, 35% and 40% crude protein) were used in this experiment. Fishmeal was used as a control with 100% inclusion and 30% crude protein level. Fifteen fishes were used in every treatment and fed at 10% of their body weight ratio twice per day for 56 days. Four fishes from each treatment were sacrificed for whole body composition. The observation suggested that a gradual increase in weight gain was found with increasing crude protein level. However, no significant differences (P>0.05) were found for food conversion ratio and specific growth rate with fish fed diet 35% and 40% crude protein. The whole body composition of crude protein, lipid and ash for the 35% and 40% crude protein also showed no significant difference (P>0.05) suggesting that the 35% crude protein diet is appropriate for African catfish fingerlings nutrition.

References:
Food intake is one of the key factors governing growth. However, larval nutritional studies have so far mostly focused on essential fatty acid requirements and there are practically no studies on the effects of diet composition on food intake in fish larvae. Furthermore, results from juvenile or adult animals cannot be extrapolated given that fish larvae have much higher requirements for energy and structural components for rapid growth and organogenesis/metamorphosis, as well as a structurally and functionally less developed digestive tract, which is likely tied to differences in metabolism. However, these types of studies are especially difficult to conduct in fish larvae for a wide number of biological and technical reasons. In this study we employed a method of labeling Artemia using fluorescent microspheres to quantify ingestion and investigate food selectivity in response to the fatty acid (FA) composition of the diet in Senegalese sole larvae and post-larvae. We tested four treatments of live prey enriched with lipid emulsions differing in the oil source that was used - cod liver oil (CLO), linseed oil (LSO), soybean oil (SBO) and olive oil (OO) – from first feeding (enriched rotifers from 2 to 8 days post hatching - dph; enriched Artemia metanauplii from 6dph onwards). The results showed that larvae fed the CLO diet had a significantly higher dry weight at 22dph and 30dph, followed by LSO (not significantly different from the SBO and OO treatments). The ingestion trials performed at 19dph and 35dph showed a significantly higher intake (number of Artemia per mg of wet weight of larvae) of the CLO diet. However, when presented with a choice of the four diets, post-larvae at 29dph and 30dph did not show a preference for any of the diets. We were therefore able to demonstrate that the FA composition of enriched Artemia can affect food intake during the larval (pelagic) and post-larval (benthonic) stages and that the most ingested diet (CLO) was also responsible for higher growth. The expression of several anorexigenic and orexigenic gastrointestinal and neuropeptides (CCK-L, CCK-M, PYY, CART, POMC, NPY and AgRP) was assessed just before feeding and at 30min, 1h 30min and 3h after feeding, in whole larvae (at 16dph) and separate head and body compartments (at 34dph). The food intake and gene expression results will be analyzed and discussed in conjunction to investigate potential regulatory pathways of appetite/ingestion that might be affected by the FA composition of the larval diet.
KEY NUTRIENTS FOR THE ENHANCED GROWTH AND DEVELOPMENT OF ATLANTIC COD (GADUS MORHUA) LARVAE FED COPEPODS, IN COMPARISON WITH ROTIFERS

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It is been demonstrated that vegetable oils in salmonid feed diminish HUFA content particularly EPA and DHA and produce a rise in the content of linoleic acid, so that the ω-3: ω-6 ratio is modified. A good alternative to diminish this effect is replacing soybean oil partially for flaxseed oil in rainbow trout’s diet due to its higher content of linolenic acid and lower linoleic acid.

The aim of this study was to evaluate EPA and DHA content recovery in rainbow trout (Oncorhynchus mykiss) fed a fish oil diet after being fed a mix of soybean and flaxseed oil in the diet during 12 weeks under commercial conditions. A commercial diet with 30% fish oil was supplied to rainbow trouts (BW 2100 g), located in 8 sea water floating cages under commercial conditions at a water temperature of 11 °C, previously fish were fed diets with different proportions of soybean and flaxseed oils for 12 weeks (Diet 1: 70% soybean oil + 30% fish oil, Diet 2: 57% soybean oil + 13% linseed oil + 30% fish oil); the control group was fed a diet based only in fish oil. The lipid content and changes in fatty acid profile in the whole fillet, including belly fat, was measured, and the effects on productive performance were analyzed.

Fish were randomly sampled every 4 weeks to measure weight, fork length, carcass weight, viscera weight, liver weight and shear strength. At the same time, 8 to 12 steaks were sampled for each treatment and control respectively, which were kept frozen at -20 °C until analysis.

It was found that EPA content in fillets increased in treatments 1 and 2 during the trial (37 and 24% respectively), but both values were lower than the control at the end of the trial (T1 0.59, T2 0.52; control 0.73) with significant differences (P-value <0.05). The DHA content diminished over time, significantly in T1, without significant differences between treatments at the end of the trial (T1 0.45; T2 0.47; Control 0.55).

The ω-3/ω-6 ratio increased over time (57% T1 and 31% T2) with significant differences. However, recovery was higher in rainbow trout previously fed the diet containing soybean oil. The final value for the ω-3/ω-6 ratio in both treatments was lower by 15% in relation to the control with significant differences (P-value <0.05) (0.58 T1, T2 0.59, 0.67 Control, expressed as grams of each fatty acid in a 100 g fillet).

It was concluded that 12 weeks feeding a fish oil diet after a diet with soybean oil and flax oil in rainbow trout, is insufficient to reach the fatty acid profile of rainbow trout reared with fish oil during the whole period in commercial conditions. There was not any significant impact on growth response and visual color of fillet, even though it is feasible to identify a residual effect of feeding vegetable oil from the previous step on the shear strength and instrumental color.

Project partially financed by domeyko project, universidad de chile and salmones antartica, chile

Keywords: Rainbow trout; EPA; DHA; flaxseed oil; Linoleic acid
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**Keywords:** Rainbow trout; EPA; DHA; flaxseed oil; Linoleic acid

Project partially financed by domeyko project, universidad de chile and salmones antartica, chile
COMPARATIVE PERFORMANCE OF A PILOT-SCALE BIOFLOC TECHNOLOGY (BFT) AND RECIRCULATING AQUACULTURE SYSTEM (RAS) FOR INTENSIVE TILAPIA CULTURE IN INDOOR TANKS
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This paper compares the production performance of two intensive indoor tank-based tilapia culture systems: (1) ‘biofloc technology’ (BFT) based on bacterial nutrient recycling and (2) a conventional re-circulating aquaculture system (RAS). Theoretical BFT advantages include; potential for higher dietary protein recovery and water productivity, operational simplicity, lower capital and operational costs and associated environmental benefits. Trials were conducted on a pilot commercial-scale in partnership with a tilapia producer in Thailand. The replicated BFT treatments incorporated two ‘low/ high’ stocking densities (SD) and two formulated-floating commercial diets with 17% and 30% crude protein (CP) contents. The RAS positive-control replicates were established at the ‘low’ SD and 30% CP factor levels alone. Results were evaluated on five biotic production factors; fish growth, survival, condition, feed and protein conversion efficiency.

Marked feeding and growth inhibition in BFT treatments associated with biofloc accumulation under closed operating conditions necessitated periodic removal of excess suspended solids. Mean specific growth rate (SGR) in the best performing BFT treatment was 68% of the RAS system and carrying capacity was limited to only 10-11 kg m⁻³ before process instability set in. This compares unfavourably with levels of 50-60 kg m⁻³ routinely achieved in aerated RAS. The magnitude of growth and yield penalties also precluded anticipated gains in feed and protein conversion efficiency for which outcomes were equal or superior in RAS. Higher condition factor and survival outcomes were also indicative of superior welfare conditions in RAS. We conclude that light-limited BFT would not represent a commercially viable alternative to intensive tilapia culture in RAS.
The concept of sustainability is grounded on pillars such as profitable production, environmental conservation and social development, and in aquaculture presents the challenge of optimizing the use of natural resources, facilities, hand labor, the introduction of integrated production systems (e.i. polyculture), the rational use of inputs, and, the utilization of waste processing. In Brazil, tilapia is the most industrialized freshwater fish with production of 155 thousand tons and upon that, its average yield of fillets is approximately 30% of harvested mass and the remaining 70% is waste, including head, bones, viscera, skin and scales, thus permitting this species to be a candidate for innovations in sustainability. A feasible option to minimize environmental problems generated by the high amount of fish waste is to produce an ingredient to be recycled from waste and use into diets of fish, studying its potential as a source of nutrients and, thus, collaborate in advancing aquaculture that requires a corresponding increase in the volume of food. The aims of this study were to optimize the use of fish waste from Nile tilapia on production of acid (A) or fermented (F) protein hydrolysates from viscera (V) or total waste (T), and to evaluate the chemical composition of the hydrolysates.

Fish waste was collected from a fish farm, grounded and ensiled till stabilize de pH. The acid hydrolysates had a pH between 3.9 and 4.2 after the addition of acids, and were stored with temperatures of 27° C to 30° C. The fermented hydrolysates were produced by anaerobic fermentation using microorganisms such as Lactobacillus sp and a carbohydrate source (molasses) to produce lactic acid, which is important to decrease the pH.

Hydrolysate from viscera showed the lowest content of crude protein (CP) and the highest content of ash, fat, and energy. However, hydrolysate from total fish waste showed higher values of CP and lower content of ash, fat, and energy. This result indicates that hydrolysate from total fish waste can support farmed fish to reach the same growth rate as obtained with commonly used fish meal. The protein hydrolysate derived from total fish waste (acid or fermented) may potentially serve as a good source of protein. This result is the most interesting topic for our further research.
The first aim of the experiment was to determine the effects of gradually replacing soybean meal (SBM) with coarsely ground (CG, 1 mm) rapeseed cake (RSC) in fish meal-free feed for tilapia (Oreochromis niloticus, PROGIFT, Generation 12). The second aim was to find out if fine grinding (FG, 0.5 mm) or a combination of FG and autoclaving (FG+A) improved the nutritional value of RSC.

A regression study was conducted to define a level of RSC to which the tilapias were highly sensitive. Five diets were formulated to contain 32% crude protein (CP), 31% gelatinized starch and 7% lipid. RSC gradually replaced SBM, diluted with cellulose to contain the same concentration of CP as the RSC. The sum of CP from RSC and SBM was 53% of total dietary CP. RSC replaced 0, 25, 50, 75% and 100% of CP from SBM. Pellets were produced in a pasta extruder, and subsequently dried at 54°C. Each diet was fed by 3 daily meals to 2 tanks with 20-g tilapia, in a recirculated system, with water temperature at 26-27°C, for 6 weeks. Daily feed intakes were assessed during the first 21 days, while pooled values for feed intake were measured during the last 3 weeks. Fish were weighed at start and week 6. Samples of whole fish, liver, thyroid, muscle, intestine, faeces and otoliths were taken for further analysis.

The fish fed the diet without RSC increased daily feed intakes from less than 3% (DM/ body weight) to 4% during the first week of feeding, and maintained this level throughout the 21-day assessment period. All diets with mixtures of SBM and RSC (25, 50, 75% SBM replacement) also reached 4% at one week of adaptation, while the daily feed intakes were reduced during the last 2 weeks. The diet without SBM had a steady increase in feed intake from 2% towards 4% during the three weeks. Cumulative feed intake and growth rates were reduced by RSC levels to 50% replacement. At higher replacement, only small reductions in growth rates were seen. Weight gains varied from 45 to 58 g per fish for the various treatments. The feed conversion ratio (FCR, g DM intake per g gain) ranged from 1.0 to 1.1 for all dietary treatments. The ratio of liver weight to whole body was increased up to 50% replacement and decreased at higher levels.

The tilapias seemed highly sensitive to the replacement levels between 0 and 50%. Thus, three diets with 50% RSC (CG, FG, FG+A) were formulated and processed under the same conditions as described above. They were fed to triplicate groups of 37-g tilapia for 3 weeks. Gains ranged increased from 38 to 45 g/fish and FCR ranged from 1.02 to 1.06. No significant differences were seen between 1.0 and 0.5 mm grinding, while feed intake and gain were significantly reduced by autoclaving the RSC.

Nutrient digestibilities and retentions, and thyroid function in the tilapias will be reported.
Two experiments were conducted to determine the digestible nutritive value of Australian canola meals, their utilization and examination of effects on biochemistry, histology and gene expression when fed to barramundi. In the first experiment, the digestibility of four Australian canola meals (three solvent-extracted meals and one expeller-extracted meal) from four crushing plants in different regions, were determined. Diets were formulated from 70% of a basal mash with a 30% inclusion of each test ingredient with another diet including (also at 30%) a lupin kernel meal as a reference ingredient and another diet as a basal reference diet. Barramundi (~390 g) were assigned to 24 mesh cages (300L) placed in 6 x 2500L tanks. Fish were acclimatized to the diets for one week before faeces were collected by stripping techniques over a three week period. Faeces and diet samples were analysed for yttrium, dry matter, protein, amino acids and energy. The second experiment was designed to determine the optimal canola meal inclusion level in diets for barramundi. A serial inclusion (0%, 10%, 20%, 30%) of two of the canola meals in diets (solvent and expeller) was formulated to the same digestible protein and energy specification for barramundi, with an eighth diet including 30% lupin kernel meal as a reference. Fish (~50 g) were allocated across 24 tanks (300 L) with three replicates per treatment and at a density of 15 fish per tank. Fish were fed to satiation once per day, and uneaten feed was collected to calculate feed intake. After 8 weeks fish were weighed and three fish from each tank were sampled for chemical composition analysis while another three fish were used for taking blood, kidney, liver, pyloric caeca, distal intestine and stomach for biochemical, histology and gene expression analysis.

The results of this study revealed that there was a variation in protein digestibility amongst canola meals. Protein digestibility of expeller canola meal (63.1%) was significantly lower than that of solvent canola meals (74.5% to 86.6%) and lupin (92.7%) while energy and dry matter digestibility were fairly consistent among canola meals and similar to that of the lupin kernel meal. Amino acid digestibilities closely followed a similar trend to that of protein digestibility. The results of the growth experiment showed that 30% inclusion of solvent canola meal in diet did not negatively affect feed intake, growth performance, and feed efficiency of barramundi, however 30% inclusion of the expeller canola meal diet caused a reduction in feed intake, poorer growth and poorer feed efficiency compared to the control and other test diets. Further study on plasma biochemistry demonstrated the plasma T3 and T4 concentrations were lower in fish fed diet containing 30% of solvent canola meal, and amongst 12 genes examined in liver the expression of CYP1A1, CYP3A, CYP2N, GST and FXR were all down regulated in fish fed this diet. The presence of higher concentration of anti-nutritional factors such as glucosinolates likely accounted for detrimental effects on fish fed 30% expeller canola meal diet.
The effects of dietary protein levels and dietary protein to energy ratios on growth and feed utilization of juvenile pompano (Trachinotus blochii) was studied. Nine experimental diets were formulated to contain three levels of crude protein (400, 450 and 500 g kg\(^{-1}\), dry mater basis) and three levels of gross energy (19, 23 and 25 MJ kg\(^{-1}\)). Nine groups of juvenile pompano (initial average weight of 31.5 g) with triplicates were fed each experimental diets for 9 weeks. The fish were cultured in 500 L tanks equipped with a biofilter system. The specific growth rate, feed efficiency and feed protein utilization were significantly affected by dietary protein and energy levels. Specific growth rate and feed efficiency were significantly increased with increasing level of dietary protein. Conversely, increasing dietary energy from 23 to 25 MJ kg\(^{-1}\) affected negatively fish growth. Highest specific growth rate, feed efficiency and protein utilization were found in fish group fed diet containing highest 491 g kg\(^{-1}\) protein and 23.2 MJ kg\(^{-1}\) (analytical results) with protein to energy ratio of 21.2 g MJ\(^{-1}\).
Research surrounding fish oil replacement has mainly focused on the incorporation of alternative oil sources of terrestrial origin such as vegetable oils and animal fats in aquafeeds. However, the main disadvantage of these sources is that they do not contain the omega 3 long-chain polyunsaturated fatty acids (n-3 LC PUFA; and specifically eicosapentanoic acid, 20:5n−3; EPA and docosahexanoic acid, 22:6n−3; DHA). Despite considerable research effort focused on trying to find and incorporate suitable alternative oil sources at levels up to 85% or total lipid, salmonid aquaculture still consumes over 65% of global fish oil supply. Marine carnivore finfish require both EPA and DHA to meet dietary n-3 LC PUFA requirements, but little is known about the potential different role of, and requirement for, EPA and DHA. Thus, considering that it is now clear that one of the ultimate goals for the future of the salmonid industry is to maximise the efficiency of n-3 LC PUFA utilisation, the need for increased knowledge about the metabolic roles of individual n-3 LC PUFA is paramount, and specifically the understanding of the relationships between dietary origin of EPA and DHA, their metabolic fate and the exact distribution over the various types of lipids. EPA and DHA are known to have considerable different physiological roles and it is envisaged, but often not considered, that the way in which they are metabolised and deposited by the fish is different. In order to optimise the efficiency of their deposition, the critical differences in how these two fatty acids are metabolised and deposited must first be understood. The focus of this study was to examine the differences in the metabolism and deposition between EPA and DHA.

Two experimental diets were formulated to contain 50% protein and 21.5% lipid, differing only in the composition of the added lipid source. Diets contained 140 g/kg of added oils, comprised of a blend of tallow and concentrated EPA or DHA, so that in diet D-EPA and diet D-DHA the amount of EPA or DHA was equivalent of 100% of what is found in fish oil, respectively. Triplicate groups of juvenile Atlantic salmon (30 per tank) were fed to apparent satiety with experimental diets at 10°C in a freshwater RAS system. Several selected tissues were analyzed for the fatty acid composition of each lipid class. Results will be presented and discussed, towards increasing the current understanding of the different metabolic fates of EPA and DHA.
Organ-specific proteomics of larval fish is limited by difficulties in dissection. Thus, method development for efficient proteomic analysis of whole fish is required to investigate changes in larvae physiology. Proteome assessment of whole fish is hampered by the presence of high-abundant proteins, which interfere with the detection of low-abundant proteomic components, and consequently hide physiologically significant changes restricted to a certain cell type. Several strategies exist to reduce sample complexity and extend the range of proteins detected. For example, in 2-D gel based proteomics, narrower pH-range IPG strips and larger gels can increase the detection of lower-abundance proteins. Our aim is to develop a robust protein extraction method from whole fish larvae to study changes in protein expression using label-free shotgun proteomics. Different protein extraction methods were compared for their ability to enhance protein enrichment in samples of Atlantic salmon larvae (~200 mg). Manual sample disruption was compared with mechanical homogenization, and direct protein extraction (Urea/Thiourea/Tris) was compared with solubility-based protein fractionation (NaCl/Tris + Urea/Thiourea/Tris). SDS-PAGE analysis of sequential fractionation revealed distinct protein profiles. Preliminary studies on whole Atlantic salmon larvae using manual disruption and direct protein extraction identified 432 proteins. Ongoing shotgun proteomic analysis will determine the effectiveness of this approach to increase proteome coverage.
This study was carried out to determine if two protein concentrates, formulated as fish meal analogues, could partially replace a salmon by-product meal in practical diets for cobia juveniles. First, a basal diet was formulated to contain 51.4% salmon by-product meal (FML) and 16.1% soybean meal (SBM). From the basal diet, four other diets were designed to replace fishmeal at 12, 25, 37 and 50% for SBM. Diets contained a mean of 48.4 ± 0.87% crude protein (CP, as fed), 11.6 ± 0.63% fat and 18.8 ± 0.84 MJ/kg gross energy (GE). Cobia of 22.80 ± 1.63 g (n = 150, CV = 7.2%) were fed for 10 weeks at 10 fish/m\(^3\) in 30 tanks of 0.5 m\(^3\) kept indoors under a closed recirculation system. At harvest, fish survival reached 99.3 ± 3.7%. There were significant differences in daily weight gain, FCR and final body weight (BW). Final BW started to deteriorate at 25% replacement of FML (201 ± 35.3 g, 193 ± 39.0 g, 184 ± 44.6, 164 ± 31.8 g and 110 ± 37.0 g for 0, 12, 25, 37 and 50% replacement, respectively), while daily weight gain dropped after 12% replacement (2.5 ± 0.2 g, 2.4 ± 0.3 g, 2.3 ± 0.1, 2.0 ± 0.1 g and 1.2 ± 0.3 g, respectively). FCR for diet with 50% replacement was significantly higher (4.5 ± 2.26) compared to other treatments (2.00 ± 0.65). Based on this data, a second study was designed with a control diet containing 44.6% FML and 22.2% SBM. From this control diet, six other diets were prepared to replace FML at 25, 50 and 75% for two protein concentrates (PC) analogue to FML. The PCs were made to contain either plant (soy protein concentrate, corn gluten meal) or animal by-products (poultry by-product meal, meat and bone meal, feather meal). Both PCs were supplemented with L-Lysine, DL-Methionine, fish oil, sardine hydrolasate and minerals. Diets contained a mean of 48.9 ± 0.57% CP (as fed), 10.6 ± 0.43% fat and 19.3 ± 0.26 MJ/kg gross energy (GE). Cobia of 14.4 ± 0.9 g (n = 300, CV = 6.2%) were stocked at 10 fish/m\(^3\) in 30 tanks of 1 m\(^3\) raised for 84 days. At harvest, fish fed the diet with the plant PC at 75% FML replacement had a drop in survival to 70.0 ± 18.7%. No other negative effect was observed on fish survival for other diets (97.6 ± 4.4%). However, both cobia final body weight and biomass significantly deteriorated when FML replacement exceeded 25%. Fish fed the control diet and diets with 25% FML replacement containing the plant and animal PCs achieved a mean daily weight gain, a percentage weight gain and a specific weight gain of 2.5 ± 0.1 g/day, 1,462 ± 38.5% and 3.3 ± 0.0% day, respectively. Final body weight for fish fed the control diet (218 ± 47.9 g) did not differentiate from fish fed diets with 25% replacement (213 ± 58.9 and 226 ± 32.7 g for plant and animal PCs, respectively), but significantly reduced at 50% (152 ± 35.3 g and 184 ± 39.7 g) and 75% (76 ± 26.2 and 144 ± 38.7 g, respectively) replacements. The present study has shown that FML inclusion in practical diets for cobia can be reduced from 51.4% to 44.6% without any detriment to fish performance. A further reduction of 25% was achieved with plant and animal by-product concentrates designed to be analogue to FML.
Increased risk of intestinal health disorders in salmonids is the consequence of feeding diets containing high levels of plant ingredients. In spite of the emerging role of plant-based diet in fish intestinal health diseases, little is known about their pathogenesis. Soybean meal-induced enteritis (SBMIE), which is a chronic inflammation of the distal intestine caused by feeding moderate and high dietary levels of soybean meal (SBM) to salmonids, has recently been used as a model to investigate the development of intestinal inflammatory responses in fish. To better understand the underlying mechanisms of plant-based diet-related intestinal disorders and their prospective solutions, recent key molecular findings associated with SBMIE and the protection against SBMIE conferred by several microbial ingredients will be presented.

