Dietary N-carbamoylglutamate supplementation enhanced the growth and the endogenous synthesis of arginine of hybrid sturgeon juveniles under dietary arginine deficiency

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N-carbamylglutamate (NCG) has been shown to increase the endogenous synthesis of arginine (Arg) and enhance performance of piglets. The present study was conducted to determine the effects of dietary NCG and Arg supplementation on growth, body composition, biochemical compositions and GH/IGF-I genes expression of hybrid sturgeon (Acipenser schrenckii ♀ × A. baerii ♂) juveniles under dietary Arg deficiency. A 2×3 (Arg levels: 0 and 1%; NCG levels: 0, 0.05% and 0.1%) factorial design was used. The results showed that the final body weight, percent weight gain and protein efficiency ratio were increased, and the feed conversion ratio was decreased by the addition of Arg or NCG (P < 0.05). Body moisture was decreased, and body crude lipid was increased by the addition of Arg or NCG (P < 0.05). Body crude protein and crude lipid contents was increased by the addition of NCG (P < 0.05). Urea contents was decreased, and total protein contents and alanine aminotransferase activity in serum were increased by the addition of Arg or NCG (P < 0.05). The concentration of Arg, NO and the activity of NOS in liver were increased by the addition of Arg or NCG (P < 0.05), and the interactions were observed (P < 0.05). Relative expression of GH and IGF-I genes in liver were increased by the addition of Arg or NCG, and interactions were observed (P < 0.05). In conclusion, dietary NCG or Arg supplementation promoted the growth and NCG increased the endogenous synthesis of Arg of hybrid sturgeon under dietary arginine deficiency, and 0.1% NCG better than 0.05% NCG.
Effect of taurine supplementation to non or low fishmeal diet on growth, intestinal morphology and cytokines gene expression of juvenile red sea bream, Pagrus major

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Plant ingredients are the most cost-effective alternative ingredients for the fish meal in the aqua-feed industry. And the taurine utilization in plant protein based diet has caused widespread concern. Therefore, the objectives of this study were to investigate the effects of taurine supplementation to plant protein based diet of juvenile red sea bream. Six isonitrogenous (46% crude protein) and isolipidic (12% crude lipid) practical diets were formulated. The diets were designated as FM (50% fishmeal); LFM (20% fishmeal diet); LFMT (20% FM with 0.5% taurine); NFMT1, NFMT1.5 and NFMT2 (non FM with 1.0%, 1.5%, 2.0% taurine). Fish were fed one of the six diets to satiation twice a day for 10 weeks. At the end of the experiment, growth parameters were evaluated. Morphology and cytokine gene expression of liver and intestine were analyzed.

The results showed that fish fed NFM showed significantly lower specific growth rate (SGR), feed efficiency (FE) and protein digestibility than those in FM group. However, as the elevation of taurine supplement to NFM diet, SGR, FE and protein digestibility were significantly improved. Fish fed LFM showed significantly lower SGR than LFMT0.5 group. Typical pathological changes were observed mainly in the intestine submucosa of fish fed LFM and NFM. However, the pathological changes were obviously improved when the taurine supplement level increased. Inflammatory cytokine genes (IL-1β, IL-8, TNF-α, TGF-β) were up-regulated in low and non fishmeal groups than that in FM group. And LFMT0.5 group showed similar expression levels to FM. Fish fed NFM showed significantly higher expression level of these genes than FM. For the NFM groups, as the taurine supplement level increased, gene expression levels tend to be significantly decreased. These results demonstrated that fishmeal replaced by plant protein might cause growth retardation and intestinal pathological changes in red sea bream. However, these negative influences can be improved by graded levels of taurine supplement.

Keywords: Taurine; Fishmeal replacement; Intestinal morphology; Red sea bream
Growth performance and metabolism of juvenile European seabass (Dicentrarchus labrax) fed diets supplemented with arginine, and two lipids levels

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Arginine supplementation may affect animal metabolism, being able to act in the modulation of genes related to lipid metabolism. Therefore, the objective of the present study was to evaluate the supplementation of arginine to juvenile European sea bass in diets with two levels of lipids. Four diets were formulated to be isonitrogenous, supplemented or no with L-arginine and containing two lipids levels. A hundred and forty-four fish (56.26 ± 0.40 g) were distributed into twelve 110 L circular tanks, with flow-through water and natural photoperiod. Fish were hand fed three times a day to apparent satiety, for four months. Dissolved oxygen saturation was maintained at 95.81 ± 4.42%, temperature at 19.63 ± 2.03 °C, and salinity at 35.21 ± 0.39‰. At the end of the experimental period fish were fasted for 24 h, and nine individual fish per tank were euthanized with MS-222 overdose (1,000 mg L-1). All fish were counted and weighed at the beginning and at the end of the experimental period. No mortality occurred during this study. Arginine supplementation, and diet lipid content, and consequently gross energy levels (21.1 and 22.5 MJ kg-1), had no effects on fish feed intake (88.30 ± 6.34 g) or feed conversion ratio (1.48 ± 0.19). Fish final individual weight (116.73 ± 5.75 g), and total weight gain (60.06 ± 6.01 g) were not influenced by dietary treatments. Also, no differences were observed in visceral fat percentage (4.59 ± 1.12%) and muscle yield (24.36 ± 1.48%) of juvenile fed with experimental diets. A lower hepatosomatic index (p < 0.05) was observed in European seabass fed with diets supplemented with arginine. There was a significant interaction of the two factors, and the unfolding showed that L-arginine supplementation leads to lower HSI (2.91 ± 0.04) than non-supplementation (3.23 ± 0.17%) and the two lipid levels (3.14 ± 0.16 and 2.97 ± 0.05%, respectively). Arginine supplementation in the diets promoted a lower metabolic overload of the liver, and probably, consequently better liver health and metabolism. Further analyses are in progress, in order to give a better insight of the lipid metabolism regulation in European seabass.
Does Dietary Fatty Acid Profile Affect Performance and Intestinal Lipid-Related Genes Expression in Gilthead Sea Bream?

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Diets with high fish oil (FO) and fish meal (FM) replacement by plant ingredients can be used without severely affecting gilthead sea bream growth, contributing to make production more sustainable. Despite this, little knowledge about the consequences of these dietary conditions in other physiological processes such as those related with nutrition exist. The aim of this study was to test the effect of FM and FO highly substituted diets in intestinal lipid-related genes expression, but also growth and feed intake in this species.

Ten isonitrogenous (46%) and isolipidic (22%) diets, named A to J, were formulated and produced by Skretting ARC (Norway) with 75% of FO replacement by a single or a blend of vegetable oils (VO; palm, rapeseed, soya and linseed oils) and 81.2% of FM substitution by plant protein sources. Juvenile gilthead sea bream growth parameters were analysed after 18 weeks of feeding twice a day with the experimental diets and samples from proximal intestine were collected for qPCR analyses.

Differences were not found in somatic parameters among fish fed either with diets A to G or with the monosubstituted diets (H, I, J). Despite this, significant changes were found in feed conversion ratio, being the values lower for those animals fed with 75% of soya oil (diet H), and for those fed with blends containing soya and/or a high inclusion of linseed oil. Regarding gene expression, changes by dietary fatty acid profiles were not found in gilthead sea bream fed monosubstituted diets. Nevertheless, in animals fed with VO blends a significant positive correlation was found with unsaturated/saturated fatty acids ratio for the intestinal alkaline phosphatase (alpi) and the fat translocase cd36a gene expression; and with the dietary n-3 content for the latter. Furthermore, a significant negative correlation was found between the fatty acid binding protein (fabp2) gene expression and the n-6/n-3 ratio, altogether suggesting a differential stimulation of fatty acids absorption.

