



Pseudo-stem by-product from Canarian banana crop (*Musa acuminata* colla): preliminary study on the inclusion for tilapia diets.

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Banana culture is an important productive activity in the Canary Islands producing high amounts of waste, principally pseudo-stem, which is normally let dried on the land as a residue. In a scenario where resources are becoming scarce and more residues are generated, re-thinking and re-designing the way we produce is urgently required for the aquaculture sustainability. The objective of this study is to evaluate the inclusion of four dietary levels of processed banana pseudo-stem on tilapia feed acceptance and growth. Pseudo-stem was processed using two specialized fibre extraction machines. The by-product obtained (BT by-product) was lyophilized and pulverized. Afterwards, it was hydrolysed for 30 min at 100°C with 2% H₂SO₄ according to Elias et al. (2014). The feeding trial was performed at the Aquaculture Research Facilities, belonging to the ECOAQUA Institute of the ULPGC. Twelve fishes per tank (3.9 ± 0.64 g), were randomly distributed in 5 recirculation systems with 3 tanks each (80 L/tank). Fish were manually fed twice a day, six days a week, for 47 days with five isoproteic and isolipidic diets with increasing levels of BT by-product (Table 1). The inclusion of BT by-product decreases significantly tilapia growth as the dietary inclusion increase (Figure 1). Growth parameters in tilapia juveniles may be affected by diets containing high ash ingredients (Köprücü & Özdemir, 2004), which could explain the significant decreasing of growth by BT by-product inclusion. Nevertheless, growth values from diets BT2.5 and BT5 are in the range of growth rate expected for this specie and size (Ahmed et al., 2016; Harmantepe et al., 2016). On the other hand, liver and intestine histopathological analysis reveal no damage caused by the dietary inclusions on these structures according to Genc et al. (2007). According to these preliminary results, BT by-products seems to be a promising raw material to be included in tilapia diets, but further processing is required on this by-product to achieve a high-quality ingredient for tilapia diets. Likewise, studies are on going to better degrade the fibre content and to deeper clarify the fibre and minerals in BT-by product and their implication in the observed tilapia performance.



Assessment of protein and phosphorus bioaccessibility of selected lupin species and varieties by simulating the gastrointestinal digestion of rainbow trout (*Oncorhynchus mykiss*)

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Legumes are considered an indispensable source of proteins and other nutrients. The interest for this type of ingredients in the formulation of diets for aquaculture has intensified in recent years due to the accelerated growth of this industry as well as the need to reduce the high dependence on fishmeal and fish oil. Lupin (*Lupinus spp*) is a large genus of leguminous plants which includes more than 400 recorded species, whose seeds differ considerably in their chemical composition and nutritive value. Different lupin species has been described as viable protein alternatives to replace both fishmeal and soybean meal in aquafeeds without significant deleterious effects on growth performance, nutrient digestibility, health condition and final product quality. In addition to high protein content and a nutritional value comparable to soybean, sweet lupine varieties are characterized by having the lowest antinutritional content among all legumes. However, new methods of evaluation and analysis to establish the nutritional value of the many species and varieties of lupin currently available are essential to optimise their use in aquafeeds.

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 protein and phosphorus bioavailability in six sweet lupin varieties: *Lupinus albus* (Rumbo, Ramses), *L. luteus* (AluProt-CGNA), *L. angustifolius* (Lila) and *L. mutabilis* (Pinta, Baer). The results were contrasted with those obtained with soybean (*Glycine max*, SBM), considered the plant protein reference for aquafeeds to explore their suitability for incorporation in fish diets.

The bioavailability of P from each ingredient after their digestion simulating the gastrointestinal conditions of the rainbow trout resulted in the following order of soluble P release: Baera>Pintaa>Lilaa>AluProt-CGNAab>Rumbobc>SBMbc>Ramsesc. On the other hand, protein bioavailability measured as the total amino acids released from each ingredient resulted in the following order of nutrient release: Baera>Pintaa>Lilab>Rumbobc>AluProt-CGNAbc>Ramsesc> SBMd (different letters indicate p-value<0.05). Based on these results, the possibility of using different lupin varieties as a protein source in aquafeeds and the way to improve their nutritional value based on the factors affecting nutrient bioavailability is discussed.