Intestinal homeostasis is regulated by a complex crosstalk between intestinal immune system, intestinal commensal microbiota, and intestinal epithelium. Intestinal microbiota is known to produce vitamins, amino acids, and enzymes, along with regulating intestinal epithelial cell differentiation, colonization of harmful bacteria, and immune responses. The intestinal epithelium is a selective permeable barrier that regulates nutrient and fluid transport, prevents bacterial translocation and influx of luminal antigens into the intestinal tissue. The intestinal immune system is fundamental for clearance of antigens and described as diverse, consisting of immunoglobulins, humoral elements, and multiple lines of immune cells. Thus, changes in the intestinal immune system, the intestinal microbiota, and the intestinal epithelium might affect the intestinal homeostasis.

There is compelling evidence of changes in these essential components of the intestinal homeostasis during SBMIE. Increased relative abundance of certain intestinal bacterial groups has been observed during SBMIE. The latter might be associated to changes in the expression of several genes coding for antimicrobial peptides, complement components, and antigen presenting pathway-related proteins. For example, suppression of properdin, a key regulator of the complement system, during SBMIE might result in deregulation of immune responses. An ongoing oxidative cellular stress has been associated with cell damage during SBMIE. Thus, expression of several genes involved in the ubiquitin-mediated proteolysis and acute phase responses is induced during SBMIE. On the other hand, the glutathione metabolism pathway, which protects against oxidative stress, is down-regulated during SBMIE, which might result in tissue damage. Tissue healing is also associated with chronic inflammatory processes as SBMIE, being characterized by increased cell proliferation and migration, and extracellular matrix remodeling. Microbes from different taxa (e.g. bacteria, yeast, and microalgae) have shown to counteract or dampen the effect of SBM on several immune and metabolic pathways and provide a similar intestinal microbiota composition to fish with healthy intestine, resulting in prevention of SBMIE in salmonids. An overview of the main molecular aspects during the development and counteraction of SBMIE will be presented.
Fishmeal and oil are expensive ingredients in commercial shrimp feed and many studies have achieved considerable success in partially or totally replacing fishmeal with other protein sources, mainly of terrestrial origin, in various shrimp species. However, these lack highly unsaturated fatty acids, the appropriate amino acid balance and cholesterol, which affects not only growth and survival of shrimp, but also its final quality for consumers. Fishmeal and oil can be replaced by other marine origin products, thus obtaining a more appropriate fatty acid and cholesterol composition to meet the nutritional requirements of the target organism.

In this study, 3 feeding trials with juvenile shrimp *L. vannamei* were conducted: two six-week growth trials for fishmeal replacement, using macroalgae meal (*Sargassum* sp.) [trial 1], and viscera from scallop (*Atrina maura*) and squid (*Dosidicus gigas*) and whole mackerel (*Scomber japonicus*) meals [trial 2]; a two-week-trial for fish oil replacement (finishing diets) using oils from different organisms: (*Sargassum* sp.), scallops (*Argopecten or Atrina* sp.), and squid (*Dosidicus gigas*) [trial 3].

The experimental meals were obtained from marine products dried in an air-forced oven at 60°C for 24h, pulverized and sieved at 250 microns. The macroalgae meal was included at 4% in a reference diet for shrimp (35% CP, trial 1) while the other meals were included at 32% into shrimp feeds, completely replacing fishmeal (trial 2). Oils were extracted from the marine products using solvents (chloroform:methanol) and included at 11% in the feeds (finishing diets).

No differences were found for survival, which was above 95% for all treatments, feed conversion ratio (1.4-1.5), or weight gain among different oil or meal treatments. However, there were significant differences in fatty acid, pigment, and sterol composition in the muscle of shrimp fed alternative marine meal or oils, compared to fishmeal or fish oil. Shrimp fed *Sargassum* sp. meal or oil had less total lipid and more 20:4n-6 in muscle compared to all other diets and a commercial feed. Shrimp fed scallop oil had more 20:5n-3 and those fed squid had more 22:6n-3 in muscle than any other diet, including fish oil. The lowest total lipid concentrations were found in shrimp fed *Sargassum* sp. oil and the highest in those fed squid, while pigments levels were higher in shrimp fed *Sargassum* sp. and lower in those fed fish oil, and lowest in the commercial feed. Cholesterol and total sterol levels were slightly lower in shrimp fed *Sargassum* sp. Marine products, and particularly local animal by-products, are excellent replacements regarding fatty acids and pigments, but processing costs need to be decreased to be a viable alternative to fish oil and fish meal.
A three months experimental trial was conducted with variable percentage levels of protein (25, 30, 35, 40, 45 & 50) diet fed to the common Indian catfish, *Clarias batrachus* to study the satiation time, maximum feed intake in different hours of the day, protein/dietary energy requirement for optimum growth in different size groups, amino acid absorption and quantitative requirement, *in vivo* protein biosynthesis etc. and finally to calculate the requirement of digestible energy for the production of one gram fish to thousand gram in weight.

It was observed that the maximum feed intake recorded with smaller size groups (5.0 g) as compared to 20.0 g and 50.0 g, satiated within one hour and highest appetite was recorded at 12.00 hours of the day. Maximum growth were observed with 45.0% protein (5.0 ± 0.3g) followed by 40% (20.0 ± 2.1 g) and 35% (50.0 ± 3.6 g) in *Clarias batrachus*, although growth continued till 50% of dietary protein but not significant at the 0.5% level. Maximum weight increase recorded with 1: 14.85 digestible energy / digestible protein ratio.

Maximum amino acid absorption takes place in the posterior serosal layer of the intestine. It was also recorded that the same 10 amino acids are essential and their requirement was almost at par with the other fish species, and as in higher vertebrates. However, the importance of cystine and tyrosine for the growth of *Clarias batrachus* should not be ignored and should be considered as two additional essential amino acids. The protein biosynthesis *in vivo* is highly correlated with the dietary protein/energy, feed intake and growth performance of fish.

POSTER 111
NUTRITIONAL ENERGETICS OF COMMON INDIAN CYPRINIDS, LABEO ROHITA
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An eight weeks experimental trial was conducted with variable percentage levels of protein (25, 30, 35, 40, 45 & 50) diet fed to the Indian major carp, Labeo rohita to study the satiation time, maximum feed intake in different hours of the day, protein/dietary energy requirement for optimum growth in different size groups, amino acid absorption and quantitative requirement, in vivo protein biosynthesis etc. and finally to calculate the requirement of digestible energy for the production of one gram fish to thousand gram in weight.

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Maximum amino acid absorption takes place in the posterior serosal layer of the intestine. It was also recorded that the same 10 amino acids are essential and their requirement was almost at per with the other fish species, and as in higher vertebrates. However, the importance of cystine and tyrosine for the growth of Labeo rohita should not be ignored and should be considered as two additional essential amino acids. The protein biosynthesis in vivo is highly correlated with the dietary protein/energy, feed intake and growth performance of fish.

Patra B.C. 2008a. Evaluating the nutritional condition of an Indian climbing perch, Anabas Testudineus (Bloch) fingerlings by the RNA/DNA, ca/p ratio in muscle and protein bio-synthesis rate in liver and muscle. Aquaculture America’08, Florida, USA FFN 10, 2
An experiment was conducted with barramundi (Lates calcarifer) juveniles to examine the partial efficiency of utilisation of methionine from both intact protein and a crystalline DL-methionine source. Three diets were produced; two based on a lupin protein concentrate (LPC) and the other containing fishmeal as the primary source of protein. Each of the diets was formulated to the same digestible protein and energy densities. One of the LPC-based diets (Diet LM) was additionally fortified with a range of crystalline amino acids in order to ensure all essential amino acids (EAA) were present in the diet well above estimated requirements, with the exception of methionine which was designed to be marginally limiting through managed addition of DL methionine. The other LPC-based diet had no additional DL-methionine and was substantially deficient in this EAA (Diet LD). Each of the diets were then fed at one of three ration levels (low, medium, high) to 10.3 ± 0.2 g fish over a 28 day period. Over this period, the fish fed the fishmeal based diet at the highest ration level grew to an average weight of 37.3 ± 0.46 g while those fed the LPC diet fortified with all EAA at the highest ration level grew to 25.4 ± 2.27 g. Interestingly, the weight of the fish fed the LPC diet with no additional methionine at the highest ration level declined over the course of the experiment resulting in a final weight of 9.2 ± 0.88g, clearly demonstrating the impact of dietary methionine deficiency.

The main objective of this study, however, was to assess the partial efficiency of utilisation of each diet and its first limiting nutrient in each case, by correlating nutrient deposition against intake of that same nutrient. Partial efficiencies of protein utilisation for each diet varied, with that of the fishmeal-based diet observed to be 54.6% and the LM diet, 36.4%, while that of diet LD was only 5.6%. When the same parameters were investigated for methionine utilisation, partial efficiencies of methionine utilisation of 21.5% and 24.9% were calculated for the LM and LD diets respectively. The fishmeal based diet had a partial efficiency of methionine utilisation of 52.5%, however this was not the first limiting EAA in this diet.

These results demonstrate that the partial efficiency of protein utilisation in diets where DL-methionine is the first limiting constraint is significantly poorer than that from an intact protein source like fishmeal. Similarly, when this is examined in terms of methionine utilisation, it can be shown that the use of this form of the amino acid is about half as efficient as that of the fishmeal protein bound form. Furthermore, while as methionine became increasingly limiting in the diet (diets LM and LD) the ability of the fish to use the dietary protein was compromised, the efficiency of methionine utilisation was largely unaffected (remaining at around 21 – 25%), however, when the data is reassessed based on only the L-methionine content of the LM diet (L-methionine constitutes 50% of the content of DL-methionine) an improvement in the partial efficiency of utilisation to around 36% was observed. These observations further demonstrate the differences in utilisation of amino acids from different sources.
A study investigated the effects of dietary lipid and protein levels on the growth performance, nutrient utilization and tissue lipid deposition in meagre (Argyrosomus regius). Triplicate groups of 64 juvenile fish (IBW: 63 g) were fed over 63 days with one of four experimental diets, varying in their crude protein content 44% and 50% and crude fat levels of 15 and 18%. Fish fed diet 50L18 showed a significantly greater final body weight, specific growth rate and protein efficiency ratio. Similarly, fish fed the 50L18 diet showed a significant increase of protein retention, and the highest daily gain of nitrogen and energy. Dietary changes had no significant effect on lipid and energy retention. The reduction of dietary crude protein levels in concomitance with an increase of dietary lipids did not support a protein sparing effect. Significant differences were found between the mean lipid deposition on liver and viscera, but not on the muscle samples. The fatty acid profile was not significantly different between treatments, and neither were the atherogenicity and thrombogenicity indexes. Overall, the results indicate that the best growth performance was observed in meagre fed the 50L18 diet, but with excessive mesenteric fat deposition in the abdominal cavity.
A study was undertaken to evaluate the effects of replacing fishmeal and/or fish oil in a practical formulation for juvenile meagre with a complementary mixture of plant proteins and vegetable oils, in terms of growth performance, nutrient utilization, intestinal structure and functionality and several haematological stress indicators. Twelve homogenous groups of 75 meagre (mean initial body weight: 55.4 ± 3.5 g) were fed one of four isonitrogenous (51% DM), isolipidic (17% DM) and isoenergetic diets (22.9 MJ·kg⁻¹ DM) over 88 days. A FMFO diet was formulated with high levels of marine protein sources (45%) and fish oil as the sole fat source. Based on this formulation, 60% of fish oil was replaced by a blend of vegetable oils (diet FMVO). Two other diets were formulated with a 50% replacement of marine proteins by plant protein sources. One of these plant protein-rich diets contained fish oil as the sole fat source (PPFO), while in the other 60% of fish oil was replaced by a blend of vegetable oils (PPVO). Growth of meagre was not significantly affected (P>0.05) by the replacement of fishmeal and fish oil by ingredients of vegetable origin. Meagre fed the plant-protein rich diets (PPFO and PPVO) showed a significant enhancement (P<0.05) of feed efficiency and retention of protein, lipids and energy. Dietary lipid source had no effect (P>0.05) on FE, PER and nutrient retention. Dietary treatments had no effect (P>0.05) on the whole-body composition of fish. Dietary changes on both protein and lipid sources did not affect (P>0.05) intestinal morphology, activity of intestinal brush border enzymes and haematological stress indicators in fish. Meagre showed a good ability to cope with vegetable based diets.
There are several knowledge components enabling the use of alternative proteins for aquaculture. This information includes: proximate and nutrient compositions of a particular ingredient, and bioavailability of nutrients. Corn protein concentrate (CPC), a co-product, comes from wet milling industry and is now available for aquaculture. CPC and its diets at varying inclusion levels (0, 10, 20, 30, and 40%) were evaluated in juvenile rainbow trout. We focused mainly on (i) the growth response, (ii) apparent digestibility coefficients (ADCs) of nutrients in CPC and its diets, and (iii) amino acid distribution in plasma at different times in the post-absorptive period.

Although, CPC has higher protein content than CGM, it appears to have lower nutrients bioavailability. We found that increasing CPC in the fish diet above 30% resulted in linearly reduction of growth, digestibility of protein and some indispensable amino acids, as well as amino acid bioavailability ($P < 0.05$). The differences in concentration and peak of plasma amino acid normally reflect the differences in quality of the protein in term of amino acid bioavailability. A close relation between plasma amino acid concentrations and amino acid digestibility was observed ($P < 0.05$). Majority of plasma amino acids of fish fed CPC diet showed peaks at 24 h post-absorption. The inclusion of CPC in the fish diets resulted in increased hepato-somatic index (HSI), as well as elevated levels of some plasma enzymes, such as GPT, CK, and CRE ($P < 0.05$). High plasma levels of plasma enzyme conjunction with decreased values for the somatic indices might indicate possible damaging effects of high dietary CPC levels on the liver in rainbow trout. As with many other corn co-product meals, CPC was good digestible protein; however, the application of CPC required the blending of some other protein and amino acid supplements to balance the nutrient requirement of the fish.
Cloning and Characterization of SREBP-1 and PPAR-α in Japanese Seabass Lateolabrax Japonicus, and Their Expressions in Response to Different Dietary Fatty Acid Profiles

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Sterol regulatory element-binding protein 1 (SREBP-1) and peroxisome proliferator-activated receptor α (PPAR-α) are the key lipid metabolism regulating transcription factors in eukaryotic cell. In this study, SREBP-1 and PPAR-α like cDNA were cloned and characterized from the liver of Japanese seabass (Lateolabrax japonicus) by homology cloning with degenerate primer and RACE techniques. The full-length of SREBP-1 cDNA was of 3797 bp, including an open reading frame (ORF) of 3594 bp encoding a polypeptide with 1197 amino acids, and the 1895bp long PPAR-α cDNA encodes a polypeptide with 477 amino acids. Gene expression analysis via real-time quantitative polymerase chain reaction revealed that the SREBP-1 and PPAR-α like transcripts were widely distributed in various tissues, with high expression levels of SREBP-1 in blood, brain, and eye, and high expression levels of PPAR-α in liver and eye, compared to other tissues. A 10-week feeding trial using juvenile fish (29.53±0.86 g) was conducted to investigate the effects of different dietary fatty acid profiles on gene expressions of SREBP-1 and PPAR-α. Six diets were prepared, each with a characteristic fatty acid: Diet PA: C16:0, Diet SA: C18:0, Diet OA: C18:1n-9, Diet LNA: C18:3n-3, Diet N-3 LC-PUFA: DHA+EPA, and Diet FO: the fish oil control. The results showed that the liver expression level of SREBP-1 significantly ranked as followed: PA, OA>SA, LNA>N-3 LC-PUFA>FO, while the PPAR-α transcript levels in liver of fish fed N-3 LC-PUFA was significantly lower than those in fish fed other diets, suggesting that dietary fatty acid profiles significantly regulated the gene expression of SREBP-1 and PPAR-α.
Palm oil (PO), rapeseed oil (RO), soybean oil (SO) and linseed oil (LO) replacing the same fish oil (FO) level in diets (with required n3 LC-PUFA) of juvenile turbot (scophthalmus maximus L) were to investigate the effects of dietary fatty acids composition on growth performance, plasma biochemical parameters, lipid deposition and hepatic lipid metabolism related genes expression. Fish (9.49(SEM 0.03) g) were fed diets to apparent satiation for 12-week. The results showed that specific growth rate, feed efficiency and apparent net protein utilization of turbot were independent of dietary fatty acids composition. Fatty acid composition of liver and muscle had a significant difference among dietary treatments, and closely correlated well with that of diets. Plasma triglyceride in SO group was significantly higher than that of RO group. Plasma high density lipoprotein cholesterol and low density lipoprotein cholesterol in PO group were significantly lower than those in FO group. Lipid content of liver in RO group and LO group was significantly higher than that in PO group and FO group, and the activity of hepatic glucose-6-phosphate dehydrogenase and malic enzyme had a reverse trend. In RO group and LO group, relative expression of lipoprotein lipase, liver X receptors and apolipoprotein B-100 were significantly higher than those in FO group, while relative expression of peroxisome proliferator-activated receptor α and fatty acid synthetase were significantly lower than those in PO group. Relative expression of microsomal triacylglycerol transfer protein in LO group was significantly higher than that of FO group and SO group. There were no significant differences in relative expression of sterol regulatory element-binding protein 1, carnitine palmitoyltransferase I and hepatocyte nuclear factor-4α among dietary treatments. These results suggested that growth and feed utilization of turbot, on basis of obtaining required dietary n3 LC-PUFA, were not significantly affected by dietary fatty acids composition. The increase of lipid deposition in liver of turbot fed RO and LO diets would be related to the up-regulation of fatty acids synthesis-related gene.
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EFFECTS OF DIETARY N-3 HIGHLY UNSATURATED FATTY ACIDS (HUFA) ON GROWTH, FATTY ACID COMPOSITION, DIGESTIVE ENZYMES AND FATTY ACYL Δ6 DESATURASE EXPRESSION OF ORANGE-SPOTTED GROPER (EPINEPHELUS COIOIDES) LARVAE
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The effects of dietary n-3 highly unsaturated fatty acids (HUFA) on growth, fatty acid composition, digestive enzyme activities and fatty acyl Δ6 desaturase expression were investigated in grouper larvae, Epinephlus coioides. The larvae were fed each diet six times daily to apparent satiation with graded levels of n-3 HUFA (0.52, 0.94, 1.57, 1.97 and 2.43%) for 4 weeks. Results showed that the specific growth rate (SGR) of larvae fed the diet with 1.97% n-3 HUFA was significantly higher than other treatments. Activities of pepsin and lipase were significantly higher in the treatment with 1.97% n-3 HUFA. The activity of trypsin increased significantly with increasing dietary n-3 HUFA, while the activity of amylase decreased significantly. The fatty acid composition of the grouper larvae was highly reflective of dietary fatty acid profile. When expressed in yeast, the putative Δ6 desaturase of orange-spotted grouper showed activities of Δ6 desaturase and Δ8 desaturase. The expression of Δ6 desaturase significantly increased with n-3 HUFA from 0.52% to 0.94% and then decreased significantly. This result strongly suggested that exogenous n-3 HUFA was needed to maintain normal grouper development, and dietary n-3 HUFA could significantly affect growth, fatty acid composition and development of digestive tract. Moreover, dietary n-3 HUFA could regulate the biosynthesis of HUFA through influencing the expression of Δ6 desaturase and Δ6 desaturase is the rate-limiting enzyme in HUFA biosynthetic pathway of grouper due to its low activity. This may contribute to understanding the effects of n-3 HUFA on physiological functions and the mechanism of HUFA biosynthesis.
Molecular Cloning and Developmental Profile of Cholecystokinin (CCK) and Effect of Size-Fractioned Fish Protein Hydrolysate on Transcription of CCK, Trypsin and TOR in Large Yellow Croaker (Larimichthys crocea) Larvae