Overall, these data corroborate that an important substitution of FO by VO is possible to farm gilthead sea bream without affecting growth, but dietary fatty acid profile must be considered to maintain a correct intestinal functionality.
OMEGA-3 FATTY ACID BIOCONVERSION IN LARGE ATLANTIC SALMON VIA THE MANIPULATION OF DIETARY SHORT-CHAIN TO LONG-CHAIN OMEGA-3 FATTY ACID RATIOS

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Oils rich in long-chain omega-3 polyunsaturated fatty acid are increasingly subject to reduced availability and enhanced market volatility. In response, significant research attention has been placed on dietary manipulation of the short-chain to long-chain omega-3 ratio in order to record the effects on endogenous omega-3 production in commercially valuable finfish species. However, to date, there is a paucity of information regarding large Atlantic salmon reared in saltwater, therefore, limiting industry relevance of novel feed formulations which aim to enhance the sustainability of aquafeed. Four experimental diets were formulated utilising three different lipid sources; poultry by-product oil, fish oil and camelina oil, to create varied ratios of short-chain to long-chain omega-3 fatty acids and hence four varied compositions of ‘substrate’ and ‘end-product’ in terms of in vivo bioconversion of omega-3 fatty acids. Atlantic salmon grew to in excess of 3000g and were analysed using the whole-body fatty acid balance method. In general, omega-3 substrate, namely 18:3n-3, was essential for significant endogenous production of 22:6n-3, however, diets with added fish oil recorded negligible endogenous production of 22:6n-3, regardless of omega-3 substrate level. Furthermore, due to in vivo bioconversion of 18:3n-3, fish fed high dietary levels of camelina oil and no added fish oil ended up having comparable levels of long-chain omega-3s in the fillet to those fish fed diets containing added fish oil and no dietary camelina oil. Additionally, bioconversion of 18:2n-6 appeared to be significantly influenced by dietary short-chain to long-chain omega-3 ratios, despite dietary levels of 18:2n-6 being relatively consistent across dietary treatments. Specifically, desaturation of 18:2n-6 appeared to be negatively affected by fish oil inclusion. However, further research is required to elucidate the relationship between added dietary fish oil in suppressing Δ-6 desaturation of 18:2n-6. The present study highlights the suitability of camelina oil in providing sufficient substrate (18:3n-3) for endogenous production of 22:6n-3 in large Atlantic salmon.
Regulation of growth, fatty acid profiles, antioxidant capacity and expression of lipid related genes by different dietary lipid in juvenile swimming crab, Portunus trituberculatus

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An 8 week feeding trial was conducted to evaluate the effect of dietary lipid levels on growth performance, antioxidant capacity, hematological characteristics, fatty acid profiles, enzyme activity and genes expression of lipid metabolism related of juvenile swimming crab. Three isonitrogenous (47% crude protein) diets were formulated to contain 5.83%, 9.91% and 15.07% crude lipid levels, respectively. Each diet was randomly assigned to quadruplicate groups of eighty swimming crabs (approximately 21.09±1.11 g). The results indicated that swimming crab fed a diets containing 15.07% lipid had significantly lower growth performance, feed utilization and survival than those fed 5.83% and 9.91% lipid diet. Fatty acid profiles of hepatopancreas and muscle reflected the fatty acid composition of the three types of diets. The content of malondialdehyde (MDA) increased both in the serum and hepatopancreas of crab fed high lipid level diets, and the highest activity of hepatic total superoxide dismutase (T-SOD) and serum glutathione peroxidase (GSH-PX) was observe in crab fed the diet containing 5.83% lipid. In high-fat group obtained the lowest activity in FAS and G6PD, and highest CPT1 than others treatment groups. The expression levels of fas, acc, g6pd, 6pgd, dgat1 and srebp1 in the hepatopancreas decreased significantly with increased dietary lipid contents. In addition, the expression levels of fabp1 significantly increased when the dietary lipid contents increased from 5.83% to 9.91%, and then decreased with the increased of lipid levels from 9.91% to 15.07%. However, cpt1 expression markedly up-regulated with the increased of lipid levels. Over all, this study indicated that crab fed diet of high-fat could reduce growth, feed utilization and antioxidant capacity. In addition, different lipid levels of diet could strongly influence on enzyme activity and relevant gene expression levels of lipid metabolism (anabolism and catabolism) of the juvenile swimming crab.
Effects of dietary fish oil replaced by soybean oil on growth, biochemical and antioxidant responses, fatty acid composition and related gene expression of inflammation of juvenile large yellow croaker (Larimichthys crocea)

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A 70-day feeding trial was conducted to investigate the effects of dietary fish oil replaced by soybean oil on growth, biochemical and antioxidant responses, fatty acid composition and related gene expression of inflammation in the liver of large yellow croaker (initial weight 15.87±0.08g). Four isoproteic and isolipidic experimental diets were formulated with 0% (the control group), 33.3%, 66.7% and 100% replacement of fish oil by soybean oil. Results showed that there were no significant differences between fish fed diets less than or with 66.7% soybean oil and the control group in specific growth rate (SGR), feed efficiency (FE) and hepato-somatic index (HSI) (P>0.05). However, fish fed with the 100% soybean oil diet had lower growth performance than the control group (P<0.05). When fish feed 100% soybean oil diet, total triglycerides, low-density lipoprotein-cholesterol contents and alanine aminotransferase significantly increased (P<0.05), whereas high-density lipoprotein-cholesterol content significantly decreased (P<0.05). Superoxide dismutase and catalase capacity were significantly lower in fish feed 100% soybean oil diet compared to the control group, while fish fed diet with 66.7% soybean oil had no significant difference (P>0.05). The fatty acid composition in the liver and muscle of the large yellow croaker was well correlated with dietary fatty acid profile. The concentrations of C18:2n-6 and C18:3n-3, except EPA and DHA in the muscle and liver, were elevated with the increasing dietary soybean oil levels. The expression of the genes related to inflammation didn’t showed a significant increase in IL-1β, IFN-γ, TNF-α and IL-6 in the liver of fish fed diet with 66.7% soybean oil compared with the control (P>0.05), but in fish fed the diet with 100% soybean oil, the IL-1β, IFN-γ, TNF-α and IL-6 mRNA relative expression were significantly increased (P<0.05). Results of the present study suggested that 66.7% of fish oil could be replaced by soybean oil without significantly influence the growth, biochemical and antioxidant responses, related gene expression of inflammation of juvenile large yellow croaker.
EFFICACY OF NOVEL LONG CHAIN OMEGA-3 CANOLA OIL TO REPLACE FISH OIL IN PRACTICAL DIETS OF PACIFIC WHITE SHRIMP

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The rapid expansion of the aquaculture feed industry, is paralleled by an increased demand for quality feed ingredients which includes fish oil. Presently the industry uses around 78% of the world fish oil supply resulting in major limitations. There are a number of reasons to use marine oils; however, the primary driver is the high level of essential fatty acids, which are not present in most terrestrial oil source. However canola has been modified to contain significant levels of long chain polyunsaturated fatty acids, which could serve as a replacement for marine oils. Hence, the present study was designed to determine the efficacy of modified canola oil to replace the fish oil in practical diets of L. vannamei. Eight test diets were formulated to be iso-nitrogenous and iso-lipidic (360 g/kg protein and 80 g/kg lipid), while the 54.5 g/kg fish oil in the basal diet was incrementally replaced (25, 50, 75 and 100%) with novel canola oil (diet 2-5 respectively) and generic canola oil (50, 75 and 100% replacement in diet 6-8 respectively). Each diet was fed to three replicate groups of 10 shrimps/diet (initial weight= 1.71 g ± 0.03) in a semi-recirculatory system for 49 days. During the experiment, shrimp were fed four times/day assuming a FCR of 1.8. There were no significant difference observed for survival and whole body lipid content between tested diets. Shrimp fed with fish oil, 25, 50, and 75% novel canola oil diets had significantly higher weight gain then shrimp fed with 100% fish oil replacement diets and 75% generic canola diet. Results reflect the potential use of novel canola oil up to 75% replacement of fish oil for the diet formulations of shrimp without compromising the growth. Fatty acid profiles of whole shrimp paralleled that of the test diets with the exception of docosapentenoic acid (DPA), which increased inversely at 50, 75 and 100% replacements of novel canola oil in diets.
Seaweeds are a renewable and non-traditional raw material that could be used as a sustainable feed ingredient to face some of the environmental challenges in aquaculture production. Seaweeds are a rich source of nutrients and, a source of various bioactive compounds, which may have health-promoting effects in both humans and animals. Zebrafish (Danio rerio) is an attractive animal model for the investigation of immune and other health-related functions in monogastric animals, including fish as well as humans. The tools available for the study of the biology of this model are numerous allowing thorough investigations of basic mechanisms.

Thus, a feeding trial was conducted with zebrafish to evaluate the health-promoting effects of two seaweeds: Saccharina latissima (Sac) and Palmaria palmata (Pal). A total of five diets were evaluated, one control diet without seaweed, two diets containing Sac products, i.e. whole Sac and a protein concentrate of Sac, and similar for the Pal. All the seaweed products had an inclusion level in the diet of 15%. The feeding experiment was run with four replicate tanks per diet for 8 weeks. At the end of the trial, fish were euthanized, body measures taken and intestinal tissues and contents sampled for evaluation of immune and stress-related genes expression, histomorphology and bacterial microbiota profiles.