European marketable lupin varieties as potential protein-rich ingredients in aquaculture

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With fishmeal supply reducing and aquaculture sector expanding worldwide, it urges to seek for alternative protein ingredients. Besides the nutritional value, digestibility and cost, the choice for an ingredient must also consider sustainability aspects. Following this, grain legumes, besides being non-GM low-cost ingredients, are crops adapted to European edaphoclimatic conditions, and can replace both protein and energy dietary sources. Among grain legumes, lupins (*Lupinus* spp.) have the highest levels of crude protein. The knowledge of the nutritional value of marketable European lupin varieties is, therefore, of utmost importance, and a starting point to better take advantage of these ingredients in aquafeeds.

The present work aimed at determining the chemical composition (proximate composition, carotenoids and organic acids profiles) of marketable raw whole dry seed varieties of white (WL, *L. albus*), narrow-leafed (NLL, *L. angustifolius*) and yellow lupins (YL, *L. luteus*), collected from different European companies. Additionally, data already published on the alkaloid content of lupin seeds are referred.

YL presented the highest average crude protein content, followed by WL and NLL (40, 37 and 30 g/100 g, respectively). Seeds fiber levels were high (25-30 g NDF/100 g) and all samples lacked starch; still, energy provided by them was high (18-19 MJ/kg). Lupins are poor sources of lipids (on average <9 g/100 g). Lutein and zeaxanthin were the carotenoids detected; therefore, these seeds provide natural pigments that can improve fish color. WL varieties presented the highest levels of total organic acids (followed by YL), being rich in citric acid; while functioning as diet acidifier, citric acid enhances fish growth and feed utilization. Only five varieties presented less than 0.05 g alkaloids/100 g (sweet), meaning they could be included in diets without any previous treatment.

YL could be interesting to include in aquafeeds given their highest protein content. Despite being also appealing for aquaculture, sweet WL and NLL are more valued for human consumption (e.g. bakery industry). Depending on formulation goals, lupins can be included in fish feed as milled seed or processed. Air-classification technology could provide a protein-rich lupin product, while extrusion could increase the utilization of seeds α -galactosides.



Review Of Recent Research Demonstrating That Low-Gossypol Cottonseed Protein Will Extend The Use Of Fish Meal In The Diets of Several Aquaculture Species

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In addition to fiber for clothing, global cotton production creates about 11 million metric tons of high quality protein. This byproduct of cotton production represents a massive, sustainable, protein resource, but unfortunately it is underutilized by the aquaculture industry due to the presence of the naturally occurring polyphenolic toxin, gossypol. Gossypol severely limits the utilization of cottonseed protein in the diets of all animals. Over the past 60 years various chemical, genetic, physical and biological approaches have been investigated to mitigate the toxic effects of gossypol. Modern molecular biology techniques were recently used to create cotton plants that produce seed containing edible (food-grade) levels of gossypol (<450 ppm). RNAi gene silencing technology and a seed-specific promoter were used to silence the production of gossypol, thus creating an opportunity to supply large volumes of high quality plant-based protein that can cost effectively extend fish meal usage. The process of “deregulation” by FDA and USDA is in the final phase. After the low-gossypol products have been deregulated and proven to be safe and effective, this technology will be deployed to other cotton growing regions. Eventually, high quality cottonseed protein will become widely available. Feeding studies with low-gossypol cottonseed products have recently been conducted with shrimp, pompano, black sea bass, flounder and hybrid striped bass. Without exception, cottonseed protein had excellent palatability characteristics for all species tested. Anecdotal evidence indicates that it most likely contains an unidentified feeding stimulant. Cottonseed protein contains a low level of lysine, compared to fish meal (56% of fish meal lysine), but with the addition of lysine, high protein, low-gossypol, cottonseed products have been shown to effectively replace more than 75% of the fish meal in the diets of those aforementioned species. The use of this sustainable, plant-based protein to extend the use of fish meal will allow the aquaculture industry to continue its rapid expansion, even in the face of declining fish meal supplies. This presentation will provide a review of recent research.