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Previous study in our laboratory has found that permeate after ultrafiltration of fish hydrolysate (PUFH) replacing 40% of fish meal (FM) was beneficial to growth and development of large yellow croaker (Larimichthys crocea) larvae, however it is still unclear about the mechanisms involved. Thus, this study was conducted to investigate the mRNA expression of CCK, trypsin and TOR in response to dietary fish protein hydrolysate (FPH) in large yellow croaker larvae and in order to fully understand the role of CCK, we also cloned the full length of CCK cDNA and investigate its molecular ontogeny. The control diet (FM) was produced using fish meal as the only protein source, and three isonitrogenous (60% crude protein) and isolipidic (14% crude lipid) diets were formulated by replacing 40% of fish meal with three different size-fractionated FPH (retentate after ultrafiltration of fish hydrolysate or permeate after ultrafiltration of fish hydrolysate or non-ultrafiltered fish hydrolysate), which were named RUFH-40, PUFH-40 and NUFH-40, respectively. Each diet was randomly allocated to triplicate groups of larvae (3.15±0.15mg, 15 day after hatch (DAH)) for 30 days. In the current study, a 859 bp full-length cDNA sequence of CCK gene was obtained by RT-PCR and rapid amplification of cDNA ends methods. Results showed that transcription of CCK increased gradually during the first 11 days, then increased significantly by about 4.0-fold from 11 to 15 DAH and thereafter was down-regulated slightly from 15 to 25 DAH. Afterward, the mRNA level of CCK decreased abruptly by about 2.7-fold from 25 to 30 DAH and remained stable until 40 DAH. Transcription of CCK and trypsin of experiment fish in PUFH-40 was comparable with that in FM but was significantly higher than that in RUFH-40 and NUFH-40 (P<0.05). No significant differences were observed in TOR gene expression of larve among four dietary treatments (P>0.05). Results the present study suggested that PUFH replacing 40% of fish meal could benefit growth and development of large yellow croaker by promoting transcription of CCK and trypsin.
THE USE OF NEAR INFRARED SPECTROPHOTOMETRY (NIRS) AND SPECIES-SPECIFIC IN VITRO PROTEIN HYDROLYSIS TO PREDICT THE QUALITY AND APPARENT NUTRIENT DIGESTIBILITY OF FEED INGREDIENTS FOR SHRIMP, LITOPENAEUS VANNAMEI

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Near infrared spectroscopy (NIRS) is a tool that can be used for process control, quality assessment, identification of raw materials and process by-products to ensure proper ration supplementation, control diet formulation, and optimize least-cost formulation. NIRS provides the opportunity to rapidly and non-destructively predict crude and digestible composition values prior to diet preparation. The predictive accuracy of NIRS relies heavily upon obtaining a calibration set, which represents the variation in the main population, accurate laboratory analyses and the application of the best mathematical procedures. The objective of this study is to develop a rapid control tool to assess the quality of feed ingredients using species-specific in vitro protein digestion as reference data to develop the NIRS calibrations.

A web-based questionnaire was submitted to animal feed companies in Brazil and selected research institutions engaged in animal nutrition and feed analysis in order to assess the actual panorama of the applications of NIRS in feed manufacturing. Results from this survey indicated that NIRS has been used extensively by the resident commercial animal feed manufacturing sector in Brazil to measure the nutrient composition of raw materials throughout the aquafeed industry. A preliminary assessment using NIRS to predict the in vitro (pH-stat) degree of protein hydrolysis (DH) with standardized shrimp hepatopancreas enzymes extracted from farmed animals (L. vannamei, juvenile) was carried out with 34 samples of soybean meal (low and high protein content), obtained from main producing countries. Correlation between the original DH results and NIRS predictions were generated by the calibrations spectra, which were measured in triplicate (n=102). Both multiple linear regression (MLR) and partial least square regression (PLS) were performed to analyze data to create the best calibration equations. The preliminary study indicated that it is necessary to include more spectra, in order to fill the entire calibration range, generating more robust and reliable models, moreover, achieve better results of correlation (r) and standard deviation (Sdev). Nevertheless, the results obtained with DH were interesting in terms of standard deviation (0.04), correlation (0.96) and sum of squared errors (0.21). NIRS can bridge the gap between advanced scientific knowledge and application in the field, improving the accuracy of feed formulation by considering additional quality criteria as hydrolysable protein in feeds.

Ongoing research includes a representative population of different feed ingredients (fishmeal, poultry by product meal, meat and bone meal, soybean meal, and wheat flour, n = 120 for each raw material) sampled from aquafeed manufacturers to be further assessed in vitro (DH) as reference to develop reliable NIRS calibrations.

The analysis of gene expression can evaluate the response of an organism to nutritional, environmental and reproductive management changes. The objective of this study was to evaluate the influence of a probiotic composed of *Bacillus subtilis* in female and male tilapia broodstocks on the gene expression of five genes related to reproduction (IGF1, IGF3, OMV4, βLH and βFSH). The experiment was conducted at APTA- São Paulo Agrubusiness Tecnology Agency - Pirassununga, SP/Brazil and had duration of 110 days. One hundred and eight females and 48 males (527.65 ± 185.98g and 30.16 ± 3.57cm) were used. The three treatments were used; T0 - control (without probiotic), T1 – continuous intake of probiotic and T2 – alternate intake of probiotic (7 day diet with probiotic and 7 days without probiotic) at a dose of 0.5 g kg⁻¹ of feed (10¹⁰ CFU g⁻¹). To quantify gene expression by Q-PCR, the liver were removed from experimental fish (male and female independently), and immediately conserved in RNA later®. The organs were homogenized and total RNA was isolated using the Gene JET RNA purification kit (Thermo scientific). Total RNA (1 ug) from each sample was reverse transcribed for converting RNA into DNA using the iScript cDNA Synthesis kit (Bio-Rad). Real-time PCR analysis carried out using an iCycler (Biorad). The amplification protocol used as follows: initial denaturation and enzyme activation for 7 min at 95 °C, followed by 40 cycles of 95 °C for 15 seconds, and 65 °C for 30 seconds. Comparisons between groups were made by one-way analysis of variance followed by Tukey's analyses (P < 0.05). Most of the literature shows that IGF1 and IGF3 genes related to reproduction and growth. The IGF3 expressed exclusively in the fish gonads but additionally, in this research, it was also detected in the liver. These two genes suppressed the expression in T2♀, while with the other treatments these genes expressed higher in both T1♂ and T2♂. The gene OMV4 is a sequence extracted from the vitellogenin gene of *Oreochromis massambicus*. The vitellogenin is a glycolipoprotien produced in the liver and carried to the ovary by blood stream. This gene expressed in all treatments, either males or females, being highest in the T1♀. Vitellogenin is considered to be a biomarker of estrogen exposure in males, young or anti-estrogen in female’s eggs. It is usually found in females, but the male fish may have induced hepatic expression of vitellogenin when exposed to environments contaminated with estrogenic substances. Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are gonadotropins produced by gonadotropic cells and play an important role in the regulation of gametogenesis and esterodogenesis, required for the development of sexual behavior and secondary sexual characteristics. These hormones are expressed in T1♀ and T2♀ and suppressed in T1♂ and T2♂. This behavior occurs in females due to reproductive cycle preparation. In males, these hormones may indicate the fish is already prepared for the next reproductive cycle and did not need produce more hormones. The use of *Bacillus subtilis* in tilapia broodstocks was efficient in enhance reproduction of the animals treated at doses used.

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SEASONAL VARIATION OF IMMUNE PARAMETERS IN FAT SNOOK (CENTROPOMUS PARALLELUUS) SUPPLEMENTED WITH PROBIOTIC BACILLUS SUBTILIS

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The aim of this study is to evaluate the respiratory burst and phagocytic activity of head kidney phagocytes of fat snook (Centropomus parallelus) supplemented with the probiotic Bacillus subtilis. For this proposal, 5756 fat snook fingerlings stocked in 12 cages of 2x2x1.8 meters, density of 36 fish m⁻³, from the lagoon-estuarine region of Cananéia-SP, Brazil were used. The experimental design was completely randomized, performing two treatments with four simultaneous replicates with the probiotic Bacillus subtilis, in doses of 5.0 g and 10.0 g /kg of diet plus the control group, totaling three treatments. All the animals were fed twice a day during seven (7) day intervals with probiotic and the remaining other seven (7) days with a normal diet without probiotic, over a period of twelve (12) months.

Bimonthly, nine fish per treatment were anesthetized, killed, head kidneys removed, macerated in RPMI-1640 medium (Roswell Park Memorial Institute), filtered and the cell suspension rich in phagocytes were counted and adjusted to 10⁷ phagocytes mL⁻¹. To measure respiratory burst, the phagocytes were set in plates of 96 wells and measured by the Nitro Blue Tetrazolium test through ROS (Reactive species of oxygen). To make the precipitate soluble a KOH and DMSO solution was used. The values were obtained spectrophotometrically on an ELISA at 630nm. Phagocytic activity was measured by placing 300μL of phagocytes solution and 300μL of Saccharomyces cerevisiae yeast solution at a concentration of 5.10⁷ yeast /mL, in a 20x20mm coverslip, covering all wells of plate. After 8 hours incubation, the coverslips washed and the adhered phagocytes counted using a phase contrast microscope, to determine the phagocytic capacity (PC) and the phagocytic index (PI). Only during spring period, did the animals of the 5.0 g and 10.0 g of probiotic diet treatments, have values of phagocytes respiratory burst statistically higher than those one of the control. In the fall, regarding to the PC, the animals in the 10.0 g treatment had presented values statistically higher than either the control or the 5.0 g treatment. It is suggested that the probiotic Bacillus subtilis, when included in the diet at concentrations of 5.0 g and 10.0 g /kg of diet works as an immunostimulant in fat snook, in intensive rearing system, increasing the resistance to pathogens that affects the animals, considering periods when survival mechanisms are compromised.

* CNPq supporting Proc nº 559760/2009-4
In most fish hatcheries in Europe an in-house developed feeding protocol is applied in which the ratio between Artemia consumption and dry feed consumption has been altered multiple times during the last two decades. During the last 15 years all changes made to the protocol were in favour of the amount of dry feed, reducing the total amount of Artemia consumed per million fry from 300 kg/ million fry to an average consumption of 105 kg/ million fry today. The reduction of Artemia consumption coincided with improved culture conditions and improved larval survival resulting in fry production with similar quality characteristics. The trend to replace even more Artemia is still ongoing, however at a much slower pace as hatcheries learn understood from the latest experiences that they might face problems in terms of fry quality and survival if not well managed. Therefore, there is still room for improvements and hatcheries can further optimize their own in-house feeding schedule, taking into account the benefits and constraints of both live feed and commercial dry feeds. Further improvements on the dry diets and feeding protocol are the driving force of the best balance concept.
POSTER 124
THE EFFECT OF EMODIN ON CYTOTOXICITY, APOPTOSIS AND ANTIOXIDANT CAPACITY IN HEPATIC CELLS OF GRASS CARP (CTENOPHARYNGODON IDELLUS)
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Presented by Mingchun Ren²

We determined the effect of emodin on the cell viability, lactate dehydrogenase (LDH) release, superoxide dismutase (SOD), glutathione (GSH), total antioxidant capacity (T-AOC), reactive oxygen species (ROS), mitochondria membrane potential (ΔΨm), and apoptosis in the hepatic cells of grass carp (Ctenopharyngodon idellus). Cultured cells were treated with different concentrations of emodin (0.04–25 µg/mL) for 24 h. We found that the cytotoxic effect of emodin was mediated by apoptosis, and that this apoptosis occurred in a dose-dependent manner. The emodin-induced apoptosis was accompanied by ΔΨm disruption and ROS generation. SOD and T-AOC significantly decreased in the emodin-exposed cells. Thus, the oxidative effect of emodin may be attributed to the loss of the cell’s ability to maintain the activity of its radical-scavenging enzymes. GSH was also significantly higher after emodin exposure, indicating that cells failed to maintain their redox balance when compensating for the increased oxidative stress. Our results suggest that emodin exerts its cytotoxic effects via apoptosis by directly affecting the mitochondria.
The Pike Silverside Chirostoma estor (PISCES: Atherinopsidae) is a promising freshwater species for aquaculture and there has been a lot of effort to develop its culture. C. estor is a stomachless species with a short gut (0.29:1 alimentary tract length: standard length) and juvenile and adult fish have a filtration system typical of a zooplanktrophic fish (Ross et al., 2006). Although hatchery management and husbandry have been successfully developed and some research has been made to know the nutritional requirements of the species, it is necessary to do more research to optimise feeding strategies and diets for culture. When conventional formulated diets are used, juvenile fish have some problems in terms of growth and survival. Although the formulation of diets for the species has been improved during recent years (Martínez-Palacios et al., 2008), artificial diets still have not led optimum fish growth and performance. The short gut and the absence of a stomach in this species make difficult the development of appropriate diets, in terms of type of nutrients and ingredients. These anatomical digestive features suggest the need of easily digestible diets; the ability of juvenile C. estor to utilise complex proteins remains uncertain. In contrast with other species, when protein hydrolysates were used to replace whole proteins in juvenile C. estor diets, no positive effects have been obtained. Since this fish is zooplanktivorous and live feeds usually contain a large fraction of soluble intact proteins, it is possible that soluble proteins could be more available for digestion and absorption than are insoluble proteins for this species. An experiment was carried out to assess the importance of both solubility and the amino acid composition on the utilization of dietary protein by pike silverside juveniles (1.56±0.004 g initial weight). Five isonitrogenous diets (439 g Kg⁻¹ protein y 124 g Kg⁻¹ lipid) with different substitution levels of whole protein (Fish meal: FM) by soluble proteins (SP) were tested, and fish performance was evaluated. Results indicate an optimum proportion of soluble protein in the diet as 25–50% of total protein, with best juvenile growth, survival and feed utilization. Fish fed the diet with 100% FM showed the lower growth and survival followed by fish fed the diet with 90% SP. The amino acid composition of the different diets showed significant differences; in diet with 100% FM, Leucine, Lysine, Threonine and Histidine seem to be limiting, but Histidine and Arginine in diet with 90% SP. Then an intermediate proportion of SP is better than higher contents in the diets. Protein body composition was not significantly affected by the different diets. In conclusion, this study confirms that the form under which protein is supplied to juvenile C. estor and the related dietary amino acid composition significantly affects fish performance in juvenile stages, similar to that found in larvae of other species.

Balanced diet constitutes approximately 50 % of costs of production in the aquaculture production units. In the intensive systems, the food provides 100 % of the nutritional requirements of organisms, generating a high dependence of the fishmeal that is the principal ingredient used in the production of balanced diet. An alternative is the use of vegetable ingredients and the use of technologies as the culture with biofloc, as food complement, this strategy helps minimize the unload of nitrogen to the environment and lowers considerably the water refills (Becerra-Dórame et al., 2012). The objective of this study was to determine the effect of substitution of fishmeal (0, 10, 20 and 30% FM) with vegetable ingredients, in the water quality and growth of tilapia (*O. niloticus x O. mossambicus*) cultivated in seawater and biofloc. Circular tanks of plastic (250 L) were used, with constant aeration, 2 kg/m³ of initial biomass and initial weight of 16±2 g. Growth assay had duration of 60 days. Every day the temperature, dissolved oxygen and pH were measured, ammonium and solid suspended total (SST) were measured weekly. The temperature was maintained in 25.30±0.06 °C, the dissolved oxygen was of 4.58±0.04, and pH of 6.47±0.01, during the experiment, without presenting significant differences (p>0.05) between the treatments. The level of ammonium was significantly lower in the treatment with 30 % of FM (0.40±0.08 mg/L), whereas the SST were significantly high with 0 % of FM (248.1±31.7 mg/L). The survival was high in all treatments varying from 83 to 100 %, no significant differences (p>0.05) were founded between treatments. The final biomass was higher in the treatment with 10 % FM (7.1±0.1 kg/m³) and the minor was with 30 % FM (5.8±1.0 kg/m³), without significant differences between the averages of the treatments. Feeding conversion rate (FCR) was of 1.2 with 10 % FM and 1.7 with 30 % FM, without significant differences (P>0.05). The results of the present study indicates that it is possible to substitute completely the fishmeal with vegetable ingredients, in the balanced diet for tilapia (*O. niloticus x O. mossambicus*) cultivated in seawater, using biofloc as food complement.

Due to small size of microalgae and low concentration in the culture medium, cost-efficient harvesting of microalgae is a major challenge. In the present study, the possibility of using electro-flocculation technique (direct current) for harvesting of microalgae Nanochloropsis oculata biomass from the culture broth was investigated. The microalgae biochemical composition and chlorophyll a content, and viability of N. oculata cells were determined on concentrated microalgal cells after 10 days of electro-flocculation. The results from the biochemical analysis and chlorophyll a measurements indicated that N. oculata flocculated cells showed no sign of degradation in biochemical composition and chlorophyll a from electro-flocculation as compared to control. Moreover, the results clearly showed that flocculation efficiency improved with increasing direct current. Between 3-10 V; the flocculation efficiency was as high as 85-97.0%. The highest flocculation efficiency, 97.0%, was obtained at 10 V, of 30 minutes and with short settling time of 2 hours. The cell viability obtained in flocculation with direct current (3-10 V) showed cells in good shape and indistinguishable from non-flocculated cells when viewed by light microscopy. This study demonstrated that electro-flocculation is an effective method to flocculate N. oculata cells; therefore, it could be considered as a cost-effective alternative for preparing microalgal concentrates for the aquaculture industry and hatcheries.