No significant diet effects were seen on growth performance and mortality. All sampled fish showed normal (healthy) intestinal morphology. On the other hand, gene expression profiling showed that both seaweed products were able to induce a mild modulation on stress and immune-related genes in the intestine. These findings suggest the presence of bioactive compounds in the seaweeds products evaluated. In order to follow up these results, an on-going experiment using similar seaweed products in a zebrafish inflammation model is being conducted and the results from both experiments will be presented at the conference.
Estimation of Gracilaria verrucosa, Enteromorpha prolifera, Algae Residue and Fungi Residue for juvenile tiger puffer (Takifugu rubripes)

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This study was investigated the effects of Gracilaria verrucosa, Enteromorpha prolifera, Algae residue, Fungi residue on growth performance, body composition, serum biochemical indices of juvenile tiger puffer (Takifugu rubripes). The control diets contained 60% and 45% fishmeal, and other four diets containing 30% fishmeal were supplemented with 10% four kinds of seaweeds or residues, which were named as FM1, FM2, GV, EP, AR, FR, respectively. Each diet was fed to three replicates of 25 tiger puffer with an initial body weight of (17.70±0.37)g for 56d. The results showed that weight gain rate and special growth ratio of EP group were significantly higher compared with that of FM1, FM2, GV and FR group (P<0.05), while there was no significant difference between EP and AR groups (P>0.05). Survival rate in fish fed AR diet was the highest among all the groups and significantly higher than that of fish fed FM2 diet (P<0.05). The feed intake decrease significantly in FM1 and GV groups, but increased in FM2 and FR groups (P<0.05). There was no significant difference in feed efficiency among FM1, EP and AR groups (P>0.05), but significantly higher than that of FM2 group (P<0.05). Hepatosomatic index and viscerosomatic index in AR group was the highest among all the groups. For amino acid composition in muscle, the levels of most amino acids increased significantly in GV group, but decreased in FM2 group (P<0.05). Compared with FM1 and FM2 groups, high density lipoprotein cholesterol decreased significantly in GV and FP group, low density lipoprotein cholesterol decreased in AR and FR group (P<0.05). The level of triglycerides in AR group was significantly higher than that of other groups (P<0.05). For serum non-specific immune, the activity of acid phosphatase in FM1 group was significantly higher than that of EP group, while the activity of alkaline phosphatase in FM2 group was significantly lower than that of GV, EP and AR groups (P<0.05). In conclusion, above results show that compared with Gracilaria verrucosa and Fungi residue, Enteromorpha prolifera and Algae residue have more beneficial effect on the growth performance and non-specific immune of tiger puffer.
Microalgae has been used over the years in the food and non-food industry due to its properties and biological activity. In aquaculture, the search for raw sources as novel ingredients for feed is a main goal due to its increased expansion and the necessity to reduce wild-caught forage fish in aquafeeds. In this work the cytotoxic activity of five different microalgae biomass (Tetraselmis suecica, Tisochrysis lutea, Scenedesmus almeriensis, Nannochloropsis gaditana and Porphyridium sp.) and a cyanobacterium (Arthrospira platensis) was evaluated. Cytotoxic activity was determined for microalgae concentration ranged from 0.0195 to 10 mg mL$^{-1}$. An MTT test was carried out on two cell lines SAF-1 (fibroblast-like culture derived from the marine fish gilthead seabream) and DLEC (derived from early embryos of the European seabass).

Tests indicated that none of the microalgae studied showed cytotoxicity for these cell lines (Figure 1). In the case of the SAF-1 cell line, the cyanobacteria Arthospira platensis, and the eukaryotic microalgae Nannochloropsis gaditana and Tetraselmis suecica stimulated cell proliferation. Same results were obtained with Arthospira platensis, Tetraselmis suecica, Scenedesmus almeriensis and Nannochloropsis gaditana for the DLEC cell line.

The concentrations of heavy metals by X-ray diffraction of the different biomasses were studied. It was observed that none of them exceeded the levels of arsenic, cadmium, fluoride, lead and mercury marked in Directive 2002/32 on undesirable substances in animal feed.

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Evaluation of microalgae biomass as feed ingredient for aquafeeds: chemical characterization

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The expansion of aquaculture requires research efforts focus on the search of novel feed ingredients. This work focussed on the characterization of the biomass of different microalgae (Tetraselmis suecica, Tisochrysis lutea, Arthrospira platensis (Cyanobacteria), Scenedesmus almeriensis, Nannochloropsis gaditana, Dunaliella salina, Chlorella sp., Porphyridium sp., and Chaetophora sp.) and their potential use as dietary ingredient for aquafeeds. Crude protein and total lipid contents, amino acid profile, polyphenolic content and antioxidant capacity were determined by standard methods. In addition, presence of protease inhibitors in microalgae biomass with capacity to inhibit digestive proteases of seabream (Sparus aurata) and Senegalese sole (Solea senegalensis) was also evaluated.

The protein content ranged from 20.0% (Porphyridium sp.) to 52.3% (D. salina), and the lipid content varied from 3.8% (Chlorella sp.) to 20.3% (D. salina) (Table 1). Amino acid profiles were similar to those from fishmeal protein, especially in A. platensis, S. almeriensis and N. gaditana, with minor difference in some essential amino acids such as lysine and methionine which are usually limiting amino acids in plant protein sources (Figure 1). The total polyphenol content was higher in T. lutea and N. gaditana. The differences in phloroglucinol and gallic acid equivalents for these species were up to two and four times greater than those found for T. suecica, A. platensis, Porphyridium sp., D. salina and Chaetophora sp., and those obtained for S. almeriensis and Chlorella sp., respectively. The highest antioxidant capacity in A. platensis and T. lutea doubled the value obtained for other strains. Meanwhile, results of the inhibition assay confirmed the absence of protease inhibitors in microalgae samples.

In general, results obtained revealed that most of the microalgae strains tested could be used as ingredient for aquafeeds. The high protein content joined to the balanced amino acid profile observed makes microalgae an interesting ingredient with possibilities to enhance the nutritional value of aquafeeds.

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Evaluation of microalgae biomass as feed ingredient for aquafeeds: In vitro protein hydrolysis by digestive proteases of marine fish

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Microalgae suppose interesting alternative ingredients for aquafeeds due to their high protein content and balanced amino acid profile. However, beyond chemical composition, nutrient bioavailability is an important aspect defining the nutritional quality of novel feed ingredients. In this regard, this work aims to assess the in vitro protein hydrolysis of different microalgae strains (Tetraselmis suecica, Tisochrysis lutea, Arthrospira platensis (Cyanobacteria), Scenedesmus almeriensis, Nannochloropsis gaditana, Dunaliella salina, Chlorella sp. and Porphyridium sp.) by the digestive proteases of seabream as reference marine fish. The in vitro protein hydrolysis assays were carried out according to Sáenz de Rodrigáñez et al. (2011). Protein degradation was monitored at different times by electrophoretic techniques for obtaining a quantitative coefficient of protein hydrolysis. In addition, total amino acids released by digestive enzymes were also quantified. Soybean meal and fishmeal were used as reference protein ingredients.

At the end of the enzymatic hydrolysis, in vitro hydrolysis values were higher than 50% in most of the microalgae tested (except for Porphyridium sp.), reaching values higher than 70% in A. platensis, D. salina and Chlorella sp. (Figure 1A). These values were similar to those obtained for regular ingredients, such as soybean meal and even fishmeal, which suggests a high bioavailability of microalgae proteins (Sultana et al., 2010; Hernández et al., 2015). Overall, seabream proteases were able to release from 4.5 to 24% of total amino acids contained in microalgae proteins. A. platensis, D. salina and Chlorella sp. reached the highest accumulative values of free amino acids (22.9, 21.0 and 23.9%, respectively) which are similar to those obtained for fishmeal. On the contrary, the lowest values were obtained for Porphyridium sp. (Figure 1B).

The results obtained confirmed that microalgae are potential protein sources for aquafeeds, although further research about the structural complexity and the amino acid bioavailability are needed to understand the differences observed during the in vitro enzymatic hydrolysis.

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Evaluation of microalgae biomass as feed ingredient for aquafeeds: cell wall disruption with exogenous enzymes

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The level of cellular wall breakdown is an important factor that can limit microalgae nutrient bioavailability within the gut of monogastric animals, including fish. The unappreciable digestive cellulose activity in the gut of marine fish hinders the efficient utilization of microalgae intracellular nutrients. Genus like Nannochloropsis or Scenedesmus presented a rigid cell wall extremely resistant, providing limited soluble protein content susceptible to be hydrolysed by fish enzymes. This work aims to assess the effectiveness of using a commercial cellulase from Aspergillus niger for cell wall disruption in two microalgae species (Nannochloropsis gaditana and Scenedesmus almeriensis). Enzymatic hydrolysis was carried out using an in vitro model. Four different enzyme to microalgae ([E]/[S]) ratios were applied. The amount of microalgae was 1.0g with increasing levels of cellulase from 0 to 80mg. The enzyme–microalgae biomass mixtures were transferred into jacketed reaction vessels containing 50mL of 100mM sodium citrate buffer solution (pH 4.5), and incubated at 37ºC under continuous agitation for 24h. Samples were withdrawn at different times and immediately immersed in a hot water bath (100ºC) for 5 min for stopping the enzymatic reaction. To understand the degree of the hydrolysis process, the evolution of total reducing sugars released from microalgae was evaluated using the dinitrosalicylic acid (DNS) method according to Miller (1959). In addition, total amino acid released was also quantified.

Quantification of reducing sugars revealed a significant increase in glucose concentration when increasing [E]/[S] ratio, reaching the highest values after 3 and 6 h in N. gaditana and S. almeriensis, respectively (Figure 1). Overall, bioavailability of aminoaicds contained in microalgae biomass increased 6 to 10% when compared with the same assay carried out in absence of cellulase.