The integrative response of Atlantic salmon to fish meal replacement: from nutrigenomics to physiology

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Aquaculture industry is under pressure to move towards sustainable practices and fish meal (FM) replacement in formulated diets is a priority in aquaculture nutritional strategy. The effects of several alternative raw materials have been under scientific scrutiny over the last decades leading to the development of new diets. In salmonids, partial replacement of FM is a common practice in the industry, but the effects of total FM replacement in the context of commercially reliable diets are still under research.

In this study, the response of Atlantic salmon (*Salmo salar*, 500gr) to different diets with progressive FM replacement was evaluated over three months on a grow out trial, using an approach that integrates molecular, cellular and biochemistry assessments. For this, fish were fed a control diet with high percentage of FM (CHP), a diet with partial FM replacement (COM), or one of the three diets with complete FM replacement differing in the alternative raw ingredients proportions being a diet with higher proportion of ingredients originated by animal by-products (T1), by plants (T2) or a balanced mixed of both animal and plant (T3). Fish intestinal and hepatic transcriptomic responses were assessed by whole transcriptome sequencing (RNA-seq analysis), integrated with tissues histological evaluation, individuals' growth performance and overall health condition determined by a comprehensive panel of biochemical indicators. To infer the capacity of fish to respond to a homeostatic challenge, fish were further exposed to an acute stress and evaluated likewise after 72 hours.

Although there were no differences in FCR among groups, specific growth rate in group T2 was significant lower. Overall, fish presented similar biochemical outputs in terms of energy substrates, enzymes, plasma electrolytes, and nitrogenous compounds. On the molecular level, intestinal transcriptomes were more reactive to new formulations than the hepatic ones, responding with regulation of several processes and pathways, but in a different manner according to the degree of FM replacement. Data indicated that partial and total FM replacement as formulated in this study does not affect fish growth performance, however, the underlying mechanisms triggered to reach the same output are different according to the FM replacement strategy.



Brewers by-products as alternative ingredients for partial substitution of fish meal in aquaculture feed

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Aqua-feeds are formulated considering all the essential nutrients fishes need to keep healthy. They are highly dependent on fish meal (FM) and oil (FO). Alternative ingredients which replace these marine components are required to result in sustainable and economical feeds. However, it depends on improving feed efficiencies and substituting away from FM and FO.

Brewers' by-products stand as a potential alternative for replacing FM in aquaculture feed due to: their availability in Europe (6 million tons of brewer spent grain (BSG) and 0.8 million tons of brewer yeast (BY)); their nutritional characteristics (high content in protein; 50% and 25% in BY and BSG) and the preliminary results which demonstrate no differences in feed intake with 15% inclusion of BY in Senegalese sole feeding.

This project, funded by Life Program (LIFE16ENV/ES/000160), aims to demonstrate the feasibility of the utilisation of BY and BSG in Sea bream and Senegalese sole (as model of sea fish) and trout (as model of fresh fish) feeding, increasing aquaculture sustainability by providing 2 new sustainable raw materials for aquaculture feed.

Firstly, the adequacy of by-products for aqua-feed production will be studied and demonstrated at industrial scale. All legal, technical and administrative requirements for each stage of the value chain will be considered. Secondly, fish growth trials and feeding efficiency will be carried out. In a 1st trial, extreme levels of substitution of the FM with BSG and BY will be tested and, based on these results, these levels will be adjusted. Then, a 2nd trial will test previously adjusted diets to ensure the best growth and health of the fish. Finally, growth in weight and length, histological examination of the intestine and liver and health status of the fish will be evaluated.

The expected result is the provision of 2 new raw materials for aqua-feed producing market, validated throughout 2 fish growth trials at semi-industrial scale.

It will improve the sustainability of the aquaculture in the future by reducing 25 % of the environmental impact of aqua-feed production by replacing at least 15 % of the FM with a new raw material from brewer by-products.



Effects on the growth and blood serum parameters of plant protein concentrates on the diets for rainbow trout (*Oncorhynchus mykiss*) fingerlings.