The aim of this study was to evaluate the effect of lysine in productive and reproductive performance, hormonal characteristics and body indices of male breeders of Rhamdia voulezi kept in cages. A population of 400 fish was used with an average initial weight of 35.18±0.25 g, identified with tags and distributed in a completely randomized design with four treatments and four replications in 16 cages with 25 fish each, over a period of 185 days. The treatments consisted of four diets prepared to contain lysine levels of 1.20 (T1), 1.40 (T2), 1.60 (T3) and 1.80% (T4) with 30% crude protein and 3,500 kcal kg⁻¹ digestible energy. During the reproductive period, 18 males were randomly selected for treatment. Samples of semen were liberated with compression of the abdominal region and taken to the laboratory where were weighed (g), measured (cm), hypophysation (with crude pituitary extract carp (EHC)) at a dosage of 2.5 mg.kg⁻¹ of the fish. After a period of 240 accumulated thermal units, semen was collected for the analyses of the following parameters: semen volume, duration and rate of sperm motility, sperm survival, sperm concentration, percentage of normal sperm, pH and osmolarity. Then, the blood was collected (analysis of cortisol and testosterone) using syringes, after, the animals were sacrificed by cervical column dislocation, dissected where the testicles, liver, fat and viscera were weighed and their estimated body indices. For productive performance it was observed effects (p<0.05) for the average final weight, weight gain, feed conversion and condition factor were observed, the level of 1.80% lysine which provided the greatest weight gain (52.62±19.33 g) and lower feed conversion (0.95±0.19). Regarding reproductive performance only seminal volume was affected (p<0.05) and greater semen volume (3.81±0.98 mL) was observed in T4. Cortisol concentrations were not significantly different among treatments (p>0.05). However, a significant quadratic effect was found (p<0.05) for testosterone. No significant effects on body indices were found (p>0.05). In conclusion the diet containing 1.80% total lysine increased weight gain, seminal volume and decreased feed conversion.

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Snakehead (*Channa striata*) is a valuable food fish cultured in Thailand. Farms generally produced their own dry-wet mixtures feed on-site each day for snakehead. Farm-made feed is combinations of dry pelleted feed (i.e. commercial feed) and a wet meal containing a variety of feed ingredients, including trash fish, in the vicinity of farms. Snakehead production is mainly integrated agriculture-aquaculture farming system (i.e. pig-fish farming). Removal of organics in wastewater can be done by using the natural property of microalgae to sequester CO$_2$ and purify nutrient-rich waste streams. Application of microalgae can be used for any wastewater having BOD less than 500 mg/L (i.e. pig-fish farming, BOD < 10 mg/L), unless the wastewater contains inhibitory chemicals and metals. *Spirulina platensis* and *Nostoc commune*, for example, ubiquitously present in the sedimentation pond, consume organics as food to grow and reproduce, in suitable conditions. Small scale microalgae production for integrated agriculture-aquaculture system probably appeared to be applicable for snakehead production.

The dry-wet mixtures feed was prepared using pond-cultured microalgae, *S. platensis* and *N. commune* as a protein supplement for juveniles Snakehead. Commercial fish feed was used as a reference diet. The experimental diets were made by graded inclusion levels of each cultured microalga at 10 and 20% in the reference diet. 800 fish were stocked and acclimated under laboratory condition using a static water system containing fifteen plastic cages (95×95×85 cm$^3$) in cement rectangular tanks (1×2×1 m$^3$). Juveniles (approximately 1.3 cm fish$^{-1}$ of body length; 1.5 g fish$^{-1}$ of body weight) at the density of 30 fish per cage were selected and distributed into the experimental units. Experimental fish were hand-fed at 5% total body weight twice a day (0900 and 1600). Water quality parameters were checked three times per week after the first feeding. Those parameters were kept within optimal range for freshwater fish; temperature 27.5–28.0 °C, dissolved oxygen (DO) 5.0–6.5 mgL$^{-1}$.

This study, all the fish were restricted-fed by the experimental diets. The optimum inclusion level of *S. platensis* and *N. commune* were 20 and 10%, respectively. An increased of *S. platensis* levels improved the growth responses of fish (i.e. FCR, SGR, and PER), while increased *N. commune* levels negatively affected the growth response of fish. *S. platensis* and *N. commune* did not influence the survival rate of fish. The carotenoid content significantly increased with increasing *S. platensis* and *N. commune* levels. Additional studies with isoproteic and energetic diets are further recommended to confirm the effect of these culture-microalgae on snakehead.
The use of high energy diets as a means to reduce the feed conversion ratio (FCR) and improve the growth performance has been widely documented, particularly in salmonid species. While digestible energy can be derived from either oil or protein (and to a limited extent by carbohydrate), oil contains approximately 60% more of the energy density per kilogram compared to protein. It therefore makes sense to derive as much energy as possible from oil as opposed to protein.

Barramundi (Lates calcarifer) is an increasingly significant aquaculture species farmed in many parts of the world. Commercial diets for the species are traditionally of relatively low energy content. This presentation will show research that demonstrates the merits of high energy diets in barramundi, whereby both fish growth and feed conversion are vastly improved, with an overall benefit to the sustainability of the farming operation.

Three feed trials (two semi-commercial and one experimental), each lasting between three and five months, were conducted in barramundi of larger than 1 kg starting weight. Diets of varying energy content (in this case varying fat content) were evaluated for their effects on barramundi performance at different growing conditions (optimal and sub-optimal temperatures). In all cases, the higher energy feeds resulted in significantly improved FCR and growth, with feed conversion reduced by up to 14% and growth increased by up to 18%.

Additionally, barramundi flesh was analysed for its composition with particular emphasis on fat content and fatty acid composition of fish fed high fat diets.

These studies highlight that, similar to work undertaken in salmonids, barramundi respond positively to increased energy density.
The development of aquaculture and animal feed industry in the last decades has increased the demand and competition for feed ingredients, which in turn has pushed up prices and reduced availability for many critical ingredients, such as fishmeal. The evaluation of feed ingredients is important to formulate compound diets that are cost-efficient, highly digestible and that provide good growth and feed efficiency. The most common method employed by researchers and feed companies is the use of in vivo digestibility techniques involving a growth performance assay. Nevertheless, such tests require long periods of time for completion, the use of large number of animals, and the use of expensive purpose built experimental rearing facilities. In vitro techniques are rapid, safe and do not have the ethical restrictions of in vivo methods. There is no information previously collected and compiled about in vitro methods and if their characteristics are suitable for the evaluation of ingredients for aquaculture. The aim of this study was to review and evaluate the in vitro methods used in the evaluation of feed ingredients for aquaculture.

In vitro digestion methods for aquatic organisms are mainly based on the methods already developed for terrestrial animals. From the early studies in the mid eighties to date about 65 studies have been published, of which 45 use fish, 16 crustaceans and 4 mollusks. The most commonly used species are shrimp (Litopenaeus vannamei) and salmonid fish (Oncorhynchus mykiss). The primary target of the majority of the studies is the nutritional quality of proteins, whereas very few study carbohydrates (production of reducing sugars) lipids (release of 14C-labelled) and minerals (release of phosphorus). In vitro digestion methods has been carried out using open or closed bioreactor systems, species-specific or commercial enzyme sources, and measuring different end-products (released aminoacids by o-phthaldialdehyde method, reactive amino groups by TNBS* and HPLC*; protein in the residue by Kjeldahl or SDS-PAGE*; number of peptide bonds cleaved by pH stat and pH drop). Simple digesters by changes in the pH have been used on nearly half of total reported nutritional studies. Simple digesters reproducing one step by mixing enzymes with the substrates and measuring end products or undigested substrate have been also used to a great extent. More complex configurations have been used less frequently. Combination with other measurement methods may improve prediction of ingredient quality. Some issues still need investigating before the in vitro digestion method can be routinely used by the aquafeed industry as a tool to assess raw materials.

*TNBS (trinitrobenzensulfonic acid hydrate)
*HPLC (High-performance liquid chromatography)
*SDS-PAGE (sodium dodecyl sulfate, polyacrylamide gel electrophoresis)
POSTER 132
DIETARY PHOSPHOLIPID TYPE AND LEVEL IN MICRODIETS FOR GILTHEAD SEABREAM LARVAE: EFFECTS ON OXIDATIVE STRESS AND HISTOLOGICAL CHANGES IN MUSCLES, INTESTINE, AND LIVER
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²Group of Investigation of Aquaculture, University of Las Palmas, Las Palmas, Spain

The objective of the present study was to determine the effect of dietary marine phospholipids (MPL) obtained from krill and soybean lecithin (SBL) on the larval performance, oxidative status and histological changes in muscles, intestine, and liver in seabream larvae. Larvae were fed from 16 to 44 day post hatching five formulated microdiets with three different levels of phospholipids and two different types (Control, 7MPL, 9MPL, 7SBL and 9SBL %). Larvae fed MPL showed a higher survival, stress resistance and growth than those fed SBL and 0KPL, regardless the dietary PL level. Inclusion of SBL markedly increased the peroxidation risk as denoted by the higher thiobarbituric acid-reactive substances in larvae, as well as a higher expression of antioxidant enzymes genes such as catalase, glutathione peroxidase, and superoxide dismutase, which may in turn increased incidence of muscular lesions in the larvae fed dietary SBL. Inclusion of SBL was associated with a relative accumulation of lipid droplets in the supranuclear zone of enterocytes compared to larvae fed dietary MPL. While, dietary MPL supplementation prevented the accumulation of lipids droplets in the hepatocytes (liver steatosis) and resulted in larger hepatocyte size compared to dietary SBL, which in turn improve the lipid utilization efficiency and ultimately larval performance. Finally, increasing dietary MPL or SBL lead to a better assimilation of polyunsaturated fatty acids in the larvae, n-3HUFA (especially DHA and EPA) or n-6 fatty acids (especially 18:2n-6), respectively. In conclusion, inclusion of MPL was more effective than SBL to improve larval performance, oxidative status and lipid utilization. Besides, MPL reduced the incidence of muscular lesions and prevented the intestinal and liver steatosis.
Aquaculture production is an important source of protein for the human consumption, since it represents 41% of the production worldwide. The principal cost of production is the balanced diet, which represents up to 60% of total production costs. Fishmeal is the principal source of protein in many balanced diets and in recent years its availability has diminished, causing an increase in the costs of formulating a balanced diet. Consequently it is necessary to search for alternative ingredients that allow the replacement of fish meal (Muzinic et al., 2006). The objective of the present work was to evaluate the effect on the postprandial oxygen consumption and growth of tilapia cultivated in seawater using four experimental diets with 0, 10, 20 and 30% of fishmeal and a commercial diet. The fish were acclimatized in tanks of 5000 L at 28 °C, increasing gradually the salinity with seawater a rate of the 10 g/L every 24 h, up to obtaining 35 g/L. For the bioassay of growth the fish were selected (7.7 ± 0.2 g) in tanks of 60 L, and were allocated at random one each of the experimental diets. The experiment lasted 60 days. Postprandial oxygen consumption was determined using a closed system as respirometer, where the water volume was kept constant. The fish were selected and placed at random (3.0±0.28 g). The results are expressed in mg O2/g/h dry matter. The information was analyzed by the software STATISTICA, using an ANOVA and Tukey’s multiple comparison of means test was used, when there existed significant difference between the averages (P<0.05). Table 1, shows the results of growth and postprandial oxygen consumption from tilapia fed with different levels of fishmeal. In the present work a significant effect (P<0.05) was found in the final weight and postprandial oxygen consumption by the diet, agree with other authors (El-Sayed, 1999) among the different diets.

Table 1. Final weight, survival, feed conversion ratio (FCR) and postprandial oxygen consumption of tilapia fed diets with different levels of fishmeal.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Final weight (g)</th>
<th>Survival (%)</th>
<th>FCR</th>
<th>Postprandial oxygen consumption (mg O2/g/h dry matter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial</td>
<td>22.1±8.3</td>
<td>80±10</td>
<td>ND</td>
<td>0.3899±0.08</td>
</tr>
<tr>
<td>0</td>
<td>17.6±2.1</td>
<td>83±6</td>
<td>1.8±0.4</td>
<td>0.4761±0.9</td>
</tr>
<tr>
<td>10</td>
<td>15.2±0.7</td>
<td>73±6</td>
<td>2.6±0.4</td>
<td>0.5095±0.10</td>
</tr>
<tr>
<td>20</td>
<td>17.0±5.0</td>
<td>73±12</td>
<td>1.9±1.0</td>
<td>0.4972±0.08</td>
</tr>
<tr>
<td>30</td>
<td>16.9±1.1</td>
<td>83±20</td>
<td>2.0±0.1</td>
<td>0.5251±0.11</td>
</tr>
</tbody>
</table>

The increasing interest in the culture of the seahorse (Hippocampus ingens), makes it necessary to increase the knowledge on the different aspects of its biology, supply and growth. This situation has led to the development of successful protocols of the culture of this species. The functional capacity of the digestive system to modulate its digestive enzymes and metabolic processes is a key step in determining the type of food that must be supplied to the larvae (Sandoval-Muy and Barón-Villa, 2007). This work describes the histology of the digestive tract and evaluates the digestive capacity of Hippocampus ingens, for its first month of life. Weekly 25 juvenile fish were collected from a commercial farm. A sample of these fish was used for the histology and another sample for the enzymatic activity. The entire fish, were homogenized in water, to obtain a proteic extract. The extracts were then analyzed for: soluble protein, trypsin-like activity using Benzoil-Arg-p-nitroanililne as substrate and amylase activity using corn starch as substrate. These analyses were undertaken at 0, 1, 2, 3 and 4 weeks. The digestive tract is described histologically in the first four weeks. It was found that enzymatic activity of the trypsin-like and amylase enzymes is present from the moment that they are liberated from the male brood pouch. This clearly supports that these fish have the capacity to digest substrates like proteins and carbohydrates. It was observed that in the first days of development, the activity of trypsin-like increases significantly (p <0.05), and that the activity of amylase diminishes significantly (p <0.05). Currently there are not previous studies that have evaluated the digestive capacity of H. ingens, but it is thought that this trend is similar in larvae of other salt-water fishes. Probably, this behavior is the result of the digestive system suffering important morphologic and physiological changes.

The observed results of this study are part of a larger project that is being developed in order to evaluate the use of lipids and carbohydrates at various levels in diets fed to juvenile tambaqui. The utilization of lipids and carbohydrates were evaluated by analysis of the means of productive performance, body composition, energy metabolism, and also isotopic turnover of nutrients in muscle, liver, and visceral fat tissues. 1,080 juveniles with an initial weight of 10.88 ± 0.13 g were utilized in this experiment and were distributed among 24, 450-liter tanks. The experiment was conducted with six treatments in a 2 x 3 factorial design which comprised of two levels of lipids (4% and 8%) and three levels of carbohydrates (41, 46 and 51%) in the diets. The fish were fed twice a day during an experimental period of 60 days. The results were subjected to an analysis of variance. When significant differences were detected, the averages were compared by the Tukey test at 5% probability.

With the increase in the level of lipids in diets (8%), there was a significant increase in the averages of protein, triglyceride and serum cholesterol. The highest means of the hepato-somatic index (HSI) occurred with the levels of 41% carbohydrates and 4% lipids. For the averages of the viscero-somatic fat index (IGVS), there was an interactive effect which showed that the 41% carbohydrate level and the 4% lipid treatment (mean 2.98 ± 0.31) differed from the treatments of 41%, 46% and 51% carbohydrate levels- all with the 8% lipid level (mean 4.11 ± 0.37, 3.97 ± 0.30, 3.87 ± 0.50, respectively). Therefore, greater visceral fat deposition occurred in animals which received the diet with 8% lipid composition. Muscle and liver tissues are also being collected for further analysis of glycogen and lipid compositions in tambaqui in order to evaluate the use of lipids and carbohydrates as protein substitutes in diets.
The observed results of this study are part of a larger project that is being developed in order to evaluate the use of lipids and carbohydrates at various levels in diets fed to juvenile tambaqui. The utilization of lipids and carbohydrates were evaluated by analysis of the means of productive performance, body composition, metabolic energy, and also isotopic turnover of nutrients in muscle, liver, and visceral fat tissues. 1,080 juveniles with an initial weight of 10.88 ± 0.13 g were utilized in this experiment and were distributed among 24, 450-liter tanks. The experiment was conducted with six treatments in a 2 x 3 factorial design which comprised of two levels of lipids (4% and 8%) and three levels of carbohydrates (41, 46 and 51%) in the diets. The fish were fed twice a day during an experimental period of 60 days. The results were subjected to an analysis of variance. When significant differences were detected, the averages were compared by the Tukey test at 5% probability.

With the increase in the levels of carbohydrates (46 and 51%) and in the level of lipids (8%) in the diets, a significant increase was shown with the averages of feed conversion ratio and the rate of protein efficiency. There was an interactive effect for the averages of specific growth rate, which differed statistically between the 41% carbohydrate and 4% lipid (mean 3.09 ± 0.29) diet, and the diets with 46% carbohydrate and 4% lipids and 8% lipids (averages of 3.66 ± 0.06 and 3.57 ± 0.22, respectively). Muscle, liver, and visceral fat tissues of tambaqui are also being collected for subsequent determination of the isotopic turnover of carbon ($\delta^{13}$C) and deuterium ($\delta^2$H) for the purpose of studying physiological assimilation of nutrients.
USE OF COLOURFUL FLOWERS FOR ORNAMENTAL FISHES

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For ornamental fishes, herbal based carotenoids are mainly derived from micro algae RED, yellow corn, corn gluten meal, marigold, Hibiscus Hallhock, red rose, red pepper extract and carrot are used for pigment development of ornamental fishes. The Use of herbs like neem buds in treatment of ornamental fishes is good as sugary form because of its impact of treatment. Neem is one of the most promising medicinal plants, all the time and ways it has wide spectrum biological activity and showed a glow in fishes. Apart from neem, jasmine flowers also attracts the fishes in bath treatment. The use of herbal medicines are more effective because they are safe, widely available and in expensive.

References:
The effects of delayed first feeding on survival and growth of laboratory-reared *Nibea albiflora* (Richardson) larvae were studied under controlled conditions for 15 days. Larvae began to feed exogenously at 3 days after hatching (DAH) and the point of no return (PNR) occurred between 5 and 6 DAH at 21-22°C. The experimental design included a conventional feeding regime with initial feeding from 3 DAH as a control, delayed first feeding for 4, 5 and 6 DAH. Morphometric characteristics (total length, head depth, body depth, eye diameter, musculature height, mouth diameter) were evaluated under different initial feeding time (3 DAH, 4 DAH, 5 DAH and 6 DAH). Larvae growth was significantly affected by the time of first exogenous feeding. *N. albiflora* larvae initiated first feeding at 4 DAH, 5 DAH and 6 DAH achieved comparatively lesser growth performance in all morphometric characteristics than that of 3 DAH at the end of the experiment. At 6 DAH, significant differences were observed between 0 day delayed first feeding larvae and 3 day delayed first feeding larvae for all morphometric characteristics except eye diameter and mouth diameter ($P<0.05$). However, there was no significant differences in total length, head depth, body depth, mouth diameter, and eye diameter between 0 day delayed first feeding larvae and 1 day delayed first feeding larvae ($P<0.05$). After 15 days rearing, significant differences in all morphometric characteristics appeared between 0 day delayed and 1 or 2 day delayed feeding and followed to the end of the experiment. Survival of the 0 day delayed first feeding larvae, 1 day delayed first feeding larvae and 2 day delayed first feeding larvae was 10%, 8% and 2% at the end of experiment, respectively, while no larvae survived up to 7 DAH for 3 days delayed first feeding larvae. The present study suggests that the first feeding of *N. albiflora* larvae should be initiated at 2 days after yolk exhaustion for achieving good growth and survival.

**Keywords:** *Nibea albiflora* (Richardson); growth; survival rate; delayed first feeding; the point of no return (PNR)
A 15-day growth trial was conducted to evaluate four kinds of diets with different combinations on spotted maigre, *Nibea albiflora*, larvae culture (from 15 to 30 days post-hatch, DPH). The diets used in present study were S-rotifer (*Brachionus* sp.) singly (T0) and substitution rotifer with Artemia (T1-T3), copepod (T4-T6) and microdiet (T7-T9) at three levels (25%, 50% and 75%), respectively. Results showed that larvae fed on rotifer solely achieved the highest survival rate and specific growth rate (SGR) at the first three days (15-18 DPH), but decreased sharply after then. However, larvae fed rotifer mixed with 50% copepod exhibited significantly higher survival, and larvae fed rotifer mixed with 75% copepod showed the greatest SGR at the end of experiment (30 DPH). The larvae fed diets containing microdiet at any level presented low survival throughout all stage. In conclusion, copepod is a suitable live food for spotted maigre and can substitute for Artemia, in addition, the optimum time for gradually weaning rotifer to copepod is about 19 DPH.