An enzymatic pre-treatment with cellulase represents a useful tool for weakening microalgae cell wall, and to facilitate the action of fish digestive proteases increasing nutrient bioavailability, although further studies are needed.

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Assessing algal biomasses as potential ingredients in microdiets for Senegalese sole (Solea senegalensis) larvae

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Algal biomasses provide a particularly interesting nutritional value (e.g. protein, LC-PUFA, minerals, carotenoids) for inclusion in microdiets for fish larvae. In this study, three trials were conducted aiming at screening the suitability and evaluating the biological efficacy of microalgae in microdiets for Senegalese sole. In Trial 1, eight species of microalgae and macroalgae were assayed during a 3 week period. Sole larval growth and survival were evaluated, together with a set of immune and oxidative stress biomarker genes. In Trial 2, sole larvae were reared from 16 to 43 days after hatching (DAH) and four treatments were tested. A commercial diet was used as Control, whereas the remaining treatments included the supplementation of the Control diet with: 5 % Phaeodactylum tricornutum (whole cells); 5 % Phaeodactylum tricornutum (broken cells); 10 % Phaeodactylum tricornutum (broken cells). Results showed that including 10 % Phaeodactylum tricornutum (broken cells) in a microdiet for sole larvae significantly increased its growth performance, although no significant differences were observed in survival. Furthermore, no significant differences were observed on larval growth and survival between Control and the remaining treatments where the dietary inclusion of Phaeodactylum tricornutum was evaluated. In Trial 3, sole larvae were reared from 16 to 65 DAH under two different dietary treatments. In the Control treatment, the same commercial diet used in Trial 1 was adopted, whereas in the second treatment this diet was supplemented with 15 % Chlorella sp. The dietary inclusion of Chlorella sp. resulted in a significant reduction of growth performance and survival in sole larvae when comparing to results obtained in the Control treatment. In overall, this study shows that microdiets for sole larvae can be improved by including 10 % of Phaeodactylum tricornutum (broken cells). This study also suggests Chlorella sp. has less potential, at least at high inclusion levels, in sole larval microdiets. The potential benefits of including algae in microdiets for fish larvae should be evaluated in a case-by-case scenario, reinforcing the need to perform a screening of different algal species at various dietary inclusion levels to assure its advantages for larval development in fish species.
Replacement of fish oil with a mixture of microalgae meal (Schizochytrium limacinum and Nannochloropsis oceanica) in diets of rainbow trout (Oncorhynchus mykiss) post-smolts: Implication on growth performance, health and product quality.

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The effect of dietary inclusion of a mixture of heterotrophic microalgae meal on growth performance, histology, muscle fatty acid composition, metabolic gene expression and fatty acid digestibility was investigated in an 8-week bioassay with post-smolt rainbow trout (Oncorhynchus mykiss) (initial body weight of 189.1 ± 4.5g) reared in saltwater conditions. Three experimental extruded diets were formulated to include 0%, 9% and 17% respectively of a mixture of microalgae meal (Schizochytrium limacinum and Nannochloropsis oceanica) (1:1 ratio) but with the same levels of total n-3 HUFA (approximately 20g/kg diet). Each diet was evaluated in triplicate groups and fed by hand, to apparent visual satiety twice a day. Faeces were collected daily from each digestibility tank by decantation. Growth performance was reduced as a result of increasing the dietary inclusion of a mixture of microalgae meal. No significant trends were observed with respect to feed utilization and liver and distal intestine histology. However, ongoing analysis is being undertaken of fatty acid digestibility, muscle fatty acid composition and SCARB1, CAT-B, CAT-L and MYO gene expression profiles that are directly associated with dietary n-3 HUFA sources. Additionally the results of this research will contribute to demonstrate that a mixture of heterotrophic microalgae meal has a potential to be included within commercial rainbow trout diets as a sustainable replacement of fish oil.
Effects of dietary fish oil replacement by microalgae, Schizochytrium sp. on growth performance, body composition and fatty acid profile of juvenile red seabream, Pagrus major

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Our former studies suggested that DHA (docosahexaenoic acid) rich microalgae Schizochytrium sp. (algae meal) can be appropriate substitute of fish oil and was suitable lipid source of non-fish meal and non-fish oil diet. The objective of this study was to determine the optimal formulation level of the algae meal in non-fish meal diet.

Six iso-nitrogenous (45%) and iso-lipidic (13%) experimental diets were prepared. Control diet was formulated with fish meal (40%) and fish oil (6%). Plant protein sources (soy protein concentrate, soybean meal, corn gluten meal) were used in the second diet as substitute of fish meal and fish oil was used for lipid source [NFM+FO]. In the third diet, fish oil of NFM+FO was replaced by rape seed oil [NFM+NFO] as negative control. In the other three diets, the rape seed oil in NFM+NFO diet was replaced with algae meal (Schizochytrium sp. powder) at 5%, 10% and 15% [AM5, AM10, AM15]. Triplicate groups of juvenile red seabream (8.8g) were fed the experimental diets for 12 weeks to near satiation.

The growth was the lowest in the fish fed NFM+NFO. It was improved by formulation of algae meal and reached to the growth level of NFM+FO in 10% algae meal group [AM10]. Lipid content of whole body in the fish on NFM+NFO group was significantly lower than the other groups. Fatty acid profile showed significant differences among dietary treatment. Fatty acid profile of liver polar lipid showed that DHA was highest in the fish fed AM10. The results might suggest that microalgae might be a candidate material for replacement of fish oil diet, and optimal level might be 5~10%.
Effects of glucose-glycine melanoidins on apparent digestibility coefficients of minerals in the rainbow trout Oncorhynchus mykiss

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Thermal treatments of animal feeds and dietary raw materials can lead to the formation of molecules affecting digestive, absorptive and/or metabolic processes in animals. Melanoidins are non-enzymatic browning products with an effect on the absorption and retention of minerals in mammals, probably due to their negative charge, but no comparable data are available for cultured fish. Thus, the present work is aimed at exploring the effects of glucose-glycine melanoidins on apparent digestibility of minerals in the rainbow trout.

Melanoidins were produced from a solution of glucose 1M, glycine 1M, and NaHCO3 0.1 M, heated to 103°C for 24 hours, were isolated by acidification and included at 0.0% or 1.2% in a basal diet prepared without heated ingredients. The two diets were isonitrogenous (~50%), isoenergetic (23.5 MJ/kg) and included 0.75% Cr2O3. Each diet was supplied to three tanks with 25 juvenile trouts (24 g) for 17 days. Settled feces were collected daily for days 11-17th, freezed at -20°C, lyophilized and stored at -80°C. Diets and feces were analyzed for chromium and minerals, apparent coefficients of digestibility (ADC’s) were calculated according to the inert-marker procedure and subjected to t-tests.

ADC’s of Na, K, Ca, Mg, Cu, and Mn showed no statistical differences between diets. ADC’s of Zn and Fe were statistically higher in the presence of melanoidins: 28.6 ± 0.7% vs. 23.2 ± 1.0% for Zn (p=0.007), 12.6 ± 1.7% vs. -11.2 ± 1.3% for Fe (p<0.001) (mean ± sem). The improvement of iron absorption in rats in the presence of bread crust high molecular weight browning products previously obtained in rats and is in keeping with the results herein reported. On the other hand, copper absorption in rats was also improved in the presence of glucose-Lys or glucose-Met melanoidins, but this variable remained statistically unchanged in the present experiment. It can be concluded, that sugar-amino acid melanodins can increase the digestibility of some cationic minerals in the rainbow trout, but mechanisms and the metabolic fate of these minerals warrant further research.

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Efficacy Of Availa®Zn And Availa®Se For White Shrimp (Litopenaeus vannamei)

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A study of Litopenaeus vannamei (initial wt. = 4 g) was conducted as a complete randomized design (CRD) with 6 replications (tanks) of each treatment, and 25 shrimp per replication. Treatments were arranged as a 2 × 2 factorial, utilizing two zinc sources [zinc amino acid complex (ZnAA) or ZnSO4] and two selenium sources [zinc-L-selenomethionine (SeAA) or sodium selenite (Na2SeO3)] (Availa®Zn, Availa®Se; Zinpro Corporation, Eden Prairie, MN, USA). Shrimp were fed pelleted diets 4 times daily, for eight weeks, at 2.5 to 3% body weight. Shrimp consuming diets containing ZnAA and Na2SeO3, along with shrimp fed ZnAA and SeAA, had greater (P < 0.05) body weight than shrimp fed inorganic sources of Zn and Se. Total hemocyte and phenol oxidase activity were increased (P < 0.001) when ZnAA or SeAA were present in the diet. Whole shrimp drip loss was decreased for shrimp consuming diets that included ZnAA or SeAA (P < 0.05). Rancidity of shrimp meat was decreased (P ≤ 0.10) by feeding either ZnAA or SeAA. Redness of fresh shrimp meat after 72-h chill, and once boiled post-72-h chill or post-14-d freeze increased (P = 0.03, 0.06, and 0.07, respectively) when shrimp were fed diets supplemented with SeAA. Overall, inclusion of Availa-Zn zinc amino acid complex and/or Availa-Se zinc-L-selenomethionine was beneficial for shrimp growth and some aspects of both immunity and shrimp meat production.
Impact of ionic composition in the intestinal fluid of salmonids and amino acids on solubility of dietary zinc in vitro

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Bio-availability of minerals in fish feeds is to a certain extent linked to their solubility as only minerals in the soluble fraction are accessible for uptake by the intestinal epithelium. In the aquatic environment, solubility and further speciation of minerals is dependent on the ionic chemistry and organic components in the water. It was hypothesised that a similar relation might prevail in the gastro-intestinal environment of freshwater (FW) and seawater (SW) adapted salmonids leading to differential solubility of dietary minerals. Therefore, in vitro tests were conducted to study the same on solubility of dietary zinc (Zn).