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Diets with high inclusion of plant protein concentrates were fed to rainbow trout (*Oncorhynchus mykiss*) fingerlings for 60 days. Two experimental diets were formulated, diet CONC contained exclusively soy, rice and corn protein concentrates (200, 200 and 150 g/kg, respectively), while the diet CONC+FM contained the mentioned protein sources (at 200, 150 and 100 g/kg, respectively) and an inclusion of fish meal at 100 g/kg. A commercial diet was used as a control. Each diet was fed to triplicate groups of 30 fingerlings of an initial weight of 0.59 ± 0.004 g (mean \pm SD). Once the feeding period was over, the growth performance (weight gain, WG expressed as % and specific growth rate, SGR expressed as %/day) was determined and then, muscle and liver samples were taken to determine the contents of lipid and protein. Finally, blood samples were taken to determine the glucose, protein and triglycerides concentrations in the serum. The data were analyzed with one-way ANOVA and when significant differences were found, a Fisher's LSD test was performed ($P < 0.05$). The WG and the SGR were higher in the fingerlings fed on the control diet than those fed on the experimental diets, but no significant differences were found (Table 1). Protein deposition in muscle was similar in all groups but significantly lower in the liver of fingerlings fed diet CONC+FM (Table 2). Lipid contents in liver and muscle were similar among the groups (Table 2). Regarding the serum parameters (Table 3) did not show significant differences among the groups, but triglycerides contents were higher in the fingerlings fed diet CONC+FM, while glucose was higher in the fish fed the diet CONC. The present data show the possibility of using the mixture of plant protein concentrates in the diets for rainbow trout fingerlings; however, the inclusion of fish meal to the concentrates did not improve the growth performance and potentially affecting negatively the triglycerides levels in the blood.



Apparent digestibility coefficients of protein feedstuffs for Nile tilapia (*Oreochromis niloticus* L.) under intensive farming conditions

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The digestibility of nutrients and energy in ingredients commonly used for aquafeeds formulation may vary substantially, depending on factors such as fish species, ingredient quality and processing conditions. However, environmental conditions and the stress associated to intensive farming should also be considered since they may affect the ability of fish to efficiently digest the nutrients and energy of the diets. Therefore, a study was conducted with Nile tilapia juveniles (60g) to determine, under intensive farming conditions, the apparent digestibility coefficients (ADCs) for dry matter, protein, gross energy, lipid and the apparent availability coefficients for P, Mg, Mn and Zn in the following ingredients: meat and bone meal (MBM), poultry by-product meal (PBM) and soybean meal (SBM). Test diets consisting of a 70:30 mixture of reference diet to test ingredient were used with chromic oxide (0.1%) as the non-digestible marker. The digestibility trials were conducted in 18-1 m³ net cages. Diets were randomly assigned to tanks of 100 fish and fed thrice daily to satiation for four days. At the end of each day, fish were transferred to adapted net cages for feces collection. Dry matter digestibility of ingredients ranged from 45 to 70%, with the highest ADC value found for PBM, followed by SBM and MBM. Crude protein digestibility was high for all tested ingredients, ranging from 72% for MBM to 92% for SBM. Lipid digestibility coefficients ranged from 68% for SBM to 73% for PBM. Digestible energy coefficients ranged from 69% for SBM to 85% for PBM. Mineral availability was usually higher for the animal products. Phosphorus availability from PBM and MBM was 45% and 44%, respectively. Soybean meal had the lowest availability for P at 28%. Similar results were observed for Mn, which values ranging from 78% for MBM to 17% for SBM. Poultry by-product meal presented significantly higher values for Mg and Zn availability. Data from this study provide more precise information concerning nutrient and energy utilization of Nile tilapia under intensive farming condition and will contribute to practical diet formulations based on levels of available nutrients.



POTENTIAL FOR USING CORN DDGS IN EUROPEAN CATFISH (*Silurus glanis*) DIETS

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European catfish (*Silurus glanis*) is an important carnivorous fresh-water species in Central and Eastern European aquaculture, thanks to their fast growth, robustness, stress tolerant capability and high market value. The protein content of its commercial feeds based mostly on fish meal (ca. 60%). The aim of this study was to assess the apparent digestibility of corn DDGS and its effects on growth performance, nutrient utilization and biochemical parameters.

A digestibility trial was performed first, where apparent digestibility coefficients of the DDGS were determined. Secondly four different practical diet were tested in the course of a nutritional experiment with 0, 10, 20 and 30 % DDGS content. Each feed included 20 % fish meal and were set to 43 % crude protein and 9 % crude fat. The fish were distributed into 12 tanks in triplicates and fed for 60 days. Growth performance parameters were calculated and samples for proximate composition, plasma biochemical parameters, liver and gut histological sections, gene expression were taken.