**Keywords:** *Nibea albiflora*; live food; microdiet; growth performance; survival
The objective of this study was to investigate the effects of partial and total replacement of fish meal (FM) with soy protein concentrate (SPC) in diets of juvenile black sea bream (Acanthopagrus schlegelii), supplementing with methionine and lysine. Six isonitrogenous and isoenergetic diets were prepared, of which FM was replaced by SPC at 0 (S1, control), 30 (S2), 47.5 (S3), 65 (S4), 82.5 (S5) and 100% (S6), respectively. Coated crystalline methionine and lysine of different ratio were added to diets S2~S6 to simulate the digestible essential amino acids (EAAs) composition of the control diet. Experimental diets were randomly assigned to six groups of juvenile black sea bream (5.50 ± 0.21g) for 8 weeks, with triplicate tanks per group, 20 fish per tank. Although negative effects were observed with fish in group S6 after the feeding trial, up to 82.5% of the dietary FM protein was successfully replaced without negative effects on growth performance and feed utilization (P>0.05). Higher moisture and lower lipid contents were recorded in the whole body and dorsal muscle of fish fed with 100% SPC replacement (P<0.05), with lower ash contents also recorded for whole body of fish fed S6 (P<0.05). Crude protein level and the EAAs profile in dorsal muscle were not affected by dietary treatment (P>0.05). Plasma total cholesterol level showed a decline trend as substitution level of SPC increased, and the concentrations in S4, S5 and S6 groups were significantly lower than that of the control group (P<0.05). Plasma aspartate aminotransferase and alanine aminotransferase activities were enhanced with increasing dietary SPC level (P<0.05). Black sea bream fed diets with high level of SPC also had higher superoxide dismutase and catalase activities compared to fish fed the control diet (P<0.05).

Keywords: Acanthopagrus schlegelii; soy protein concentrate; fish meal; replacement
An 8-week feeding trial was conducted to assess the protein-sparing capacity of dietary carbohydrate in juvenile black sea bream, Acanthopagrus schlegelii. Six isonitrogenous and isoenergetic diets were formulated by adjusting inclusion levels of corn oil and corn starch to contain various CHO/L (carbohydrate/lipid) ratios ranging from 0.81 to 2.81 with equivalent and sufficient n-3 high unsaturated fatty acids supplemented by fish oil. Each diet was fed to triplicate groups for 8 weeks. Growth performance (SGR and WG) and feed utilization (FE, PER and PPV) were significantly improved by CHO/L ratio (P...
Temperature has been considered as an abiotic master factor. Physiological processes of fish such as food consumption, digestion, immunity, etc. are influenced by water temperature. Temperature beyond the optimal limit of a particular species adversely influences fish health. Low environmental temperature showed profound immunosuppressive effects on freshwater fishes (Singh et al., 2013). The present experiment aimed to study the effect of selected amino acids and vitamins in freshwater carp *Catla catla* exposed to low temperature. *Catla catla* were fed with six experimental diets containing amino acids arginine, leucine and tryptophan and vitamins C and E. Diet without amino acids and vitamins served as control. Fish were acclimated at 25 °C and then exposed at 15 °C. Blood and tissue samples were collected. Various assays showed the positive effect of these amino acids and vitamins to overcome the cold stress in *Catla catla*.

References:
Protein ingredients vary considerably in their protein concentration. This makes it difficult to replace a high protein ingredient with one that contains a lower concentration. For example, fishmeal can contain in excess of 70% protein, while soy bean meal may contain 40 % and soy protein concentrates near 80%. In some experiments, diets are formulated with various combinations of soybean meal and soy protein concentrate replacing fishmeal. Another option that is frequently used in alternative protein experiments is the addition of an ingredient with a low protein concentration to the reference diet that contains the protein ingredient that is being replaced. In either case, the use of a third ingredient may make interpretation of the experimental results difficult due to possibility of ingredient interactions. This problem was pointed out by Ruohonen and Kettunen (2004, Aqua. Nutr., 10 (3): 145-151). In their work they discussed the utility of using mixture modeling to evaluate studies when three ingredients are varied. Unfortunately, their advice has not been widely adopted, so that the interpretation of many published studies is questionable.

I will present the results of a study that was designed as a $4^3$ factorial (fishmeal, wheat, and fish oil, each at four levels). I will discuss the advantages of using a mixture model analysis as opposed to a Three-way ANOVA.
The aquaculture sector faces great challenges due to consumers’ demand for high-quality, sustainable and competitively-priced products. While feeding constitutes a large share of the total costs (usually representing 20-50% of the operational costs), most commercial fish farms still apply suboptimal feeding formulations and regimes, which often result in poor feed conversion rates, increased feeding costs and deleterious effects in terms of environmental impact. Within this context, understanding fishes’ requirements and metabolic processing of essential amino acids and fatty acids is essential for an objective, evidence-based optimization of feeding formulations and regimes. Of particular importance is the study of the processes involved in conversion and retention of polyunsaturated fatty acids (PUFA), such as docosahexaenoic, eicosapentaenoic and α-linolenic acid, in fish, given that they constitute the major source of these nutrients for human nutrition and current trends in sustainable fish feed formulations (i.e. high replacement of marine ingredients for vegetable ingredients) are bound to affect fishes’ PUFA profiles.

The main objective of this work is to develop and validate a computational tool to predict the effect of changes in feed formulation, feeding regime and physical parameters (e.g. temperature) on downstream performance (e.g. growth, FCR, feed costs), environmental impact (e.g. total released nitrogen and phosphorous) and consumer-value parameters (e.g fatty acid profile of the edible portion) of reared fish. This dynamic simulation model is being designed and calibrated to model the physiological behavior of both gilthead seabream and rainbow trout in an aquacultural context. Specifically, it consists of a quantitative mechanistic model of fish metabolism where the complete set of metabolic fluxes is expressed as a system of ordinary differential equations (ODE). This model has been implemented in Modelica, using the OpenModelica modeling framework. At a high-level, it consists of a set of functionally distinct modules representing: 1) digestive processes; 2) protein metabolic processes; 3) lipid metabolic processes; and 4) central (i.e. energy-related) metabolic processes.

For this presentation, the results of preliminary simulations will be exposed and discussed, demonstrating the usefulness of such a numeric models for the optimization of feed formulations and practices towards, on one hand, an increase in the quality and nutritional value of fish products and, on the other hand, a reduction in the costs and environmental impact of aquacultural activities.
Animal by products (ABPs) in particular from poultry, feather and blood-products are considered valuable ingredients around the world and are therefore extensively used in aquafeeds. Because of its low price and high nutritive value poultry by-products have been extensively used in salmon feeds in Chile and Canada. So far the Norwegian aquaculture industry is not using ABPs because they fear the consumer’s perception.

Animal fats contain high levels of saturated fatty acids (SAFA), ranging from 28.5% in poultry fat to 47.5% in beef. Together with the high levels of monounsaturated fatty acids (MUFA), animal fats are good sources of dietary energy. Although vegetable oils (VO) are completely lacking in n-3 HUFA, animal fats are reported to contain these fatty acids (Moretti & Corino, 2008) though in limited concentrations.

Some studies have shown that animal fats when incorporated at no more than 50% of the dietary lipid level have no negative effects on fish growth performance as long as the EFA requirements are met (Turchini et al., 2003; Bureau & Gibson, 2004). However, the available information is too limited to establish the safety limits of replacement. The present study was therefore conducted to evaluate effects of increasing levels of poultry oil on growth performance and flesh quality of Atlantic salmon.

Three extruded isonitrogenous and isoenergetic diets (37% DM protein, 25kJ g\(^{-1}\) DM energy) were formulated: a reference diet containing fish and rapeseed oils (30:70) as lipid sources (Diet C) and other 2 diets in which the rape oil fraction was partially (Diet RO/PO) or totally (Diet PO) replaced by poultry oil. Triplicate groups of 80 fish with a mean initial body weight of 2.7 kg were randomly distributed and fed, ad libitum, the 3 experimental diets using automatic feeders for a period of 19 weeks. Increase in whole body mass and feed distributed were recorded in order to evaluate the effect of the diets on feed intake and growth parameters. Moreover, feed utilization, nutrient digestibility, flesh quality and sensory analysis were assessed.

At the end of the experiment mean body weight doubled in all treatments (4.9 to 5.1 kg) and no differences were observed on growth (TGC = 2.8 – 2.9), feed utilization (FCR = 1.01), PUFA digestibility, muscle total fat and pigments content, and flesh odour and flavour of salmon fed with the different dietary treatments.
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DIETARY PHOSPHOROUS SUPPLEMENTATION DURING FRESHWATER REARING REDUCES THE OCCURRENCE OF SPINAL DEFORMITY IN TRIPLOID ATLANTIC SALMON
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Currently reports indicate that triploid Atlantic salmon may exhibit poor growth during seawater rearing due in part to a higher incidence of vertebral deformities which may be nutritionally linked to freshwater feeding. Phosphorous (P) deficiency is a causative agent of malformation in diploids, however, few studies to date have examined triploid dietary requirements. In the first study we examined the effects of varying dietary P supplementation (A – low, B – medium and high total P) applied from first feeding to smoltification on growth, mineral retention, and vertebral pathologies in sibling groups of diploid and triploid smolts. Triploid high P diets initially showed faster growth to ~5g, but final smolt weight was significantly less than triploid fed a low P diet (45.6 vs. 48.6g). In contrast, diploids showed a consistently slower growth rate with increasing P supplementation. Prevalence of radiologically detectable vertebral deformities was higher in triploids than diploids, but decreased with increasing dietary P in both ploidy (Diploid: 70, 65.2 and 58% and Triploids: 98.6, 77.1 and 83.6% for diets A, B and C respectively). Furthermore, incidence of severely deformed individuals (>10 deformed vertebrae) remained low in diploids (0-1.5%) irrespective of diet, and decreased in triploids with increasing dietary P (10.1, 5.7 and 0% for diets A, B and C respectively). Triploids fed high P diets had the highest retention of whole body minerals (P, Ca, V and Zn), but all treatments did not differ in mechanical vertebral strength. Current findings would suggest that triploid Atlantic salmon require higher dietary P to support bone formation, reduce severity of pathologies and support their faster growth compared to diploids. It was also evident that there is a specific window in early freshwater rearing where elevated P has a beneficial effect, and a second experiment is currently being conducted which examines the duration of feeding P supplemented diets on spinal malformation. Triploid groups have been fed elevated P diets either from hatch to 5g, hatch to 20g or hatch to smolt. Results will be discussed in terms of triploid dietary P requirements and windows in relation to spinal development and environmental discharge, and work towards the development of specific triploid salmon aquafeeds.
COMPARISON OF NUTRITIONAL VALUE OF HYDROLYZED AND VITAL WHEAT GLUTENS IN EXTRUDED DIETS FOR RAINBOW TROUT

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An experiment to compare nutritional value of Vital wheat gluten (VWG) with hydrolyzed wheat gluten (HWG) in extruded diets for rainbow trout was carried out based on a 2²³ factorial model. Factors were dietary WG inclusion in extruded diets (providing 25 or 50% of dietary crude protein (CP), replacing CP from high-quality fish meal) and WG source (HWG, VWG or a 1:1 mix of the two). Each diet was fed to 0.4-kg rainbow trout in 2 tanks, for 8 weeks.

The use of HWG in diet resulted in decreased SME and torque during extrusion, and higher breaking force and lipid loss in uncoated pellets when compared to VWG. The fish nearly doubled their weights, and the feed conversion ratios (FCR) ranged from 0.8 to 0.9 g DM feed in take (g gain)⁻¹. No differences (P>0.05) were observed for growth rate, feed intake, FCR, retention of N, or organic material load to the environment per ton of trout produced.

Apparent digestibilities (AD) of nitrogen (N) and amino acids (AA) of both WG sources were high. AD of N and energy were higher in the VWG diets than in the two HWG diets. The AD of phosphorus was higher in the HWG diets than the gluten mix diets, while the AD of VWG diets was intermediate. The VWG and gluten mix diets gave significantly higher AD of total amino acids, Ile and Phe than the HWG diets. Retentions of gross energy and digestible energy intakes were significantly higher for the HWG diets than for the VWG diets and the gluten mix diets. The fecal N loss per ton of fish produced was low, and significantly lower for HWG diets than for VWG diets or gluten mix diets.

The AD of N was higher (P<0.05) for 50% inclusion than for 25%, while the opposite pattern was found for AD of energy and starch. For most essential amino acids, and for total amino acids, diets with 50% of CP from WG gave higher AD values than the diets with 25% inclusion. The diets with 25% of CP from WG resulted in higher retention of digestible energy intake than the diets with 50% of CP from WG.

In conclusion, both HWG and VWG were highly digestible, even in extruded feeds, and when supplemented with essential amino acids and taurine, both sources of gluten protein supported high feed intake, rapid growth and efficient feed conversion in rainbow trout.
RAPID ESTIMATION OF SPECIES-SPECIFIC DNA DIGESTIBILITY BASED ON DIFFERENTIAL QPCR

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Metagenomic analysis of fecal DNA has been gaining increasing attention in studying feeding ecology of various organisms in various habitats. This method, however, is based on an assumption that DNA digestibility is similar among various prey species, which has been questioned by some researchers. Thus, the present study was conducted to estimate/compare DNA digestibility of selected prey species using a novel qPCR-based methodology.

Digestibility of prey DNA was estimated by an in vivo feeding trial using six test diets. Each diet contained one of the following: (A) goldfish, (B) goldfish + citric acid, (C) goldfish + CaCO₃, (D) shrimp, (E) snail, and (F) goldfish + shrimp + snail. The diets were fed to rainbow trout and fecal samples were collected. Mitochondrial DNA was extracted from both diet and fecal samples, and quantified by qPCR using two sets of species-specific primers for each prey species. The first set of primers amplified a short stretch of DNA (51-80bp), whereas another set amplified a longer stretch (126-162bp). DNA digestibility was estimated based on the ratio between short and long amplicons using the following formula: Digestibility (%) = log((Mt-S)/(Mt-L))/(S/L) / (0.01×Mt), where Mt:16K (bp), S:length of short amplicon (bp), L:length of long amplicon (bp), [S]:relative quantity of short amplicon, [L]:relative quantity of long amplicon. DNA extracted from the Diets A-F were successfully quantified for all species tested by both long and short PCR. Fecal DNA, however, were more difficult to amplify, especially by long PCR. Calculated fecal digestibility of goldfish DNA was 2.07% (A), 1.12% (B), 1.95% (C), and -1.05% (F). Digestibility of shrimp DNA was 10.79% (D) and 12.61% (F). Digestibility of snail DNA was 1.88% (E) and 2.06% (F). These results suggest that the digestibility of dietary DNA may be estimated based on the ratio between long and short fragments.
REPLACEMENT OF ANIMAL PROTEIN WITH VEGETABLE PROTEIN IN THE DIETS OF ASTYANAX ALTI PARANAE

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The effect of replacing animal proteins (AP) with vegetable proteins (VP) on the production performance of Astyanax altiparanae was assessed through an experiment using a completely randomized design (5 x 4). Experimental diets were formulated with increasing replacement levels of AP by VP (VP0, VP25, VP50, VP75, VP100; diet VP0 contained 100% animal protein and diet VP100, 100% vegetable protein). A total of 9,000 A. altiparanae (initial weight = 1.18 ± 0.12 g, initial mean length = 2.1 ± 0.3 cm) were homogeneously distributed in 20 net-cages of 1 m$^3$ and 5 mm mesh with a density of 450 fish m$^{-3}$. These net-cages were randomly placed in an excavated nursery that was 180 m$^2$, 1.5 m deep and had 10% water renewal per day. The experiment lasted a total of 63 days; at the end of this period, the total count and individual biometrics were taken from 20% of each experimental unit. The performance was evaluated using the variables of mean weight (WG), length (LT), survival (SU), feed conversion (FC) and biomass weight gain (BG), as well as the proximate composition of the fish. There was no significant variation between treatments for WG, LT, SU and FC. However, BG was negatively affected by the replacement of AP with VP. In practical terms, this result may be considered to be of little importance because the higher cost of AP diets does not justify the observed difference. The use of VP in the diets resulted in a greater lipid deposition in the fish, suggesting future exploration of A. altiparanae as a functional food.
Marine fish larvae have insufficient digestive capacity to handle complex ingredients in compound diets as compared to juvenile fish. This study investigated the effects of incorporating fish protein hydrolysate (FPH) and exogenous enzyme, Pancreatin®, in the usage of two carbohydrate sources in microbound diet for Asian seabass. Enzyme activity and mRNA level of the main digestive enzymes were examined in parallel with the nutrient utilization. The FPH products were incorporated into the diets to replace fish meal (FM) protein at 0, 6.25 and 12.50%. At each FM replacement level, either dextrin or wheat flour was used as a carbohydrate source, and Pancreatin® was supplemented at 0 and 0.05% of diet. Seventeen day-post hatch larvae in triplicate groups were fed with experimental diets for 30 days. FPH at 6.25-12.5% FM replacement improved the maturation of brush border enzyme, leucine aminopeptidase and alkaline phosphatase, resulting in improved survival and growth rate but, it depressed the activity of trypsin and α-amylase. Dextrin is a better carbohydrate source in the diet for Asian seabass larvae than wheat flour because a complex molecule of carbohydrate seemed to depress the amylase mRNA level which decreases the activity of α-amylase causing lower carbohydrate utilization with a consequential low survival rate. The supplementation of Pancreatin® at 0.05% of diet improved survival rate in fish fed the diet containing native protein but did not improve the utilization of complex carbohydrate molecule.
Early weaning to compound feed is important for reducing reliance on live feed in fish hatchery production. The present study was therefore carried out to examine histological changes of the digestive systems in Asian seabass larval stages (0-30 days post hatch, DPH) in order to develop suitable weaning diets using different levels of round scad mackerel hydrolysate (MH) and two types of binders.

In the histological study, samples of larvae were randomly collected before morning feeding every day at first hatch to 7- DP H and every 2 to 3 days at 8-21 and 22-30 DPH, respectively. At hatching, the digestive system was a simple tube with a large yolk sac. During yolk sac absorption (1-2 DPH), the oral cavity and pharynx were developed but stomach and intestine were not differentiated until 4 DPH. The epithelium lining of digestive tract was folding and goblet cell well developed from 3 DPH. At 5 DPH, yolk sac was depleted and the swim bladder developed. Teeth and taste buds were developing at 6 and 11 DPH. The digestive organs were well developed with extension and increase in size paralleled with the growth of fish larvae. At 17 DPH, gastric glands appeared in submucosa of interior stomach indicating the full functional digestive system.

The 16 DPH seabass larvae (initial body weight of 0.0031-0.0042 g fish^{-1}) were used to study utilization of MH to replace fish meal protein at 0, 15 and 30% having either gelatin or carrageenan as a binder. The experimental diets contained protein and lipid at 50% and 20%, respectively. Minced fresh mackerel and commercial diet were included as references. Each treatment consisted of three replicate groups of fish that were fed with respective diets to satiation for four weeks. During the third week, all fish in the reference groups died. After four weeks, survival was not significantly different among treatments (p>0.05). Weight gain and specific growth rate were higher in the groups fed 0% and 30% MH than those fed 15% (p<0.05). Pepsin and trypsin activity were influenced by both the level of MH and binder types (p<0.05) that the activity level in fish fed 0 and 30% MH diet using carrageenan as the binder were higher than those fed diet containing 15% MH and the groups fed diets using gelatin exhibited lower activity.