Enzymatic and non-enzymatic assays were undertaken: for the enzymatic assays, feed samples (0.5g) were treated with alkaline protease, phytase or both in FW or SW buffer (reaction volume, 8 ml; pH: 7.3; time, 18h) at room temperature (21-22°C); for the non-enzymatic solubility tests, feed samples (0.2g) added with radiotracer (65Zn) of known specific activity, dissolved in FW or SW buffer (reaction volume, 3 ml; pH: 7.4; time, 30 min) to study the solubility of supplemented Zn and the impact of amino acid chelation. The soluble and residual fractions were separated by centrifugation at 3000 rpm for 10 min. The concentration of Zn and activity of 65Zn in the fractions were measured by ICP-MS and gamma teller, respectively.

Solubility of ingredient-bound Zn was significantly reduced by 3-4 fold in SW gut ionic condition compared to FW. Solubility of supplemented 65Zn decreased with increasing ionic strength of the reaction buffer and the relation was quadratic. Presence of amino acids increased the solubility of supplemented 65Zn in a dose-dependent manner and was higher in FW compared to SW gut ionic conditions. Cysteine, histidine and lysine improved 65Zn solubility in both FW and SW; whilst methionine was effective only in FW and the contrary was observed for glycine. Langmuir adsorption model showed that the equilibrium constant (Keq) for Zn-cysteine complex formation was 4-fold higher in SW gut ionic conditions, compared to FW. Overall, in vitro solubility of dietary Zn varied under simulated conditions of ionic composition in the gut lumen and in the presence of amino acid ligands.
Dynamics of the Digestion of Phosphorus in Rainbow Trout (Oncorhynchus mykiss) with Emphasis on Phytate and Bone Phosphorus

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Phosphorus (P) digestibility varies considerably as a function of the dietary chemical P forms present and interactions with other dietary components (e.g. calcium) and additives (e.g. phytase). There is a need to develop a better understanding of dynamics of digestion of different P forms and model this process to eventually develop more effective dietary strategies.

A study was conducted to examine the time course of P digestion in different compartments of the gastro-intestinal tract (GIT) of rainbow trout. Triplicate groups of 15 fish (IBW=140g/fish) were fed nine diets supplemented or not (basal diet) with different P forms (0.15%phytate-P; 0.30% phytate-P; 0.15%phytate-P+phytase(2,000FTU); 0.30%phytate-P+phytase(4,000FTU); 0.25%tricalcium-P; 0.50%tricalcium-P; 0.25%tricalcium-P+0.1%Ca; 0.50% tricalcium-P+0.4%Ca) for 28 days. Tricalcium-P was used as an analogue of bone-P. Yttrium oxide was included at 100 ppm as digestion indicator.

Prior sampling, fish were fed a diet containing 0.8% digestible-P but devoid of phytate-P, bone-P and inert marker for three days. Fish were then fed one meal of their respective experimental diets. Digesta from the stomach (ST), proximal intestine (PI), and distal intestine (DI) were sampled at 0.5, 1, 2, 4, 8, 16, and 24h postprandial. The concentration of plasma phosphate (plasma-Pi) and pH in ST, PI, and DI were also evaluated during the same sampling times.

Digestion indicator appearance was observed at 0.5, 8 and 16h postprandial in ST, PI, and DI, respectively. The results show that phytase increased the apparent digestibility coefficient (ADC) of P (ADC-P) in PI at 8, 16, and 24h postprandial, but not in other compartments of the GIT. The supplementation of 0.4% Ca reduced the ADC-P of tricalcium-P in PI at 24h postprandial. In addition, both levels of Ca supplementation decreased ADC-P in PI at 8, 16, and 24h postprandial when compared to the basal diet. An increase in plasma-Pi concentration was observed at 8h and this increase continued until 24h postprandial. Gastric pH decreased linearly, whereas intestinal pH increased linearly, with time.

These results strongly indicate that PI is the main site for P digestion and absorption in rainbow trout. These observations will be used to calibrate a dynamic model of P digestion in development.
Effect of bile salt supplementation on the fat digestibility of non-starch polysaccharide containing diets in rainbow trout (Oncorhynchus mykiss)

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A previous study (unpublished) with rainbow trout showed that fat digestibility of a fish meal-based diet fed ad libitum decreased with increasing non-starch polysaccharide (NSP) level. This decrease in fat digestibility was negatively correlated with faecal bile salt loss, which led to the hypothesis that endogenous bile salt synthesis might become limiting for the replenishment of the total bile salt pool in high NSP diets fed ad libitum. This study investigated the effect of bile salt supplementation on fat digestibility under both restricted and ad libitum feeding conditions. It hypothesised that reduced fat digestibility, caused by a reduction of the total bile salt pool, can be remediated with dietary bile salt supplementation. The experiment was setup in a 2x2 factorial design, with NSP level and emulsifier level as independent factors. The bile salt taurocholate was chosen as emulsifier. Four isolipidic diets were formulated; diet 1: fish meal-based diet 0% NSP + 0% emulsifier, diet 2: fish meal-based diet 0% NSP + 0.2% emulsifier, diet 3: fish meal-based diet 16% NSP + 0% emulsifier and diet 4: fish meal-based diet 16% NSP + 0.2% emulsifier. Fish were fed one of the experimental diet for 4 weeks restrictively (1.2% BW/d), followed by 3 week of ad libitum feeding. The apparent digestibility of fat and the faecal bile salt loss were calculated. For the non-emulsified diets, the NSP level significantly (P<0.05) reduced fat digestibility in fish for both feeding periods, although numerically much less for restricted feeding. A negative correlation (Pearson: -0.908; P<0.05) between fat digestibility and faecal bile salt loss was found only for ad libitum feeding of the non-emulsified diets. This could indicate that only during restricted feeding, fish are able to replenish the bile salt pool for the loss with endogenous synthesis. For restricted feeding, fat digestibility of both emulsified diets was similar to that of diet 1. For ad libitum feeding, fat digestibility was higher compared to diet 1. From this study, it can be concluded that bile salt supplementation is an effective solution to restore or even increase fat digestibility hampered by presence of NSP in rainbow trout.
Aquaculture is a promising solution to deal with the increasing world population and the consequent overexploitation of the natural fish stocks. However, most of the farmed species are carnivorous, requiring a high-protein content diet, which increase the production costs and the fishmeal dependence. Substitution of fishmeal for other components, such as vegetable-based diets is then of utmost importance to overcome these issues. Barramundi (Lates calcarifer) is an important aquaculture species but its production is highly dependent on fishmeal, which makes it interesting model for this studies.

In this context, we developed a study to evaluate the effects of two diets: a high-protein content diet (P) and a high-starch content diet (S), in the fish muscle and liver metabolite composition of juvenile Barramundi fish. A Nuclear Magnetic Resonance-metabolomics approach was followed to evaluate metabolome variations. Fish were randomly assigned into the experimental groups (P: n=6; S: n=6), fed with the experimental diets, and kept at 4% 2H-enriched water tanks during 6 days.