Results of the present study showed that the apparent digestibility of DDGS is auspicious for European catfish. No significant differences were found between the experimental groups in aspect of growth performance and plasma biochemical parameters. The liver histopathological observations showed that in 20 and 30 % DDGS groups had less vacuolized hepatocytes than the other groups.

In conclusion, our study indicates that the European catfish, as a freshwater carnivorous species have the skill to utilize the diets with DDGS up to 30 %.



Evaluation of proteases and carbohydrases in tilapia diets

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Ten practical tilapia diets were formulated to contain 32% protein and 6% lipids. Six diets were formulated to contain low level of fiber (LF) and included free protease (FP), protected protease (PP), free carbohydrase (FC), protected carbohydrase (PC), and a mix of free protease and carbohydrase (MFPFC). Four diets were formulated to contained high fiber (HF) and included dried distillers grains as a source of fiber and reduced protein digestibility. Treatments included a basal diet and a basal diet supplemented with free protease (FP), free carbohydrase (FC), and a mix of free protease and free carbohydrase (MFPFC). The level in the diet of free protease (FP) and protected (PP) was 175 g per metric ton, the level of free carbohydrase (FC), protected carbohydrase (PC) and the mix of free protease and carbohydrase (MFPFC) was 125 g per metric ton. The diets were offered to juvenile tilapia (9.29 g initial mean weight) over a 10-week growth trial. Four replicate groups of 20 fish/aquaria were offered the test diets at near satiation levels. At the conclusion of the growth trial, survival was near 100% and weight gain was around 1000%. In general, fish maintained on the high fiber diet performed slightly poorer than those on the lower fiber diet. Concerning enzyme supplements, there were no clear statistical differences among the treatments in protein retention efficiency, however in low fiber diets lipid retention efficiency and energy retention efficiency was improved with the enzyme inclusion. When considering planned comparisons, there were some significant differences or possible trends in final weight and feed efficiency. Overall, there were no clear advantages detected to the protected enzymes. Digestibility data is being collected.



Paecilomyces variotii as a replacement for soy protein in salmonid diets, a suitability study

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Aquaculture is globally, the fastest growing food production industry and continued expansion will be necessary to ensure future food security. For aquaculture to meet this production demand while remaining economically feasible and without compromising sustainability, aquafeed ingredients must be revolutionised. Atlantic Salmon, *Salmo salar* has a high protein and strict essential amino acid requirement, particularly during early developmental stages, making it an ideal candidate to assess the suitability of novel aquafeeds.

This suitability study evaluated the effects of up to complete replacement of dietary soy protein by a single celled protein (SCP), the fungus *Paecilomyces variotii* on growth performance and fillet composition of juvenile *Salmo salar* during a 5-week feeding trial. The SCP produced for this study had a suitable amino acid profile for salmonid. Five experimental diets with graded levels of SCP were formulated to be isocaloric and isonitrogenous. A commercial standard diet was also assessed for thorough comparison. Treatment groups were assigned in a completely randomised design.

No significant differences to survival or fillet composition were observed across treatments. Growth performance was not significantly different between the commercial standard diet and up to 15% replacement soy protein replacement with *Paecilomyces variotii*. This study represents the first assessment of soy protein replacement with *Paecilomyces variotii* in aquafeeds and provides promising results that encourage the continued investigation and optimisation of this fungal protein for salmonid diets.



High incorporation of plant protein in the diet of Nile tilapia, *Oreochromis niloticus* using exogenous protease