It could be concluded that the microbound diets containing MH up to 30% to replace fish meal protein using either gelatin or carrageenan as a binder were effective diets showing good acceptability in 16-17 DPH Asian seabass with fully developed digestive system.
Currently reports indicate that triploid Atlantic salmon may exhibit poor growth during seawater rearing due in part to a higher incidence of vertebral deformities which may be nutritionally linked to freshwater feeding. Phosphorous (P) deficiency is a causative agent of malformation in diploids, however, few studies to date have examined triploid dietary requirements. In the first study we examined the effects of varying dietary P supplementation (A – low, B – medium and high total P) applied from first feeding to smoltification on growth, mineral retention, and vertebral pathologies in sibling groups of diploid and triploid smolts. Triploid high P diets initially showed faster growth to ~5g, but final smolt weight was significantly less than triploid fed a low P diet (45.6 vs. 48.6g). In contrast, diploids showed a consistently slower growth rate with increasing P supplementation. Prevalence of radiologically detectable vertebral deformities was higher in triploids than diploids, but decreased with increasing dietary P in both ploidy (Diploid: 70, 65.2 and 58% and Triploids: 98.6, 77.1 and 83.6% for diets A, B and C respectively). Furthermore, incidence of severely deformed individuals (>10 deformed vertebrae) remained low in diploids (0-1.5%) irrespective of diet, and decreased in triploids with increasing dietary P (10.1, 5.7 and 0% for diets A, B and C respectively). Triploids fed high P diets had the highest retention of whole body minerals (P, Ca, V and Zn), but all treatments did not differ in mechanical vertebral strength. Current findings would suggest that triploid Atlantic salmon require higher dietary P to support bone formation, reduce severity of pathologies and support their faster growth compared to diploids. It was also evident that there is a specific window in early freshwater rearing where elevated P has a beneficial effect, and a second experiment is currently being conducted which examines the duration of feeding P supplemented diets on spinal malformation. Triploid groups have been fed elevated P diets either from hatch to 5g, hatch to 20g or hatch to smolt. Results will be discussed in terms of triploid dietary P requirements and windows in relation to spinal development and environmental discharge, and work towards the development of specific triploid salmon aquafeeds.
The study was conducted to determine the maintenance energy and protein demands, the efficiencies of energy and protein utilization of snakedhead (*Channa striata*). Three experiments were run for 28 days in the College of Fishery and Aquaculture of Can Tho University. Experiment 1 determined the energy and protein loss of experimental fish after starvation, four sizes classes of fish with average weight of 10 g, 50 g, 100 g, 200 g and 500 g were stocked in the 500-L tanks without being fed. Experiment 2 was conducted to assess the energy and protein digestibility of reference diet (42% crude protein and gross energy 19.3 kJ/g). Experiment 3, mean fish size of 38.5 g were fed the reference diet at a ration of 0%, 1%, 2%, 3% and 4% of initial live-weight each day to evaluate the efficiencies of energy and protein utilization at various energy and protein intake levels. Firstly, the results of experiment 1 showed that the exponent of weight for energy and protein metabolism in snakedhead (*Channa striata*) at 29°C, were 0.77 and 0.83, respectively. The energy and protein digestibility values of the reference diet were 88.6% and 86.1% respectively. Maintenance energy and protein demands and energy and protein utilization efficiencies were evaluated at experiment 3.
Membranes and specific membrane components have been suggested as possible pacemakers of the main processes taking place inside mitochondria. Therefore, changes in phospholipid content and composition or in phospholipid fatty acid compositions could play a central role linking different mechanisms involved in cumulative damage to cell molecules and dysfunction during periods of high stress, such as rapid growth and aging. Specifically, changes in mitochondrial lipids could modify the properties of phospholipids and membranes and lead to altered mitochondrial function and cell viability. Nutrition and feeding conditions such as ration and diet composition are parameters currently actively studied due to their possible implications on animal metabolic rate, oxidative stress, disease and aging. The effects of these factors can be largely attributed to the impact that the feed lipid and fatty acid composition has on cellular lipids, particularly those of mitochondria.

Materials and Methods
A population of rainbow trout, *Oncorhynchus mykiss*, was maintained from eggs to 3-years under the same rearing conditions. One- and 3-year-old trout (of 72.4 and 625.1 g average body weight, respectively) were used in two separate feeding trials. Each age group was split into three treatment groups and each of them fed, in duplicate tanks, an experimental diet consisting of the same base formulation but with lipid supplied either by fish oil, rapeseed oil or high DHA oil. After 6-weeks, fish were sacrificed and heart, liver and brain collected for analyses. A portion of each tissue was immediately taken into RNALater for molecular analysis (expression of mitochondrial genes), while the remainder of the tissue sample was processed to obtain enriched mitochondrial preparations. The purified mitochondrial isolates were analysed to determine lipid composition (phospholipid content and class composition, and phospholipid fatty acid composition) and oxidative status (SOD, aconitase, TBARs) of the organelle.

Results
The fatty acid composition of the diet significantly modified the mitochondrial membrane lipid composition of rainbow trout tissues and affected the susceptibility of the membrane lipids to oxidation by free radicals. Changes in mitochondrial membranes occurred at both phospholipid class and fatty acid composition levels, with this being associated with metabolic rate, free radical production and molecular damage. These changes varied depending on the fish tissue and also differed between the two age groups.

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This study evaluates the effects of dietary mannan oligosaccharides (MOS) supplementation in soybean oil (SBO) and fish oil (FO) based diets on feed utilization, growth and health of European sea bass (*Dicentrarchus labrax*). For that purpose, diets containing soybean oil (SBO) and fish oil (FO) as unique oil source were supplemented with 4 g·kg\(^{-1}\) dietary MOS (SBOMOS and FOMOS, respectively) and fed for 8 weeks. Body weight and total length, tissue proximate composition and fatty acid profiles, intestinal and liver morphology and gut health were examined.

Inclusion of MOS in FO diets significantly increased specific and relative growth and length, and reduced (P<0.05) viscerosomatic index (VSI) in comparison to fish fed SBOMOS diets. Muscle of fish fed FOMOS showed higher (P<0.05) lipid deposition compared to fish fed FO, SBOMOS and SBO dietary treatments. Fish fed SBOMOS, FOMOS and FO presented higher (P<0.05) liver protein and moisture percentages than fish fed SBO in expenses of lipid content (P<0.05). Inclusion of MOS increased (P<0.05) lipid content in fish feed SBO diets. Fatty acid profiles of tissues analyzed reflected those of the diets supplemented. However, MOS inclusion induced a higher n-3/n-6 ratio in muscle of fish fed FO diets. MOS supplementation in SBOMOS diet induced a lower (P<0.05) DHA/EPA deposition in anterior intestine compared to fish fed SBO. Fish fed SBOMOS diet showed a lower (P<0.05) accumulation of the saturated fraction in expenses of a higher (P<0.1) accumulation of the monoenoic and n-9 in posterior intestine compared to fish fed the SBO diet. Liver of fish fed FOMOS showed a higher (P<0.05) n-3, n-3/n-6 ratio, n-3HUFA, DHA, EPA and ARA content than fish fed FO diets in expenses of lower (P<0.05) monoenoics fraction deposition.

Inclusion of MOS significantly reduced hepatocyte size in terms of maximum and minimum length and area, particularly in FOMOS diet. Besides, it enlarged the length of intestine folds in fish fed SBO (P<0.05). Dietary MOS supplementation markedly up-regulated (P<0.05) the expression of IgM, COX-2, lymphocytes T CD4+ and CD8α+, major MHCI, TCRβ and TNFα, particularly when fish was fed FO based diets. MOS inclusion down-regulated (P<0.05) the expression of IL-8, IL-6 and TGFβ when included in FO based diets. However when included in SBO based diets the expression levels trend to be up-regulated, denoting again the interaction between SBO and MOS in relation to gut associated lymphoid tissue. IL-1β and IL-10 expression levels were down regulated (P<0.05) by SBO inclusion, however MOS inclusion tend to increase the expression of these genes.
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EFFECTS OF DIETARY PURIFIED MANNAN Oligosaccharides SUPPLEMENTATION ON GROWTH, GUT MUCOSAL IMMUNE SYSTEM AND LIVER LIPID METABOLISM OF EUROPEAN SEA BASS Dicentrarchus labrax juveniles
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The study assesses the effects of dietary purified mannan oligosaccharides (pMOS) on growth, proximate composition, fatty acid profile, liver lipid metabolism and intestinal health of European sea bass (Dicentrarchus labrax). Specimens of 20 g were fed 1.6 g·kg⁻¹ dietary pMOS for 8 weeks in a commercial sea bass diet. Fish performance, biochemical composition, fatty acid profile, gut and liver morphological structure, as well as gene expression of selected genes involved on the intestinal immune response and liver lipid metabolism were evaluated.

Fish fed pMOS presented higher (P<0.05) weight gain, total length and specific and relative growth than fish the control diet. No effects on condition factor, viscerosomatic, hepatosomatic and intestinal somatic indexes were detected. Proximate composition of anterior and posterior intestine, liver and muscle were not affected by pMOS inclusion. However, fatty acid profile analyses of fish fed pMOS revealed a reduction (P<0.05) in n-6 fraction and an increase (P<0.05) on arachidonic acid (ARA) deposition in muscle, a higher (P<0.05) ARA/ eicosapentaenoic acid (EPA) level in anterior intestine and a higher (P<0.05) ARA, docosohexaenoic acid (DHA)/EPA, ARA/EPA, ARA/DHA and n-3/n-6 accumulation ratios in liver after 8 weeks of supplementation. No differences were found on liver and gut morphometric analyses as well as in posterior gut goblet cells density. Dietary pMOS reduced (P<0.05) liver gene expression of fatty acid desaturase 2 (FADS2) and glycerol kinase (GCK), however no effect was detected on lipoprotein lipase (LPL), fatty acid-binding protein 7 (FABP7), angiopoietin-related protein 3 (ANGPTL3) or 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGRC). Dietary pMOS supplementation affected gene expression levels of posterior gut by up regulating immunoglobulin M (IgM), major histocompatibility complex class II (MHCII), T cell receptor β (TCRβ) and Caspase 3 and down regulating transforming growth factor β (TGFβ), however no effect was detected on the expression levels of cyclooxygenase-2 (COX2), lymphocytes T CD4+ and CD8α+, major histocompatibility complex class I (MHC1), Caspase 9 or interleukins IL-10, IL-1β, IL-8, IL-6 and tumor necrosis factor α (TNFa).
GLUCOSE UTILISATION IN THE BARRAMUNDI, LATES CALCARIFER
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Barramundi is one of the most commercially valuable cultured fish species in Southeast Asia. The inclusion of approximately 20% digestible carbohydrate in fish diets has led to poor growth performance and prolonged elevation of blood glucose levels. More recently, the metabolic response of barramundi has been measured over a time course after a single feeding event, using low levels of digestible carbohydrates. Combined, these data suggest that the barramundi are able to tolerate carbohydrates at low inclusion levels, although there is a poor understanding of the effect of different carbohydrate sources on blood glucose levels, or how dietary carbohydrates and blood glucose are being metabolised.

To further understand glucose utilisation in barramundi, this study performed a glucose tolerance test (GTT) using fish injected with one of four separate treatment groups: saline (5 ml kg⁻¹); glucose (1 g kg⁻¹); insulin (10 IU kg⁻¹) or glucose + insulin. For each treatment, 9 fish were sampled at each of 8 time points post injection: 0, 30 min, 60 min, 2 hrs, 4 hrs, 8 hrs, 12 hrs and 24 hrs. Blood, liver and muscle were collected and frozen for analysis of blood glucose levels and changes in the expression of key metabolism genes. Experiments were conducted subject to approval by the CSIRO Animal Ethics Approval AEC-A15/2012.

Intraperitoneal injection of 1 g kg⁻¹ glucose caused prolonged elevation of plasma glucose levels. There was a sharp rise in plasma glucose levels up until 2 hrs post feeding, with a peak of 12.98 mM at 4 hrs post injection. By 12 hours, blood plasma glucose readings were no longer significantly elevated, and had returned to resting levels by 24 hrs post injection. Intraperitoneal injection of 10 IU kg⁻¹ insulin caused a reduction in blood glucose levels in the first hour post-injection. These low levels were maintained throughout the rest of the time course. The simultaneous injection of glucose and insulin prevented the sharp rise in plasma glucose levels exhibited in the glucose treatment group. Plasma glucose levels were maintained near basal levels throughout the time course.

This data suggests that although barramundi are not tolerant of glucose at high levels, insulin is active in regulating blood glucose levels in this fish. However, simple carbohydrates are either not a preferred energy source, or do not directly stimulate an effective insulin response in barramundi. The expression of genes that regulate glycolysis, gluconeogenesis, fatty acid synthesis and glycogen metabolism are being investigated and the results of this study will be presented. Many aspects of glucose utilisation remain unanswered in this species. Currently, the precise metabolic fate of absorbed glucose is not clear, nor is there a complete understanding of the molecular pathways that utilise glucose in this fish.
Formulated feeds are now widely used for farming Asian seabass (*Lates calcarifer*) in Vietnam and other countries throughout the Asia-Pacific. The nutritional values of the digestible protein and energy value of feed ingredients are foundational for cost-effective diet formulation. This paper described one experiment was conducted on barramundi juveniles of 100 g each to determine digestible protein and energy value of a range of feed ingredients in southern Vietnam.

A diet containing 572 g/kg DM protein and 109 g/kg DM fat (20.7 MJ/kg DM gross energy) was used as a reference diet. Test diets were prepared using the reference diet and test ingredient with a ratio of 70:30, respectively. Five test ingredients were studied including: Vietnamese whole soybean meal, Argentinean soybean meal, Vietnamese (CaMau) fishmeal, European poultry meal and Vietnamese (Tay Ninh) cassava meal. The experiment had four replicates. Each replicate had 20 juvenile in one tank. The experimental duration was 26 days. Faeces were collected by the stripping method. Faeces were collected over a three-week period and pooled within tanks and faecal samples were kept frozen prior to analysis. For analysis, the faeces were dried and both faecal and feed samples ground and analysed for chromium, nitrogen, lipid, starch, ash and dry matter.

The results showed that the Barramundi juveniles can digest protein and energy well from a wide range of feed ingredients. These data will be useful in enabling the formulation of a range of nutritionally-efficient, cost-effective feeds for this species.
The flesh quality of Senegalese sole fed diets containing increasing levels of plant protein blends to replace fishmeal was evaluated using sensory attributes, muscle cellularity and gene expression. A control diet (CTR) was compared with three isonitrogenous (54%) and isolipidic (9%) diets with increasing levels of plant protein (PP) blends (50% PP50, 75% PP75 and 100% PP100). By the end of the experiment sole fed PP50 and PP75 had a final body length similar to the CTR (25 cm), but fish fed PP100 were significantly smaller (23 cm). Muscle cross sectional area (CSA) of fish fed CTRL, PP50 and PP75 diets were similar, and significantly larger than those fed PP100. White muscle fibre diameter varied significantly among treatments with PP50 having significantly larger fibres (72 µm) than PP100 (60 µm). Fibre density of PP50 was the lowest. No significant differences were observed in the total number of fibres among dietary treatments. FM replacement by PP sources significantly reduced the expression of several key genes involved in myogenesis and muscle growth (mrf4, fgf6, myHC and mylc2), resulting in a significant decrease of fibre diameter when the level of substitution was total (PP100). These changes in muscle cellularity could not be associated with the expression of texture-related genes (capn2, ctsb, ctsd), since no significant differences were be observed among diets. Fillet texture instrumentally evaluated was significantly affected by the total substitution of FM by PP, since PP100 diet showed the highest Modulus of Elasticity or flesh stiffness compared with the other groups. Muscle fibre density was moderately related (r=0.640) to the Modulus of Elasticity and negatively correlated with the expression of lysyl oxidase (r=-0.969).

The present results point towards a modulation of the expression of several muscle growth related genes by increasing levels of PP sources that alter muscle cellularity and textural properties of Senegalese sole.

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SUPPLEMENTATION OF TILAPIA DIETS WITH ULVA MEAL UP TO 10% INCREASES SKIN PIGMENTATION WITHOUT AFFECTING GROWTH PERFORMANCE OR FLESH QUALITY

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Increasing levels of a mixture of Ulva spp (U. rigida and U. lactuca), produced in an integrated multi-trophic aquaculture (IMTA) system, were evaluated in Nile tilapia by determining carotenoid content in muscle and skin, as well as by sensory evaluation. A control diet (CTRL) was compared with two experimental isonitrogenous (36%) and isoenergetic (20kJ/g) diets containing 5 % (U5) and 10 % (U10) of Ulva spp. meal. Duplicate groups of 18 fish (255 g initial body weight) were reared at 25±1 °C and fed each diet for 68 days. By the end of the trial, all groups of fish showed similar final body weight and specific growth rate. Whole body composition was also similar among treatments. The dietary incorporation of Ulva meal increased total carotenoid content in the skin, with fish fed diet U5 displaying significantly higher levels (6.5 µg g⁻¹) than the CTRL (1.4 µg g⁻¹). This was mainly due to increased deposition of astaxanthin and, in a lesser extent, violoxanthin identified by HPLC-DAD. No carotenoids could be found in tilapia muscle. Muscle colour determined using a colorimeter registering on the CIE L*a*b* colour scale, indicated that tilapia fillets form fish fed U10 showed the highest lightness (L* value) and yellowness (positive b*value), but the lowest redness (positive a* value). This could not be related to carotenoid deposition so it might be related to muscle fat deposition. Furthermore, sensory attributes of flesh showed no significant effects of dietary treatments on visual, olfactory, flavour and texture attributes, with the exception of sour attribute that was lowest in U10 fed tilapia.

The inclusion of Ulva meal spp. in diets for Nile tilapia seems to be possible up to 10% without major effects on growth performance or on organoleptic properties of the flesh. Ulva meal increased skin carotenoid content of tilapia due to increased deposition of astaxanthin and violoxanthin that might contribute to a better external appearance of this fish.

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USING MUSCLE CELLULARITY, TRACER STUDIES AND GENE EXPRESSION TO UNDERSTAND THE EFFECT OF DIETARY PROTEIN COMPOSITION ON PROTEIN DIGESTIVE CAPACITY AND MUSCLE GROWTH
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There is evidence that prenatal nutritional supply affects skeletal muscle development in a variety of vertebrates, with possible long-term consequences on growth potential and flesh quality. The amino acid (AA) imbalance is one of the causative factors for increased catabolism and possible loss of some essential AA to energy catabolism and other metabolic purposes. The dietary protein content and quality, may therefore, determine fish larvae capacity to digest protein and utilize it for muscle growth. In particular, the availability of certain amino acids seems to play a major role in stimulating protein accretion and skeletal myofibre numbers. However, the knowledge about the influence of early nutritional programming on muscle development and growth is still rudimentary in fish. Even more unclear are the mechanisms underlying such possible influence.

For that reason we decided to investigate whether the dietary protein composition could influence skeletal muscle cellularity and growth in a fish species that undergoes a complex metamorphosis. In Senegalese sole larvae fed inert diets from mouth opening, increased dietary protein content showed a positive effect on larval growth from 2 to 51 DAH overcoming the effect of an imbalanced AA profile. However, no major influence was found on the larval capacity to digest and retain protein. Although the proliferative capacity of myogenic cells and the growth potential of Senegalese sole larvae remained unaffected at the metamorphosis climax (19 DAH), at juvenile stage (51DAH), the dietary protein composition did affect muscle cross-sectional area. The expression levels of growth-related genes such as the myogenesis regulatory factors (MRFs) were studied in an attempt to highlight the main physiological mechanisms through which the dietary protein affected muscle growth during the metamorphosis climax and at the juvenile stage.

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POSTER 162

EFFECTS OF Xylanase AND PHYTASE IN PLANT-BASED DIETS ON DIGESTIBILITY AND GROWTH IN TILAPIA

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Future aquaculture growth will depend in-part on finding effective plant-based dietary substitutes for fish meal (FM) and more effective means of dealing with endogenous anti-nutritional factors (ANFs) present within plants. This study evaluated the potential for increased inclusion of lower-cost plant ingredients in tilapia diets through mitigation of deleterious growth effects of two ANFs; phytate-P and non-starch polysaccharides (NSP) using exogenous enzymes. The trial employed a fully-randomised replicated bi-factorial design with dietary FM inclusion level (0% FM, 3% FM and 5% FM) and enzyme inclusion (with or without combined xylanase and phytase) as independent variables, and growth (specific growth rate, condition factor) and nutrient utilisation indicators (feed conversion ratio, protein efficiency ratio, apparent net protein utilisation, hepatosomatic index and gut morphology) as dependent variables.