Multivariate analysis revealed differences between the muscle-metabolome compositions from S- and P-fed fish. In liver, this analysis showed some similarities between the metabolomes of fish from both groups. Regarding univariate analysis, muscle showed less metabolites with variations between diets than liver. Variations in muscle amino acids composition of S-fed fish could be related with the lower protein-content of the diet. The higher concentration of alanine in this tissue could be indicative of variations on energy availability, essential for growth. In S-fed fish liver, were also observed a decrease in some amino acids, possibly related with the reduced protein intake. The heightened acetate, together with the lower succinate concentration, could be indicative of an increase in lipid oxidation for energy production in these animals. Acetate levels could also be indicative of variation in the microbiota profile. In general, S diet seems to promote variation in the energy-production pathways in liver. However, results appoint to a lower impact of S diet in muscle (fillet) overall composition, which can be indicative of few variations in its final characteristics.
The effects of amylose and amylopectin levels on glucose metabolism of pacu Piaractus mesopotamicus

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Starch is the main digestible carbohydrate source in fish diets. It contains two types of glucose polymers, amylose, a linear chain of glucose units joined by alpha-(1,4) glucosidic linkages, and amylopectin, a branched-chain structure with glucose units connected by both alpha-(1,4) and alpha-(1,6) links. Pacu is a very interesting model on carbohydrate metabolism studies due to its omnivorous – frugivorous feeding habit. Six isonitrogenous and isoenergetic diets were formulated with 39% starch, containing different amylose and amylopectin levels: 0/39, 7/32; 10/29; 20/19; 27/12 and 29/10 (% in the diet). Each diet was fed to four replicate groups of pacu, until apparent satiation, three times a day, for 90 days. At the end of the feeding trial, the fish were anesthetized with eugenol (0.1 g/L) and blood was collected and liver was removed and immediately stored in nitrogen liquid. Blood glucose and hepatic activities of hexokinase (HK; EC 2.7.1.1), glucokinase (GK; EC 2.7.1.2) and pyruvate kinase (PK; EC 2.7.1.40) were determined. GK hepatic activity was higher (P<0.05) in fish fed diets containing more than 19% of amylopectin (diets 0/39; 7/32; 10/29 and 20/19) than in animals fed with other diets. Blood glucose ranged from 4.6 to 5.6 mmol/L without any significant difference. No significant differences were observed in hepatic activity of HK and PK, among dietary treatments. Considering that GK expression and activity in several fish species is under nutritional control, our results suggest that the amylose and amylopectin levels affect the glycolytic pathway in pacu and it could be related to the greater glucose availability from amylopectin. We hope that other physiological indicators could confirm our findings.
Effects of taste components on growth performance and digestive function in Red seabream (Pagrus major) fed free fishmeal soybean concentrate-based diet

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Soy protein concentrate (SPC) is a purified product that has low anti-nutrient factors, so it is a good candidate of fish meal (FM) replacer. However, a high inclusion of SPC in diets results in reduction of feed intake and growth performance.

This study revealed that low growth performance of red sea bream (Pagrus major) fed SPC diet was not only simply related to a reduction of feed intake but also low feed utilization possible associated with digestive enzyme functions.

In a growth study, two dietary treatments, satiation feeding of SPC diet and pair-fed feeding of fish meal diet (same amount of FM diet based on daily feeding rate of SPC fed fish) of fish were fed for 6 weeks. Growth performance, viscera fat content and carcass lipid content of SPC diet fed fish was significantly lower than paired-FM diet fed fish (P<0.05). Lipase activity in digesta of SPC diet fed fish was significantly lower than paired-FM diet fed fish (P<0.05). Low lipase activity in digesta indicated abnormal digestive function in SPC diet fed fish that related to low lipid utilization and low growth. In a digestive enzyme study, effect of feeding stimulant either synthetic (SFS) or natural (NFS) on digestive enzyme secretion was examined. The SPC diet with SFS fed fish had a higher digestive enzyme secretion than without SFS diet fed fish. Moreover, the SPC with NFS diet fed fish had greater and faster digestive enzyme secretion than with SFS diet fed fish. NFS is more effective than SFS due to it could stimulate digestive function via both gustatory and olfactory systems.
Effects of dietary astaxanthin on growth performance and lipid accumulation of juvenile tiger puffer
*Takifugu rubripes*

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A 74-day feeding trial was conducted to investigate the effects of dietary astaxanthin on growth performance and lipid accumulation of juvenile tiger puffer. Four experimental diets differing only in astaxanthin concentration (0, 50, 100, and 500 mg kg\(^{-1}\)) was formulated, designated as C, ASTA50, ASTA100, and ASTA500, respectively. Each diet was fed to triplicate tanks of 30 fish. The results showed that group ASTA50 has significantly higher fish weight gain than group ASTA500, while groups C and ASTA100 had intermediate values. The feed efficiency ratio and survival in group ASTA500 was significantly lower compared to other groups. The crude lipid content in liver was significantly higher in group ASTA50 compared to other groups, while group ASTA500 had the lowest lipid content. However, group ASTA500 showed the highest lipid content in muscle, which was significantly higher compared to groups C and ASTA100. Compared to group C, groups ASTA100 and ASTA500 had significantly lower glucose contents in serum. The activities of glutamic-pyruvic transaminase and glutamic oxalacetic transaminase in group ASTA500 were higher than those in other groups. Group ASTA50 tended to have higher contents of saturated fatty acids but lower contents of n-3 polyunsaturated fatty acids in liver and muscle compared to other groups. In conclusion, supplementation of 50 mg kg\(^{-1}\) astaxanthin in diet for juvenile tiger puffer resulted in the highest growth performance, while excess dietary astaxanthin (500 mg kg\(^{-1}\)) could have adverse effects. Supplementation of 50 mg kg\(^{-1}\) astaxanthin in diet induced higher lipid accumulation in liver, but 500 mg kg\(^{-1}\) dietary astaxanthin resulted in a high lipid content in muscle.
Effect of automatic feeding system in productivity of White shrimp (Litopenaeus vannamei) farmed in semi-extensive ponds

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Automatic feeding systems could be a good alternative for optimizing personal and management in production of shrimp. Previous works in intensive ponds have shown a positive effect in growth and feed conversion using automatic feeders (Napaumpaiporn et al., 2013).

The objective of current trial was study the performance of White shrimp (Litopenaeus vannamei) fed three feeding systems: manual, time feeder and sound feeder, in a commercial semi-extensive ponds of Ecuador.

Data from one hundred commercial lots were used for the analysis. Size of ponds ranged between 3 and 18 hectares, initial weight of shrimp was 0.1–0.2 grams, and density was around 10 shrimp per square meter. Final average survival was 69%.

Differences in specific growth rate and food conversion ratio were obtained for several feeding treatments. Mean of SGR was 3.58, 3.63 and 4.50 %/d, respectively for manual, time feeder and sound feeder system. Mean of FCR was 2.28, 2.07 and 1.70 respectively for manual, time feeder and sound feeder system.

Results of current trial were in agreement with data obtained by Napaumpaiporn et al. (2013) with White shrimp in intensive pond (Thailand), although these authors reported better growth (0.21 grams per day in comparison with 0.165 g/d in current study) and FCR, probably due to a higher control of feeding in smaller ponds (1 hectare).

Automatic feeding is a good alternative for improving growth and conversion of White shrimp in semi-extensive ponds, although some improvements would be required to improve growth and conversion.
Mechanism on feed intake regulation of Lateolabrax japonicus when fishmeal was replaced by plant protein

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Lateolabrax japonicas, a carnivorous fish, is given fishmeal-based diets and does not thrive when fed plant proteins. Voluntary anorexia is one of the important factors for limiting utilization of plant protein in carnivorous fish species. However, the key factor and mechanism of feeding preference are not clear. Two isonitrogenous and isoenergetic diets were formulated. A basal diet containing 54% fishmeal was used as the control (FM), whereas 100% of the fishmeal was replaced with a plant protein blend (PPB). The fish fed PPB showed anorexia in the first 2w followed by feeding adaptation during 5-8w and compensation during 9-12w. The negative energy balance resulting from anorexia in fish given PPB diet induced up-regulation of ghrelin and down-regulation of leptin transcription in the stomach, along with high plasma ghrelin concentration, which indicated that the peripheral organs were effective at sensing the nutrients deficiency. However, activation of mTOR in the central nervous system (CNS) decreased the phosphorylation of downstream S6K1, accompanied by further up-regulation of POMC and down-regulation of AgRP expression in anorectic stage, indicating that the CNS failed to respond to peripheral hungry. In 4w-3h, a reversal expression of AgRP was occurred, indicated that Lateolabrax japonicas have adapted the plant protein. In 12w-24h, the significantly lower ghrelin and higher leptin in plasma indicated that fish in PPB group were in a relatively lower appetite status compared to the FM group, however, the significant lower POMC mRNA level in hypothalamus could be an important reason for compensatory feeding during this stage. Meanwhile, higher mRNA levels of mTOR, POMC and leptin as well as lower mRNA expression of AgRP and phosphorylation of S6K1 were shown at 3d-3h than fasting 3w treatment. Therefore, the voluntary anorexia induced by plant protein was under different regulation mechanism from feeding inhibition caused by fasting. In conclusion, ghrelin/leptin-mTOR-S6K1-AgRP/POMC pathway can efficiently regulate the feeding behavior of Lateolabrax japonicas from anorexia to adaptation and compensatory even fasting. The results are of great significance to promote the future breeding of a genotype with high tolerance for plant protein.
Dissolved oxygen (DO) level in water is a key parameter to be monitored and controlled in aqua farms since it can impact on survival, health, feeding behavior of farmed fish, and consequently on economical results. In order to study the impact of DO level on growing tilapia, a trial was conducted with normal or low DO levels that can be easily encountered in commercial farm conditions. Tilapia of 71g were reared during 39 days in tanks of 200L with a water flow rate of 2L/min. Four groups with six replicates were compared. In two groups, fish density was 25 fish/tank (D25) whereas it was 50 fish/tank (D50) in the two other groups. For each density, two levels of DO (NORMAL and LOW) were monitored by adjusting the number of aerators. Fish were fed to apparent satiety twice a day and uneaten feed was collected 15 minutes after each meal. For NORMAL DO level, DO reached in average 5.0 and 5.4ppm in the morning and 4.3 and 4.9ppm in the afternoon for D50 and D25, respectively. DO reached in average 3.4 and 3.9ppm in the morning and 2.6 and 3.2ppm in the afternoon for D50 and D25 respectively in LOW DO groups.