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A 12-week feeding trial was conducted to evaluate the effect of replacing fish meal (FM) with cottonseed meal (CSM) without or with inclusion of protease on growth, hematology, blood parameters, digestibility and gene expression of Nile tilapia diets. The experimental diets categorized into three groups; the first group CSM1 which contained fish meal protein: cotton seed meal protein (FM: CSM = 2:1), the second group CSM2 which contained FM: CSM = 1:1 and the third one CSM3 contained FM: CSM = 1:2. All groups were supplemented with exogenous enzyme (protease) at 0 and 2500 U kg⁻¹ diet. All diets were fed to fish (initial body weight 11.62±0.03 g/fish) in triplicate aquaria twice daily. The best WG, SGR, FCR, PER and PPV were recorded by fish fed CSM1 and CSM2 and supplemented with 2500 U kg⁻¹ diet. The best ADC of crude protein, crude lipid and digestible energy was obtained by fish fed CSM1 without supplemented with protease (0 U protease/ kg diet) and CSM1 CSM2 supplemented with protease (2500 U protease/kg diet), with insignificant differences between means. The highest mean values of Hb, Htc, RBCs, ALT and AST were recorded by fish fed CSM1 and CSM2 and supplemented with protease enzyme (2500 U protease/ kg diet). ALT, AST and ALP improved due to dietary protease (2500 U protease/ kg diet) supplementation. group fed CSM1 and CSM2 supplemented with protease enzyme showed the highest values of gene expression of insulin like growth factor I (IGF-I) in brain and liver of tilapia compared to others groups. Results above showed that supplementation of protease can improve growth, nutrients digestibilities, hematology and gene expression of GH and IGF-I of Nile tilapia.



The effect of non-starch polysaccharide composition and enzyme supplementation on growth performance and nutrient digestibility in Nile tilapia

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High levels of non-starch polysaccharides (NSP) and phytate in aquafeeds can affect the performance of fish through reduced nutrient digestibility. Results of studies assessing the effect of exogenous enzymes to enhance nutrient digestibility and improve growth are not always consistent. This could be through a mismatch between the supplied enzymes and the diet composition (e.g. type of enzymes specific to the substrate and their ratio). The presented study investigated, whether nutrient digestibility is affected by enzyme supplementation and if this effect is depended on the NSP composition.

An experiment with a 2 x 4 factorial design was used to test the above. Four diets were formulated differing in type of NSP by adding wheat bran (WB, 25.5%), sunflower meal (SFM 27.5%) and citrus pulp (CP 20%) to the reference diet (REF). The reference diet was formulated to be low in NSP and used as control diet. The ingredients were chosen for their high NSP content and their contrast in NSP composition; WB being relative rich in hemicellulose, SFM in cellulose and CP in pectins, respectively. These four diets were either supplemented with a placebo (demi water) or enzymes (phytase, 1000 FTU/kg and xylanase, 4000 U/kg). In total 24 tanks (3 replicates/treatment) were used with 30 (mean initial body weight 41g) fish each. Fish were restrictively fed the experimental diets by hand twice daily for 43 days. Hereby measuring growth and nutrient digestibility.

Factorial analyses showed that diet and enzyme supplementation affected growth ($P < 0.05$). The nutrient digestibility was affected by the NSP composition ($P < 0.05$) except for the starch and calcium digestibility. The NSP, energy, ash, phosphorous and calcium digestibility improved with enzyme supplementation. There was an interaction effect on growth, as well as the digestibility of energy and phosphorus ($P < 0.05$). This indicates that the effectiveness of the applied enzymes was dependent on the diet and thus the composition of the NSP fraction. The growth of the fish fed the WB and SFM diets was improved, while the REF and CP diets did not benefit. Therefore it is important that the enzyme supplementation is complementary with the diet composition.



Better Performance, Nutrient Digestibility and Digestive Enzyme Production in Yellow Catfish Fed Fishmeal Free Diets Supplemented with A Protease Complex

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Poorly-characterized and less-standardized protein sources used in aquafeed usually possess imbalanced amino acid profiles, may contain some anti-nutrients, and as a result, are poorly digestible. Their quality may also vary considerably by season, region and even between batches from a single manufacturer, creating significant risks in maintaining the feed quality. Use of enzymes in animal feed are therefore, increasing worldwide to reduce the risk of deteriorating feed quality and thus, enabling formulators to increase the use of these protein sources. In this study, efficacy of a dietary protease complex was tested in an 8-week growth trial conducted at Suchow University, China with yellow catfish *Pelteobagrus fulvidraco* followed by a digestibility trial. The main objective was to determine the effects of the protease complex in diets where a portion of fish meal (FM) was replaced by a combination of soybean meal (SBM) and cottonseed meal (CSM).