Juvenile tilapia (480 fish; 48.85g ± 13.96, mean ± STD) were cultured in 650L static circular tanks for 80 days, an initial stocking density of 1.5 g l⁻¹ with four replicates per treatment. Digestibility, multivalent-cation (Ca²⁺, Zn²⁺ and Fe³⁺) utilisation, carcass N and P retention assessments were conducted (also allowing evaluation of potential environmental benefits).

Cumulative growth and nutrient utilisation improved with exogenous xylanase and phytase suggesting synergistic action on the targeted ANFs. Feed intake (FI) decreased with declining FM levels (P < 0.05), stock consumed 2.09 – 2.11, 1.86 – 2.06, 1.74 – 1.79 g fish⁻¹ day⁻¹ for 5%, 3% and 0% FM diets respectively. Improvements in FI contributed to decreased FCR at higher FM levels and with enzymes. SGR, ADG and AWG trends were as expected yet no effect were observed between the enzyme and control diets, nevertheless stock fed enzyme 3% FM and control 5% FM performed comparably (P < 0.05). No treatment effects were observed for DM and protein digestibility. Lipid digestibility improved with enzymes with the exception of the 3% FM diet while P digestibility improved by 9% except at 0% FM level. The enzymes had no apparent influence on N retention and loading contrary to previous studies but higher retention for P, Ca and the trace elements (Fe and Zn) were observed. The findings were mixed regarding effects of NSP on villi length of the proximal intestine. Villus length decreased with declining FM levels due to higher NSP content yet no counteractive effect were seen in sections from tilapia fed enzyme diets contrary to expected.

Keywords: fishmeal, tilapia, anti-nutritional factors, phytate, non-starch polysaccharide, enzymes, nutrient retention, Thailand
A feeding trial was conducted to assess the potential of using green macroalgae (*Ulva rigida*) blooms as a feeding supplement in Atlantic salmon (*Salmo salar*) diets. The feed trial was conducted at NUI Galway’s Carna Research Station, Ireland using either medium or high *Ulva* inclusion. A total of five experimental feeds were formulated: basal control (with Astaxanthin, Asx); MedUlva; MedUlva+Asx; HiUlva+Asx and HiUlva. Experimental diets were formulated to be iso-nitrogenous (40 %), iso-lipidic (25 %) and iso-caloric (25 MJ kg⁻¹).

Assessment of physical muscle colouration was carried at the end of the experiment, using Konica Minolta CR-410 colorimeter and expressed in CIE L* a* b* colour space. Results show a significant enhancement in all colour space dimensions with both levels of *Ulva* supplementation. Separation, identification and quantification in the muscle carotenoid was also carried out.
THE EVALUATION OF BROWN AND RED MACROALGAE AS SUPPLEMENTS IN ATLANTIC SALMON (SALMO SALAR) DIETS

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A 12 week feed trials were conducted at Carna research station, Ireland to evaluate the potential of macroalgae inclusion in formulated Atlantic salmon smolt (Salmo salar) diets. Four experimental diets were formulated with the inclusion of either brown or red alga: Laminaria digitata, Saccharina latissima, Chondrus crispus and Porphyra dioica. A control diet was also formulated to make basal comparisons. All experimental diets were formulated to be iso-nitrogenous (40 %), iso-lipidic (25 %) and iso-caloric (25 MJ kg⁻¹).

Throughout the feeding trial morphometric data was taken to monitor growth performance. The results revealed that macroalgae inclusion had successfully maintained comparable performance to that of the control diets. At the end of each trial, blood samples were taken to determine potential adverse effects. These included basic haematology (packed cell volume, total erythrocyte, blood proteins and glucose levels), biochemical haematology (lipid peroxidation, lipid and liver function panel) and immunological response (alternative complement activity, lysozyme and leukocyte counts). The blood examination has provided evidence that there has been no measurable adverse effect on the fish, when fed with diets including algae.
THE EFFECTS OF HYPERGLUCIDIC STIMULUS AT FIRST FEEDING AND DIETARY CARBOHYDRATE ON GLUCONEOGENESIS PATHWAY IN SIBERIAN STURGEON (ACIPENSER BAERII) DURING STARVATION AND REFEEDING

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The effects of hyperglucidic stimulus at first feeding and dietary carbohydrate on gluconeogenesis pathway of Siberian sturgeon (Acipenser baerii) to face a short starvation period and subsequent refeeding were assessed. From first feeding to yolk absorption (8–12 days post-hatching), the Siberian sturgeon (Acipenser baerii) larvae were fed with a high-glucose diet (S, 57% glucose) as hyperglucidic stimulus and fed with a carbohydrate-free diet (F) as control. At 13 dph, each group of fish was assigned to two treatments. One treatment was fed with a high-carbohydrate diet (H, 35% dextrin), and the other treatment was fed with a carbohydrate-free diet until 20th wk. At the end of the 20th wk, each group was starved for 21 days, and then refeared for 21 days. Fish were sampled at starvation 6 h (served as pre-starvation) and 1, 7, 14 and 21 d and refeeding 1, 7, 14, 21 d postprandial 6 h. The levels of plasma glucose were significantly decreased from starvation 1 d and restored to pre-starvation levels form refeeding 1 d in each group, and significantly higher than pre-starvation from refeeding 14 to 21 d when the fish fed with high-carbohydrate diet. The contents of hepatic glycogen were significantly decreased from starvation 7 d and returned to pre-starvation level from refeeding 14 d when the fish fed with high-carbohydrate diet, whereas remained unchanged during fasting and refeeding period when the fish without carbohydrate intake. The muscle glycogens remained unchanged in each group during fasting, and exceed pre-starvation level from refeeding 7 d when the fish fed with high-carbohydrate diet. The activities of PEPCK in each group were significantly enhanced from starvation 7 d and refeeding 7 d respectively. The activities of G6Pase in fish, which did not experience hyperglucidic stimulus at first feeding, were significantly depressed at the beginning of starvation. The mRNA expressions of PEPCK-C at starvation 7 d and refeeding 14 and 21 d, and PEPCK-M at refeeding 21 d and G6Pase at starvation 7 and 21 d were significantly inhibited by high carbohydrate intake. The FBPase activities were significantly inhibited by high carbohydrate intake but independent of nutritional status. The activities of PEPCK-M and PEPCK-C in each group were significantly enhanced form starvation 7 d and refeeding 7 d respectively. The activities of G6Pase in fish, which did not experience hyperglucidic stimulus at first feeding, were significantly depressed at the beginning of starvation. The mRNA expressions of PEPCK-C in H groups and G6Pase in F groups at starvation 7 d, and PEPCK-M in F groups at refeeding 21 d were significantly inhibited by the hyperglucidic stimulus at first feeding. The activities of FBPase in F groups at refeeding 6 h and 7 d, and PEPCK-C in H groups at refeeding 21 d were also significantly inhibited by the hyperglucidic stimulus at first feeding. In conclusion, the Siberian sturgeon can increase the activities of key gluconeogenic enzyme and breakdown the glycogen when feeding with high-carbohydrate diet, while mainly increase gluconeogenesis pathway when feeding with carbohydrate-free diet to maintain plasma glucose concentrations during starvation. The hyperglucidic stimulus at first feeding and high carbohydrate intake both inhibit the gluconeogenesis pathway under starvation and refeeding.
Several research studies have demonstrated that high dietary fat intake may result in increased adipose tissue that is believed to act as a negative feedback control on appetite and feed intake for farmed salmonids. Bearing in mind the changes in feed composition where the proportion of lipid inclusion has approximately doubled in past 20 years, we questioned how high fat diets in the post smolt stage of production affects biological performance. The objective of the study was thus to test the effects of dietary protein:lipid ratio on biological performance and production efficiency of post smolt Atlantic salmon.

S1 smolt (95g) were stocked in 8 cages (5x5x5m) and fed two experimental diets (N=4 cages/diet) for a period of six months (April-September). Iso-energetic diets were used where the high protein diet (HP) had 4% higher portion of protein than the low protein diet (LP), where the latter had higher lipid content (2.3-2.5%). Dietary protein and lipid content changed each time pellet sizes were increased in line with increased fish weight (3mm: APR-JUN, 4mm: JUN-JUL, 6mm: JUL-SEP). The 4% difference in dietary protein and iso-energetic level was maintained within in all pellet sizes. The two experimental diets were based on the same raw materials. At each shift in pellet size, all fish were bulk weighed and feed utilization (FCRb) and growth rate (TGC) were calculated. Visceral weight and dressed out weight of 10 fish representing the average weight per cage (BW) was measured to estimate these as relative to BW expressed as viscerosomatic index (VSI) and dressed out percentage (DOP) respectively. Muscle fat content of the same 10 fish was also analyzed.

After feeding the 3 and 4mm diets, when the fish were 286 ± 1.3g, the fish fed HP had a tendency towards lower VSI compared to fish fed the LP diet. VSI coincided with observed differences between the fish groups in DOP, which increased throughout the experimental period in the HP group. No differences in bodyweight, feed conversion (FCRb) or growth were observed in the two first periods. In July the fish fed HP had significantly lower muscle fat content than fish fed LP diet. During the 6mm feeding period, there was a significantly higher feed intake and growth amongst fish fed HP diet. This resulted in 15% higher bodyweight (945g vs. 824g) together with a significantly higher muscle fat content (13.5% vs. 11.6%) for the HP fish group. The group fed HP diets also attained significantly lower VSI and significantly higher DOP compared with LP fed fish in the last feeding period.

These results demonstrate that increased dietary protein:lipid ratio fed during the early seawater phase can be used as a tool to stimulate subsequent growth of farmed S1 salmon through the modulation of body composition. Key parameters such as faster growth and more efficient feed conversion in addition to increased dress out yields in the S1 post smolt production can be greatly optimized by maintaining a high dietary proportion of protein as well as keeping a high dietary protein:lipid ratio.
THE LOWEST FEED PRICE IS NOT A PRECURSOR FOR RELATIVE LOW FEED COST - A DEMONSTRATION OF FEED COST EVOLVEMENT THROUGHOUT A POST SMOLT ATLANTIC SALMON (SALMO SALAR L.) PRODUCTION

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Lipids are generally a cheaper energy source compared to protein, and increased lipid inclusions in feeds for Atlantic salmon have generally reduced feed prices. Dietary effects on fish performance are primarily a combination of nutrition and feed intake with subsequent factors such as environment and general fish health affecting the biological results. Understanding the feed cost development and overall economic yield of production is thus likely to be more complex than merely taking feed prices and feed conversion into account. This topic addresses the question whether low feed prices lead to low feed costs.

To demonstrate evolvements in feed cost differences between two dietary strategies, all feed prices were recorded through a 6 month production period. S1 smolt (95g) were stocked in 8 cages (5x5x5m) and fed two experimental diets (N=4 cages/diet) for a period of six months (April-September). Isoenergetic diets based on the same raw materials were used where the high protein diet (HP) had 4% higher portion of protein than the low protein diet (LP), where the latter had higher lipid content (2.3-2.5%) resembling the low price alternative. Dietary protein and lipid content changed each time pellet sizes where increased in line with increased fish weight (3mm: APR-JUN, 4mm: JUN-JUL, 6mm: JUL-SEP). Feeds were maintained with a 4% difference in dietary protein and isoenergetic levels within each pellet size. Biometric analysis were incorporated into economic feed cost calculations in an effort to demonstrate production efficiency from a bioeconomic perspective.

Throughout the trial the costs of producing the HP diet were higher than for the LP diet. Average difference in feed price for the whole period was 0.19 DKK/kg with LP being the lower price alternative. When prices were multiplied with feed conversion ratio in the respective periods, the average difference in feed cost was reduced to 0.04 DKK/kg, still with LP being the lower price alternative. The fish's dress out weight represents the basis of the contribution margin and revenue for the fish farmers and after incorporating dress out yields in the economic feed cost comparison, the HP fish group had a total biological feed cost that was 0.22 DKK/kg lower compared with LP fed fish. Profitability drives production decisions and input cost is a primary factor in profit/loss predictions. This may lead to a monotonous focus on whole body FCR as basis of cost, which does not include the increased margin of extra dress out weight. In addition to lower feed costs, higher dress out yields will generate higher revenues, enhancing the overall economic performance of a high protein diet.

Results demonstrate when a fish farmer is given the choice between two isoenergetic feeds; the cheapest feed alternative may not lead to lower feed costs. Incorporating margin of the final product is needed when evaluating feeds with different protein:fat ratios and prices, to develop a higher understanding of feed choice and its effect on biological performance as a precursor of gaining higher economic yields.
Salmonids, have limited capacity to synthesize long-chain polyunsaturated fatty acids (LC-PUFA) from 18-carbon precursors. Consequently, alternative strategies are required to improve the LC-PUFA composition of salmonids fed diets containing vegetable oils (VO). Stearidonic acid (SDA)-rich vegetable oils have been investigated as alternatives to conventional VO. Given that SDA is the product of delta-6 desaturase, the rate limiting step in the conversion of \( \alpha \)-linolenic acid (ALA) to docosahexaenoic acid (DHA), it is generally assumed that feeding SDA to salmonids will result in increased levels of DHA in fish tissues. However, previous studies have reported mixed results. Furthermore, the source of SDA-rich oil has been echium (\textit{Echium plantagineum}), which, is not a commercially produced crop. Recently, genetically modified SDA-enriched flax oil has become available. This would allow the production of SDA-rich oil in a commercial crop. Therefore, an experiment was conducted to test the effect of feeding SDA-enriched flax oil on the growth and fatty acid composition of rainbow trout. One hundred and eighty triploid rainbow trout (initial weight 350g; \( N=15 \)) were randomly allocated to 12 tanks. The fish were fed one of three diets containing either: 1) fish oil (FO, positive control), 2) conventional flax oil (negative control) or 3) SDA-enriched flax oil. Diets were formulated to contain 17.6 MJ/kg DE; 386 g/kg digestible crude protein; 137 g/kg crude fat and met or exceeded the nutrient requirements of rainbow trout. Fish were fed to visual satiety twice per day. Following the 78-day feeding period, growth performance, nutrient digestibility and fatty acid composition of fish were measured. There were no significant differences in specific growth rate, feed conversion ratio or feed consumption between the dietary treatments. Conventional flax oil and SDA-rich flax oil fed fish had significantly higher ALA and linoleic acid tissue concentrations than FO fed fish (\( P < 0.05 \)). SDA-rich flax oil fed fish had higher SDA, eicosatetraenoic acid (ETA) and docosapentaenoic acid (DPA) tissue concentrations compared to those fed conventional flax oil. FO-fed fish had significantly higher EPA, DPA and DHA compared to fish fed the other 2 diets. There was a numerical increase in concentrations of EPA in the SDA-flax fed fish (7.74 mg/g DM) compared to conventional flax fed fish (6.73 mg/g DM). However, there were no significant differences in DHA concentrations between the fish fed either flax oil. The EPA and DHA concentrations in fish fed the FO diets (16.91 mg/g DM and 34.19 mg/g DM, respectively) were significantly higher than in fish fed conventional (6.73 mg/g DM and 22.86 mg/g DM, respectively) or SDA-rich (7.74 mg/g DM and 22.66 mg/g DM, respectively) flax oils. These results indicate that while feeding SDA-rich flax oil increases precursors to DHA, it had no significant effect on EPA and DHA concentrations in the present study.
A 56-day growth trial was conducted to investigate the dietary arginine requirement and arginine utilization of gibel carp at different growth stages using diets containing casein and precoated crystalline amino acids (CAA) as the main sources of protein. Six isonitrogenous and isoenergetic diets (310 g kg⁻¹ crude protein and 16.8 MJ kg⁻¹ gross energy) containing graded levels of arginine from 5.4 to 26.6 g kg⁻¹ (in dry matter) at about 4 g kg⁻¹ increments. Triplicates of 30 fish (51.6±0.3 g) or 20 fish (147.8±0.5 g) were fed to apparent satiation with one of the six experimental diets by hand four times a day in net cages. During the trial, water temperatures were 27.6±2.6°C (small fish experiment) or 30.5±2.5°C (big fish experiment). The results indicated that both small and big fish could effectively utilize CAA. Specific growth rate (SGR) and feed efficiency (FE) significantly increased with the increase of dietary arginine levels from 5.4 g kg⁻¹ to 17.6 g kg⁻¹ (small fish) or from 5.4 g kg⁻¹ to 13.0 g kg⁻¹ (big fish) (P<0.05), and thereafter, kept stable (P>0.05). Feeding rate showed an opposite trend. Condition factor presented a similar variation with SGR, but other morphological indices like vicerosomatic index (VSI), hepatosomatic index (HSI) and gonadosomatic index (GSI) were not significantly affected by arginine levels. In both of these two growth stages, whole body and dorsal muscle protein content and protein retention efficiency (PRE) significantly increased till arginine level up to 17.6 g kg⁻¹, and then reached to a plateau or slightly declined. Also, muscle arginine content markedly increased with the increase of dietary arginine levels. The concentration of plasma growth hormone (GH), insulin like growth factor-1 (IGF-1) and NO, and the activity of hepatic NO synthase (NOS) were significantly affected by arginine levels, while plasma ammonia, urea nitrogen and hepatic alanine aminotransferase (ALT), aspartate aminotransferase (AST) activities had no significant differences in both fish. In small fish fed 5.4 and 8.6 g kg⁻¹ arginine diets, GH, IGF-1 concentration and lysozyme activity were significantly lower than other groups and with the level of arginine increased, plasma NO concentration and hepatic NOS activity ascended, but hepatic arginase activity was not significantly affected by arginine levels. In big fish, GH concentration and lysozyme activity were not markedly affected by arginine levels, while hepatic arginase activity showed an obvious difference among groups. Other indices in big fish showed the same trend with small fish. Comparing this two growth stages, significantly differences were found in all tested indices except plasma lysozyme and hepatic ALT and AST. Broken-line analysis of SGR demonstrated that the dietary arginine requirement of this two growth stages were 16.5 g kg⁻¹ (small fish) and 12.8 g kg⁻¹ (big fish) of the dry matter, corresponding to 53 g kg⁻¹ and 41 g kg⁻¹ of dietary protein, respectively. Principle Component Analysis and Factor Analysis indicated that the differences of arginine utilization of different fish growth stages were related to the secretion of growth hormone, arginine catabolism and protein utilization.
Glutamine is important factors which influence the growth, function and development of intestine, α-ketoglutarate (AKP) has a potential value as precursors of glutamine in the nutriology and intestine metabolism. This experiment studied the effects of dietary AKP on growth performance, intestine enzyme activity and anti-oxidation ability of Songpu mirror carp. Songpu mirror carp with an average initial body weight of (61.28±3.40) g were randomly divided into 12 groups with 3 replicates in each group and 20 fish in each replicate. 12 experimental diets were formulated to contain four protein levels (28%, 31%, 34% and 37%), each of which had three AKP concentrations (0, 0.75% and 1.5%). The experiment lasted for 8 weeks. Under 28%, 31%, 34% protein level, AKP supplementation showed a tendency to increase weight gain rate(WGR), protein efficiency ratio (PER) and decrease feed conversion ratio (FCR) of carp ($P>0.05$) and WGR under the 34% protein level increased significantly($P<0.05$). The interaction of AKG and protein had significant effect on intestinal digestive enzyme activity, SOD activity in foregut, GSH in midgut and MDA in hindgut($P<0.05$). Under 28% protein level, fold height of midgut in AKG groups were significantly higher than the control group($P<0.05$). The results indicated that: supplementation of AKG could improve the growth performance, the intestine enzyme activity and anti-oxidation ability of carp, AKG has important application value on the nutriology of fishes.