Survival was not impacted by DO level or stock density and reached 99.3% in average. Nevertheless, the decrease of 1.6ppm of DO in average in both D25 and D50 led to a decrease of 13% of feed intake in the low density and 24% in the high density (P<0.0001). Growth was impacted in the same proportion (P<0.0001). FCR was not impacted. The trial shows that a decrease of 1.6ppm of DO, even with no observation of mortality problem, can strongly impact feed intake, growth and economical results.
Hardness and disintegration stability of extruded feed affects fish performance

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In salmon farming, high lipid, energy dense extruded feeds are commonly used. Extrusion is a thermomechanical process where technical feed quality is controlled by steam and viscous dissipation of mechanical energy (heat), moisture level, and the physicochemical and rheological properties of the feed ingredients. Technical feed quality may affect fish performance. In the present study we have produced feeds with different hardness and disintegration stability by influencing extruder viscous heat dissipation with the use of different lipid levels in the feed mix. The feeds where thereafter dried and coated to similar lipid levels. Feeds extruded with high lipid levels had lower pellet hardness and higher disintegrating rate compared to feeds extruded with intermediate lipid levels. The feeds were used in stomach in vitro studies, and feeding studies with salmon. Feeds with high disintegrating rate had a faster gastrointestinal (GI) passage rate, significantly from stomach 30min and 1.5h after a meal, and after 9-24h to mid- and hindgut. Stomach in vitro studies showed that pellets with high disintegrating rate required larger supply of stomach acid to maintain a stable pH at 4.5, which created a lower viscosity of the content. The model also showed increased release of water-soluble components and hydrolysis of larger peptides (>500 Daltons) to increase the pool of small peptides for easy transportation, hydrolyzation and absorption in the proximal intestine. Feed entering the stomach and transport of nutrients to the proximal intestine stimulates signals to the brain that regulates appetite and satiety in animals. Feeds with high disintegrating rate fed to 0.5-1.5 kg salmon significantly increased feed intake during 70 days of feeding (Figure 1). Although increased intake of feed with high disintegrating rate is correlated with increased growth and condition factor, it is also observed decreased nutrient digestibility in trout fed similar feed types (Aas et al 2011). The GI passage rate, in combination with an increased GI filling, can affect the ability to hydrolyze and absorb nutrients. The results from the present trials will be presented to elucidate the importance of feed disintegration as a physical pellet measurement in fish feed.
Effects of an unprecedented summer heatwave on the growth performance, flesh colour and plasma biochemistry of marine cage-farmed Atlantic salmon (Salmo salar).

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Global seawater temperatures are increasing and becoming more variable, with consequences for all marine animals including those in food production systems. Farming of Atlantic salmon (Salmo salar) in Tasmania occurs towards the upper end of the thermal tolerance window for this species, and marked effects of temperature on salmon production during Tasmanian summers have been experienced but never empirically investigated. This project aimed to track the effects of elevated seawater temperatures on two different cohorts of fish in farm cages during and following an extreme summer heatwave. The farm site experienced an unprecedented high water temperature event over the 2015-2016 austral summer period, with a peak water temperature of 22.9°C (measured at 5-m depth) and 117 days above 18°C. Fish experienced a temperature-induced voluntary cessation of feeding for approximately 2 months, as well as compromised osmoregulatory, liver and renal function during high temperature periods. Flesh colour declined along the dorsal and ventral regions of the fillet and secondarily along the midline, with over 20% of fish sampled so severe that they completely lost flesh colour during the months of March and April. A return to feeding in autumn occurred faster in some fish and caused a marked bimodal size distribution to appear within each cohort as autumn progressed. There was a strong relationship between fillet colour recovery and residual condition index (RCI). Plasma alkaline phosphatase levels were positively linked with RCI, and thus may represent a useful non-destructive indicator of feeding performance. These findings shed light on the physiological consequences of marine heatwaves on fishes, and they help to direct future research endeavours aimed at mitigating the negative impacts of climate warming on cultured Atlantic salmon.
INTEGRATED MULTITROPHIC AQUACULTURE SYSTEM (IMTA) FOR EUROPEAN SEABASS AND SEA URCHIN PRODUCTION VERSUS MONOPRODUCTION OF EUROPEAN SEABASS

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Integrated multi-trophic aquaculture systems (IMTA) is an environment-friendly model of aquaculture production, involving the simultaneous production of fed and extractive species, recycling the wastes and converting them into valuable products. The present study aims to test the feasibility of a pilot scale outdoors IMTA combining the production of fish (European seabass, Dicentrarchus labrax), invertebrate (sea urchin, Paracentrotus lividus) and seaweed (Ulva sp.), comparing it with monoproduction of European seabass. Six outdoor, independent IMTA systems (2400 L) were implemented, composed by 4 tanks, in a closed recirculation system, each one supplied by a continuous water flow from the previous tank (flow direction: fish, sea urchin, seaweed, returning to fish unit and so on). Trial was conducted for 9 weeks, during winter conditions (October to December), under natural abiotic conditions (average temperate 12ºC; ranging from 6-16ºC). At the beginning of the trial 2 seaweed stocking density were established, in duplicates: 2 and 3 kg/m3; fish initial stocking density was 6 kg/m3 and sea urchin initial stocking density was also 6 kg/m3. In the seabass monoproduction system, no seaweed or sea urchin were included. Fish were fed daily with a commercial diet (42%Prot;18%Lip) and sea urchin were fed twice a week with an agar-based diet (36%Pro;2%Lip). Seaweed stocking density was maintained throughout the trial. At the end of the trial, mono and IMTA seabass production was similar. Fish growth and feed utilization efficiency of monoculture production was similar to that of 3 kg/m3 seaweed IMTA system, and higher than in the 2 kg/m3 seaweed IMTA system. Sea urchin total diameter gain was similar irrespective the seaweed stocking density. Simultaneous production of seabass and sea urchin under IMTA system is feasible, providing seaweed stocking density is adequately established to guarantee maximum growth of fish. This study needs to be performed repeated under Summer conditions to confirm its repeatability under fast growth conditions.

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Insect meal in aquafeeds has just been approved by EU. Insects are highly sustainable nutrient sources and often part of the natural fish diets. Among the most promising insect species identified for industrial production are the black soldier fly (BSF, Hermetia illucens) and the yellow mealworm (TM, Tenebrio molitor) larvae. Defatted meals may result in increased protein content and are increasingly available in the market. Yet, their use has been poorly investigated in European seabass (Dicentrarchus labrax). The apparent digestibility coefficients (ADCs) of two non-defatted (TM, 56\% of crude protein (CP) and 30\% of crude lipid (CL) and BSF, 46\% CP and 21\% CL) and defatted insect meals from different producers (TMd, 69\% CP and 12\% CL and BSFd, 55\% CP and 20\% CL) were evaluated in juveniles. A commercial-based extruded diet with 48\% CP and 21\% CL was used as reference diet (REF) and 1\% chromic oxide was added as inert marker. The test diets were obtained by replacing 20\% of the REF diet by each insect meal. Homogeneous groups of 15 European seabass (mean initial weight 30g) were subjected to a 12-hour light/12-hour dark photoperiod regime and kept in a recirculating salt water system (salinity 35\‰, 20±1°C) with a Guelph system to collect feces. Each diet was randomly assigned to two of these tanks, being the experiment divided into two periods of fifteen days, for replication of results (n=4). All diets were very well digested by seabass. Protein ADC of TM was similar to that of the REF diet (93\%), and all other diets displayed significantly higher values (95\%). TMd was better digested than TM, but BSF showed similar protein ADC values in either non-defatted or defatted. Results show that defatted Tenebrio molitor and Hermetia illucens melas can enhance protein digestibility, and consequently have a huge potential as feedstuffs for seabass diets. Future studies are required to assess the effect of such ingredients in growth performance, nutrients utilisation and immune status of this species. 

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Evaluation of sacha inchi oil as alternative lipid resource in diets for juveniles of rainbow trout, Oncorhynchus mykiss

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EVALUATION OF MARINE BACTERIA THROUGH IN VITRO AND IN VIVO ASSAYS AND THEIR POTENTIAL USE AS FEED ADITIVE AND PROBIOTIC AGENT AGAINST PATHOGENIC Vibrio parahaemolyticus (AHPND) IN WHITE SHRIMP Litopenaeus vannamei

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The shrimp farming industry has faced huge economic losses with bacterial diseases, currently shrimp vibriosis and acute hepatopancreatic necrosis disease (AHPND) are the most relevant and had caused severe mortalities. Recently, it has been studied the potential use of marine bacteria as bioactive compounds producers or probiotics, as an alternative to control aquatic disease. The aim of this study was to evaluate in vitro antagonistic activity of marine bacteria and determine the survival of white shrimp Litopenaeus vannamei fed with a diet covered with bacteria in a challenge bioassay with Vibrio parahaemolyticus (AHPND).

Three isolates were collected from marine environment from the south coast of Sonora, México. The bacteria were characterized (API 20NE, 50CH) and identified (16S rRNA) as Gram (+) spore forming rods, oxidase (-) and catalase (+), belonging to Bacillus pumilus (36R) and B. safensis (13L); and Gram (-) rods, oxidase (+), catalase (-) related to Pseudoalteromonas piscicida (36Y). Protease activity were present in all isolates and none of them present chitinase activity. In vitro antagonism were evaluated by cross streak (CSM), double layer (Dopazo) and broth co-culture, with V. parahaemolyticus strains (ATCC 17802; and MC32, B25, E14V2 AHPND). The biofilm production on microplate revealed that isolates 13L and 36Y (OD 595; 0.192, 0.528, respectively) have good adherence properties. For bioassay, during 21 days shrimps were fed with bacterial covered diet (commercial), 6 treatments by triplicate with 8 shrimps (3g) by 30 l aquaria. For the immersion challenge (48h) the pathogenic strain MC32 V. parahaemolyticus (AHPND) was applied (1.4 X 108 UFC/ml dose). The cumulative mortality was recorded: T1-36R (45%), T2-13L (54%), T3-36Y (54%), T4-Mix 36R-13L (25%), C+ (88%), C- (4%); statistically significant differences were showed between T1, T2, T3 y T4, respect the control groups. The pathogen detection was confirmed by real time PCR, histopathology, and the statistical used was ANOVA.

Shrimp fed with bacteria showed improvement in survival compared with control. This Bacillus and Pseudoalteromonas could be an effective probiotic or biocontrol agent against harmful bacteria in shrimp culture.

Figure 1. Cumulative mortality among the 6 treatments.
European sea bass (Dicentrarchus labrax) quality fed diets containing porcine spray-dried plasma, and tilapia and shrimp protein hydrolysates

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It is generally accepted that the incorporation in larval microdiets of hydrolysates processed from a high-quality protein source and containing a high proportion of short- and medium-sized peptides is beneficial to larval development in terms of enhancement of the gut maturation, reduction of skeletal deformities, and ultimately, improvement of larval growth and survival. In this study, four types of ingredients were tested as alternative sources of the commonly used fish protein hydrolysate CPSP (Control diet), hydrolyzed porcine protein rich in free amino acids and functional proteins (PEPTEIVA, APC Europe SA), a protein product composed of pure porcine albumin and globulin proteins (APPETEIN GS. APC Europe SA) and protein hydrolysates obtained from shrimp (Actipal HP1, Diana Aqua) and tilapia (Actipal HP2, Diana Aqua). All these ingredients were incorporated at 10% (CPSP replacement). Microdiets (100-600 µm) were manufactured by Sparos Lda (Portugal) and contained 63-65% crude protein, 19% crude fat (8% phospholipids) and DHA/EPA = 1%. Diets were tested for 60 days with four replicates per diet at 18 ºC (initial density: 1,000 larvae/L); during the first 30 days larvae were also offered enriched Artemia metanauplii under a co-feeding regime, whereas after then, larvae were solely fed microdiets. At the end of the trial, fish were measured for evaluating their growth in length and weight, and the activity of pancreatic and intestinal enzymes measured for evaluating the impact of tested diets on the maturation and functionality of the digestive system. Finally, the effect of diets on the incidence of skeletal deformities affecting the head, trunk and tail, as well as their severity was also evaluated. The results of different diets in terms of larval performance and quality will be discussed and proper recommendations regarding the use of the tested ingredients provided.
Specialty feeds towards the functional seafood of tomorrow

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Due to the rising demand for nutritious, safe and sustainable seafood products (fish and shellfish), aquaculture is predicted to be a major contributor in fulfilling the nutritional needs of future generations. Over the last decades, a continued research effort led to significant progress on lowering the use of fishmeal and fish oil in aquafeeds. However, this trend is altering the nutritional value of edible fish, conditioning the expected beneficial effects for consumers. Some of the strategies available today to counteract this potential loss of nutritional value in fish comprise the use of novel sustainable feed ingredients, enhance n-3 LC-PUFA (EPA and DHA) retention efficiencies and feed fortification with selected healthy nutrients.

Several trials undertaken with rainbow trout and gilthead seabream evaluated the use of several emergent raw materials (microalgae, macroalgae, yeasts) as tools to fortify fish fillets with health valuable nutrients. In trout, the dietary incorporation of an iodine-rich macroalgae (Laminaria digitata) and a selenised yeast, at the maximum iodine and selenium permitted levels in feed, resulted in a six-fold increase for iodine and a 2.9-fold increase for selenium contents in trout fillets, without altering sensorial traits. The fortified trout presented a nutritional contribution of 12.5% DRI for iodine, 78% DRI for selenium and 80% DRI for vitamin D3. One meal portion of seabream fed a diet with 10% Laminaria digitata covered 84% DRI of iodine and 60% DRI of vitamin D3. Market-size seabream fed a diet with 2.5% of Phaeodactylum tricornutum, a fucoxanthin-rich microalgae, showed a significantly higher lightness in ventral skin and a more vivid yellow pigmentation in the operculum and scored higher than the control, in terms of external appearance and brightness assessed by a panel of 100 consumers. A new perspective integrating consumers’ dietary needs and expectations must be considered when designing aquafeeds.

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Catabolism of Branched-Chain Amino Acids in Tissues of Hybrid Striped Bass, Morone chrysops x M. Saxatilis

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Glutamate in diets is extensively degraded by the small intestine of fish. However, it is among the most abundant amino acids in the body and is likely synthesized by various tissues of fish. The present study was conducted with hybrid striped bass (HSB) to test the hypothesis that leucine (LEU), isoleucine (ILE) and valine (VAL) are degraded in these tissues to generate glutamate. Slices of 10 tissues were obtained from juvenile HSB and incubated at 26 oC for 2 h in oxygenated (95% O2/5% CO2) Krebs-Henseleit bicarbonate buffer (pH 7.4, 5 mM D-glucose) containing for 2 mM [1-14C]leucine, [1-14C]isoleucine, or [1-14C]valine, respectively. Production of 14CO2 and each branched-chain ketoacids (BCKAs) was determined with the use of our established methods. Furthermore, the activities of branched-chain amino acid (BCAA) transaminase, BCKA dehydrogenase and the synthesis of amino acids from leucine in HSB tissues were determined to provide bases for understanding BCAA catabolism. All the fish tissues actively transaminate leucine, isoleucine and valine with α-ketoglutarate to form glutamate and BCKAs, with the highest rate (nmol/g tissue tissue) in the kidney. The highest activity of BCAA transaminase was observed in the heart (P < 0.05), whereas the highest activity of BCKA dehydrogenase in the kidney. In HSB, rates of glutamate synthesis from BCAAs may vary greatly with tissues and further catabolism of BCKAs may involve interorgan cooperation to provide ATP, glucose and lipids.
Effects of fish protein hydrolysate and taurine, alone or combined, on the taurine transport and metabolic profile of juvenile turbot (Scophthalmus maximus L.)

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The objective of the study was to investigate the effects of fish protein hydrolysate (FPH) and taurine, alone or combined, on the expression of taurine transporter (TauT) and metabolic profile in liver and muscle of juvenile turbot (Scophthalmus maximus L.). Firstly, the real-time PCA assay were used to measure the mRNA level of TauT in brian and eye tissues based on the result of tissue-differential expression in turbot. Then, the changes of the metabolic profile in the liver and muscle was analysed using 1H NMR-based metabolomics approach. Results indicated that TauT mRNA was distributed in all examined tissues (liver, spleen, stomach, midgut, heart, headkidney, gills, eye, skin, muscle and brain), and TauT mRNA levels were particularly high in brain and eye among those tissues. TauT mRNA levels in eye and brain tissues of fish fed taurine supplemented diets (FPH0+T and FPH10+T) were significantly lower than that in no added taurine treatments (FPH0 and FPH10). There were 43 metabolites identified in liver. Changing metabolites of liver tissue included taurine, alanine and choline and were mainly involved in taurine and choline metabolism. In muscle tissue, 43 metabolites were identified using 1H NMR-based metabolomics. Changing metabolites included taurine, creatine, glycine, lactate, TMAO (Trimethylamine-N-oxide). And, the metabolites were mainly involved in glycolysis and gluconeogenesis and taurine metabolism. In conclusion, optimal level of dietary FPH may not affect taurine transport and metabolism in liver and muscle tissues when high plant protein based diets whether supplemented with or without taurine, while the energy metabolism of muscle may be affected through up-regulate the level of TMAO metabolites and down-regulate the level of creatine and lactate. In addition, taurine supplemented diets affect the metabolism of taurine by decreased expression of TauT gene, improved the level of taurine metabolite in muscle and liver tissue, either alone or in combination with appropriate amounts of FPH.