**Keywords:** α-ketoglutarate; songpu mirror carp; growth performance; intestinal morphology; digestive enzyme activity; antioxidant capacity

**References:**
A 62-day growth trial was conducted to determine the dietary methionine (Met) requirement of the large size gibel carp (Carassius auratus gibeilo) (initial weight of 51.0±0.02g). Six isonitrogenous and isoenergetic diets using fish meal, blood meal and pea protein concentrated as protein sources supplemented with crystalline amino acids, were formulated to contain graded levels of Met (0.44%, 0.66%, 0.86%, 1.09%, 1.28% and 1.51% of dry matter, respectively) at a constant dietary cystine level of 0.4%. The results showed that weight gain (WG), specific growth rate (SGR) and protein productive value (PPV) significantly increased with the Met supplementation in diets. The morphometric parameters and body composition were not affected by dietary Met levels (P>0.05). Plasma high density lipoprotein cholesterol (HDL-C) and total cholesterol (TC) were increased with higher dietary Met levels. Fish supplied with 0.44-0.86% methionine showed lower plasma ALT level than that of other groups. Plasma triacylglycerol, LDL-C and the activity of AST kept relatively constant among treatments. The activities of intestine r-glutamyl transpeptidase (r-GT) was increased, which intestine creatine kinase (CK) and and hepatic lipase was decreased with higher level of Met. On the basis of WG and PPV that the optimum dietary Met levle of the large size gibel carp were 0.73% and 1.06%, respectively (0.16% and 2.48% of dietary protein) in presence of 0.40% cystine at 95% best growth using broken-line model. Based on the liver lipid content, dietary Met requirement of gibel carp was 1.28% (2.98% of dietary protein).

Keyword: Gibel carp (Carassius auratus gibeilo), growth-up, methionine requirement, lipid metabolism
THE EFFECTS OF DIETARY L-CARNITINE ON GROWTH PERFORMANCE, BODY COMPOSITION AND HEMATOLOGY INDICES OF JUVENILE ACIPENSER NUDIVENTRIS
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An 8-week feeding trial was carried out to evaluate the effects of dietary L-Carnitine on growth performance, body composition and hematology indices of juvenile Acipenser nudiventris. Experimental diets were formulated to contain 500, 750 and 1000 mg L-Carnitine. A total of 120 Acipenser nudiventris averaging 49.36±1.27 g (mean ± SD) were randomly distributed into 12 fiberglass tanks of 500 L capacity. There were no significant differences in fish weigh at the beginning of the rearing period. Experimental diets were formulated and prepared to contain 40% crude protein and 20 MJ/kg gross energy. Fish were fed 3% of body weight daily at 20 ± 2 °C.

Individual determination of length and weight was carried out on a monthly basis. At the end of the feeding trial, 30% of fish population from each treatment was selected and blood samples were collected. Carcass samples were transferred into laboratory in order to investigate the effects of dietary L-Carnitine on body composition. Based on the results obtained from this study, there were no significant differences (P>0.05) in final weight, body weight increase (%BWI), specific growth rate (SGR) and feed conversion ratio (FCR). However, the highest body protein were observerd in 500 and 1000 mg L-Carnitine but carcass protein content improved with increasing dietary L-Carnitine levels (750 and 1000 mg L-Carnitine) (P<0.05). Total protein content and LDL of fish fed 500 and 750 mg L-Carnitine were higher than those of fish fed other experimental diets. Significant increases in hemoglobin and hematocrit were recorded in fish fed 750 and 1000 mg L-Carnitine (P<0.05. (The results obtained from this study indicated that the dietary L-Carnitine had positive effect on fish biochemical index and body composition and suggested a dietary 750-1000 mg/kg for juvenile Acipenser nudiventris.

Key words: Acipenser nudiventris, L-Carnitine, growth performance, body composition, hematology indices

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THE USE OF ANTIOXIDANT FROM BLACK GLUTINOUS RICE BRAN (ORYZA SATIVA L.) IN NILE TILAPIA (OREOCHROMIS NILOTICUS) DIET

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The study in the use of antioxidant from black glutinous rice bran in Nile tilapia diet was divided into two experiments: 1) Study on Phytochemical and antioxidant properties of black rice bran. 2) Study of using black glutinous rice bran in Nile tilapia diet on digestibility, growth performance and health in tilapia. The results to experimental 1 found that of black rice bran has total anthocyanin, total phenolic, total tannin and phytic acid by 52.42±0.23, 3.77±0.03, 0.32±0.08, 0.14±0.01 mg/100g, respectively. The anthocyanin stability in different strong condition at temperature 4 and 25 °C and storage time at 0, 7 and 14 days was significantly different (p<0.05). The properties of rice bran act as an antioxidant determined by Thiobarbituric acid reactive substances (TBARs) analysis method was 8.0 µM malondialdehyde, and the percentage of inhibition of 2,2-diphenyl-1-picrylhydrazyl (DPPH, 0.05 mM of Trolox). The crude extract of anthocyanin from black glutinous rice bran storage at 4 °C with significantly highest antioxidant activities analyzed by DPPH and TBARs tests (p<0.05). Thus, the best fixed at 4 °C was storage condition for anthocyanin in black glutinous rice bran.

The study present of using black glutinous rice bran in Nile tilapia diet, digestibility, growth performance and health in tilapia with initial weight of 8.76 g/fish was fed with experimental diets for 8 weeks. Fish were fed the diets supplement with black rice bran at 0, 5, 10, 15 and 20%. All diets contained 30% crude protein and gross energy at 3,000 Kcal/kg. The results showed that significant differences (p<0.05) in average daily gain, specific growth rate and protein efficiency ratio. Fish were fed with 15 and 20% of black rice bran in diet were better than other groups. Survival rate and feed conversion ratio were similar in all groups (p>0.05), but feed conversion ratio of tilapia diet with possibly up to 20% trending the better than other groups. Digestibility co-efficiency and protein digestibility were not significantly different (p<0.05), digestibility co-efficiency in range 97.14-97.36% and protein digestibility of diet in range 98.73-99.12%. In the part of fish health found that, hematocrit, total protein, albumin, total bilirubin, AST, ALT, ALP and hepatosomatic index were not significantly different (p>0.05). The study indicated that the dietary black glutinous rice bran contains ingredients that could be used for tilapia diet with possibly up to 20% without negative effect on growth, protein efficiency ratio, digestibility and health for tilapia.
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MIR-17 IS INVOLVED IN THE REGULATION OF HUFA BIOSYNTHESIS IN RABBITFISH SIGANUS CANALICULATUS
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Long-chain polyunsaturated fatty acids (LC-PUFA) such as arachidonic (ARA), eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids play important roles in maintaining the normal growth, development as well as physiological functions of humans and other animals. In vertebrates, the biosynthesis of LC-PUFA requires introduction of double bonds catalyzed by fatty acyl desaturases (Fad). Δ4 Fad was first discovered in vertebrates from the marine teleost rabbitfish Siganus canaliculatus by our lab in 2010, which was characterized to convert 22:5n-3 to DHA directly and thus another “Δ4 Fad” pathway exists in vertebrates for LC-PUFA biosynthesis besides the traditional “Sprecher” pathway (Li et al., 2010, PNAS, 107: 16840-16845).

Recently, increasing studies indicate that microRNAs (miRNA or miRs) are involved in the regulation of lipid metabolism in mammals. However, little is known about their function in LC-PUFA biosynthesis of teleosts. In this study, we investigated the regulatory role of miR-17 in Δ4 Fad gene expression and biosynthesis of LC-PUFA in rabbitfish. First, 1958 bp of miR-17-92 gene cluster was cloned. By sequence alignment, the miR-17 precursor (pre-miR-17) (89 nt) and mature (22 nt) were ascertained, which located at the forepart of miR-17-92 gene cluster. The pre-miR-17 showed 92 % sequence identity to that of zebrafish, and 82-85 % identity to that of human, mouse, chicken and frog. The sequence of mature miR-17 was highly conservative among different species. Rabbitfish miR-17 displayed relatively high expression in gill but lower expression in liver, eyes. Dual luciferase reporter assays demonstrated that miR-17 targeted the 3’UTR of Δ4 Fad directly. In vivo, the expression level of miR-17 was inversely correlated with Δ4 Fad mRNA and protein quantity in livers of rabbitfish raised in 32 ppt and 10 ppt water. In vitro, incubation of rabbitfish primary hepatocytes with LNA (18:3n-3) promoted the expression of miR-17 and Δ6/Δ5 Fad, but suppressed the expression of Δ4 Fad. In contrast, LA (18:2n-6) and EPA decreased the expression of miR-17 and Δ6/Δ5 Fad, but had no effect on Δ4 Fad. However, the expression of miR-17, Δ6/Δ5 Fad and Δ4 Fad were all down-regulated by DHA. The results indicated that miR-17 was involved in the regulation of LC-PUFA biosynthesis in rabbitfish liver by targeting Δ4 Fad, and may act as a switch to orchestrate the “Δ4 Fad” pathway in response to PUFA supplement. These data are helpful for us to reveal the regulation mechanisms of LC-PUFA biosynthesis in rabbitfish.

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DAILY FEEDING BEHAVIOR AND DIGESTION PATTERN IN SENEGAL SOLE LARVAE: A TOOL FOR IMPROVING FEEDING PROTOCOLS

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Both the improvement of current feeding protocols of marine fish larvae based on live prey and the design of suitable inert feeds requires a good knowledge of the complexity of larval feeding behavior and of factors modulating food processing. Some of these factors affecting net efficiency of the digestive process are: the species, developmental stage, and diet composition. On the other hand, daily feeding ration and number of meals per day affect the absorption and assimilation of nutrients, and hence final growth. Senegalese sole (Solea senegalensis) has become a species with high commercial interest for aquaculture. The rapid physiological changes that Senegalese sole larvae undergo throughout development, including a pelagic pre-metamorphic and a benthic post-metamorphosis phase, complicate the determination of their feeding needs during larvae and post-larvae rearing.

In this study, the daily feeding behavior and digestive enzymes activity in S. senegalensis were analyzed in pre-metamorphic (3 and 6 days post hatching; dph) and post-metamorphic larvae (20 and 30 dph) during a 24 h cycle, with a photoperiod of 12 light:12 dark. Gut content was measured by counting rotifers mastax in pre-metamorphic larvae and by weighing the dissected gut in post-metamorphic larvae. Enzymes activity (trypsin and lipase) was analyzed individually per larvae using fluorogenic substrates. Results showed that Senegalese sole larvae have a diurnal feeding behavior during the pelagic phase, reaching the highest gut content at the end of the light period. On the other hand, metamorphosed larvae showed feeding activity during the whole daily cycle, although the gut content tended to be higher during nocturnal hours. Trypsin activity at pre-metamorphic stages was closely related to the amount of food present in the digestive tract while in post-metamorphic larvae the activity kept almost constant during the whole day, with the exception of first and last hours in the day, when activity levels were lower. Regarding to lipase activity, results point to a continuous production of lipase, although activity levels tended to be higher during dark phase in all stages.

These results clearly confirm that moving from pelagic to benthic life involves a change in the processes related to feeding and digestion by sole post-larvae. Future experiments will explore whether this pattern may continue changing beyond the first month of life up to acquiring a definitive juvenile feeding strategy. This knowledge will allow the design of more appropriate feeding protocols during the first weeks for a better nutrient utilization and therefore enhanced growth and development.

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EFFECTS OF DIETARY ASTAXANTHIN/XANTHOPHYLLS RATIO ON GROWTH AND SKIN PIGMENTATION OF LARGE YELLOW CROAKER PSEUDOSCIAEA CROCEA R

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Large yellow croaker Pseudosciaena crocea is the 3rd largest mariculture species in China. The quality of farmed large yellow croaker is inferior to the wild one. One of the main quality problems is the skin color. The aim of this study was to analyze the effects of dietary astaxanthin/xanthophylls ratio on growth and skin color of large yellow croaker. Five carotenoid supplemented diets were formulated to contain 75/0, 50/25, 37.5/37.5, 25/50 and 0/75 mg/kg astaxanthin/xanthophylls, and named as Diet-1, Diet-2, Diet-3, Diet-4, Diet-5, respectively. The diet without carotenoid supplementation served as control diet, and named as diet-0. Fish of similar size (13.80±0.03g) were cultured in floating sea cages for eight weeks. Ventral and dorsal skin color were quantified using the $L^*$, $a^*$, $b^*$color scale in the night. Carotenoid content and melanin content of ventral and dorsal skin were also determined. The results showed that survival, the specific growth rate (SGR) and feed conversion ratio (FCR) were not significantly affected by dietary treatments ($P>0.05$). There were no significant differences in ventral skin lightness ($L^*$) among all treatments ($P>0.05$). However, the dorsal skin lightness of fish fed with Diet-0 was significantly lower than those fed with the carotenoid supplemented diets ($P<0.05$). The lowest yellowness ($b^*$) values both in ventral skin and dorsal skin were found in fish fed with Diet-0. As to fish fed with carotenoid supplemented diets, the highest yellowness values in dorsal skin and ventral skin were found in fish fed with Diet-1, while the lowest values were observed in fish fed with Diet-5. The higher reddish ($a^*$) of ventral skin were found in fish fed with Diet-2 and Diet-3. As to dorsal skin, the highest reddish was exhibited in fish fed with diet-4. Fish fed with Diet-0 showed higher melanin content in dorsal skin than those fed with carotenoid supplemented diets, though no significant differences were observed among all treatments ($P>0.05$). The significant lowest carotenoid content in ventral skin and dorsal skin were showed in fish fed with Diet-0 ($P<0.05$). Fish fed with higher ratio of xanthophylls in diet obtained higher carotenoid contents. Significantly linear relationship between parameters ($b^*$ and $L^*$) and carotenoid content were found both in ventral skin and dorsal skin. In conclusion, fish fed with Diet-2 or Diet-3 obtained better skin color, though no significant differences in growth and feed efficiency were found among all treatments.

Key words: large yellow croaker; astaxanthin; xanthophylls; skin color
This study evaluated the effect of dietary thiamin on growth performance, feed utilization and non-specific immune response for juvenile Pacific white shrimp, *Litopenaeus vannamei*. Six isonitrogenous and isolipidic practical diets were formulated with graded thiamin levels of 6.9, 32.7, 54.2, 78.1, 145.1 and 301.5 mg kg\(^{-1}\) of dry diet, respectively. Each diet was randomly assigned to triplicate groups of 30 juvenile shrimp and provided 4 times each day to apparent satiation. Weight gain (WG) and specific growth rate (SGR) of the shrimp were significantly influenced by the dietary thiamin levels; the maximal WG and SGR occurred at 54.2 mg kg\(^{-1}\) dietary thiamin level. However, with further increase of dietary thiamin level from 54.2 to 301.5 mg kg\(^{-1}\), the WG and SGR significantly decreased. Shrimp fed the 54.2 mg kg\(^{-1}\) thiamin diet exhibited higher feed efficiency, protein efficiency ratio and protein productive value than those fed the other diets. Dry matter and protein content in whole-body were significantly affected by the dietary thiamin levels. Thiamin concentration in hepatopancreas significantly increased when the dietary thiamin level increased from 6.9 to 145.1 mg kg\(^{-1}\). The total protein, glucose, triacylglycerol and cholesterol contents in hemolymph were not significantly affected by the dietary thiamin levels. Dietary thiamin had significantly influenced superoxide dismutase, catalase and lysozyme activities in hemolymph. Results of this study indicated that the optimal dietary thiamin requirements estimated using a two-slope broken-line model based on WG and thiamin concentration in hepatopancreas were 44.66 and 152.83 mg kg\(^{-1}\), respectively.
A twelve-week feeding trial was conducted to evaluate the effects of dietary vitamin C on growth performance, hematologic parameters and innate immune responses in juvenile yellow catfish, *Pelteobagrus fulvidraco*. Six practical diets were formulated to contain 1.9 (as the basal diet), 18.9, 36.8, 78.4, 156.5 and 316.0 mg ascorbic acid equivalent kg\(^{-1}\) diet. Each diet was fed to triplicate groups of juvenile yellow catfish with initial body weight of 2.0 g in 300-L cylindrical fiberglass tank. The results of present study showed that typical vitamin C deficient signs such as retarded growth and sluggish response were observed in the fish fed the basal diet. Weight gain (WG) and specific growth rate (SGR) were significantly influenced by dietary vitamin c levels, fish fed the basal diet had significantly lower WG, SGR than those fed the diets supplemented with vitamin C. However, survival, protein efficiency ratio (PER) and feed efficiency (FE) were not significantly affected by the dietary vitamin C levels. Fish fed the basal diet had higher hemoglobin, serum glucose and triglyceride concentrations than those fed the vitamin C supplemented diets. The activities of serum superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) significantly increased when dietary vitamin C levels increased from 1.9 to 156.5 mg kg\(^{-1}\), while fish fed the basal diet had higher malondialdehyde (MDA) than those fed the diet supplemented with vitamin C. The activities of serum lysozyme, total complement activity (CH50), phagocytosis index (PI) and respiratory burst (RB) of head kidney were significantly influenced by the dietary vitamin C levels, fish fed the basal diet had lower serum lysozyme, phagocytosis index (PI) and respiratory burst (RB) than those fed diets supplemented with vitamin C. The challenge experiment with *A. hydrophila* showed that lower cumulative survival was in fish fed the unsupplemented diet, the cumulative survival were significantly increased with increase of the dietary ascorbic acid levels from 18.9 to 36.8 mg kg\(^{-1}\), while the cumulative survival reached plateau when dietary ascorbic acid levels increased from 78.4 to 316.0 mg kg\(^{-1}\). Analysis by broken-line regression of SGR indicated that the dietary vitamin C requirement of juvenile yellow catfish was estimated to be 114.5 mg kg\(^{-1}\) diet. These results suggested that vitamin C did significantly influence on growth performance, antioxidant ability, immune response and disease resistance of juvenile yellow catfish.
We investigated the effects of incorporating chitinase (ChiB565)-hydrolyzed shrimp shell chitin into the diet of hybrid tilapia (\textit{Oreochromis niloticus} $\times$ \textit{O. aureus}) with regard to production, intestinal immune status and autochthonous bacteria, and protection against bacterial pathogen \textit{Aeromonas hydrophila}. Five experimental diets were formulated by supplementing the basal diet with the hydrolyzed shrimp shell chitin (0.0%, T1 control; 0.08%, T3; 0.16%, T4; or 0.24%, T5) or 0.1% commercial chitosan-oligosaccharides as commercial recommendation dose (T2, positive control). After a 35-day feeding trial, we found no significant difference in weight gain, feed conversion ratio or survival rate in tilapia among groups T1–T5. However, the levels of mRNAs encoding the pro-inflammatory protein tumor necrosis factor-$\alpha$ and the stress-response protein heat shock protein 70 were much lower in groups T2–T5 ($p < 0.001$). The levels of transforming growth factor-$\beta$ were higher in groups T2 and T4 ($p < 0.001$ and $p < 0.0001$, respectively). In addition, groups fed lower amounts of chito-oligosaccharides (groups T3–T4) changed marginally their intestinal autochthonous bacteria ($0.60 < C_S < 0.80$). When challenged with \textit{A. hydrophila}, the mortality of groups fed chito-oligosaccharides was lower than controls, especially in those fed higher amounts of chito-oligosaccharides (groups T4–T5; $p < 0.05$). These results indicate that dietary intake of chito-oligosaccharides can improve intestinal health, changed intestinal autochthonous bacteria, and improve resistance to infection by \textit{A. hydrophila}, even with higher efficiency than commercial chitosan-oligosaccharides at its recommended dose.
We formulated experimental diets for hybrid tilapia to investigate the effects of replacing dietary soybean meal (SBM) or cottonseed meal (CSM) by completely hydrolyzed feather meal (defatted rice bran as the carrier; abbreviated as CHFM), with emphasis on fish growth, the composition of adhesive gut bacteria, intestinal and hepatic immune responses, and disease resistance. A series of four isonitrogenous (33% crude protein) and isolipidic (6% crude lipid) diets were formulated to replace the isonitrogenous percentages of CSM or SBM by 6% or 12% CHFM. Quadruplicate groups of healthy and uniformly sized hybrid tilapia were assigned to each experimental diet. Fish were hand fed three times a day for 8 weeks at a rearing temperature of 25–28°C. The growth performance of hybrid tilapia fed diets with partial replacement of dietary SBM or CSM with CHFM was comparable to the group of fish fed the control diet. The CHFM-containing diets affected the intestinal autochthonous bacterial community in similar ways. All CHFM-containing diets stimulated the expression of heat shock protein 70 in the intestine but suppressed its expression in the liver. Only the CHFM6/SBM diet stimulated the expression of interleukin-1β in intestine, and no effects were observed in all diets to the expression of interleukin-1β in liver. Thus, regarding the immune response in the intestine and liver, CHFM is a good alternative protein source that induces less stress in the host. CHFM did not affect disease resistance to Aeromonas hydrophila infection in hybrid tilapia. These data suggest that CHFM is a good alternative to partially replace SBM and CSM in tilapia feed.