Five isoproteic (38.3% CP) and isoenergetic (19.3 kJ/g GE) diets were formulated: a positive control (PC – 15% FM, 24.5% SBM, 10% CSM), a negative control (NC – 11% FM, 26.5% SBM, 14% CSM), and three other diets NC125, NC150 and NC 175 where NC diet was supplemented with 125, 150 and 175 ppm of the dietary protease complex. The growth trial was conducted in triplicates in randomly assigned 15 tanks with 15 fish per tank. Yttrium oxide (0.01%) was added to all diets as indigestible marker for the digestibility assay.

Despite no differences in growth performance among the dietary treatments, in PC, NC125, NC150 and NC175 treatments, apparent digestibility coefficient (ADC) of CP (0.972, 0.962, 0.972 and 0.963, respectively) and gross energy (GE – 0.932, 0.907, 0.920 and 0.908, respectively) were significantly higher than those of the NC treatment (0.945 ADC CP and 0.859 ADC GE). In case of the ADC of lipid, although not significant, they were numerically higher in treatments supplemented with the protease complex than the NC diets and similar to the PC diets.

It can be concluded from the study that the dietary supplementation of the protease complex increased the availability of nutrients in the yellow catfish diets.



Fishmeal replacement with plant and animal proteins and supplementation of exogenous phytase and protease in diets for juvenile cobia (*Rachycentron canadum*)

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Cobia is one of the most promising species for tropical marine fish farming, with excellent performance, growth rates that reach 6 kg in one year and feed conversion of 1.5, but as most carnivorous tropical fish species is still largely dependent on elevated fishmeal inclusion in diets. The present study reared juvenile cobia at the nursery phase (initial ind. weight 14 g until ca. 100 g, 32 x 500L tanks, recirculated system) with eight dietary treatments (quadruplicate) to verify performance, apparent digestibility and nutrient retention of fish fed diets with partial fishmeal (FM) replacement by locally available feed ingredients: soy protein concentrate (SPC, at 28% dietary inclusion) or poultry by-product meal (PBM, at 30% dietary inclusion) - in comparison with a reference practical diet (49% FM inclusion: 47.4 CP/14.3 lipid/2.94 phosphorus, % as-is). The supplementation of inorganic phosphate (Pinorg = DCP, 1.4%) or microbial phytase (Phy, 100 ppm = 1000 FTU.kg⁻¹) and/or protease (Pro, 600 ppm) was compared in five SPC diets: SPC (non-supplemented), SPC+Pinorg, SPC+Phy, SPC+Pinorg+Pro, and SPC+Phy+Pro; only protease was tested in PBM diets. Composition: SPC diets - 47.3-48.0/12.2-13.6/1.59-1.97, PBM diets 49.3/15.0/2.30; % crude protein/lipid/phosphorus (as-is). At >94% survival, final weight was significantly lower only in SPC+Pinorg+Pro. Total feed intake was higher for FM and PBM diets. On the other hand, FCR was significantly lower in diets SPC, SPC+Phy and SPC+Phy+Pro. Apparent digestibility: Phy supplementation increased dry matter, crude protein (CP), energy and phosphorus digestibility among SPC diets (Pro was also positive for P digestibility), while SPC+Phy+Pro showed the highest digestibility of CP and energy among all tested diets. Whole-body composition: Pinorg, Phy and Phy+Pro improved P and ash content with SPC diets. Crude protein, phosphorus and ash retention efficiency in fish increased significantly in SPC diets compared to FM and PBM diets, and SPC+Phy and SPC+Phy+Pro showed the highest nutrient retention among tested diets. In conclusion, FM could be partially replaced by SPC or PBM in cobia nursery diets with potential benefit of phytase or phytase + protease supplementation in SPC diets upon FCR, digestibility and nutrient retention.



THE EFFECT OF EXOGENOUS ENZYMES IN THE DIET OF COMMON CARP (C. CARPIO) ON GROWTH PERFORMANCE AND FEED UTILIZATION.

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Introduction

When using high inclusion levels of plant based ingredients in fish feed, the presence of Anti-Nutritional Factors (ANF) needs to be taken into consideration. Non Starch Polysaccharides (NSP) are recognized as true ANF, causing impaired growth performance and feed utilization. NSPase could counteract these negative effects by breaking down the NSP.

Objective: to investigate the effect of NSPase in a practical common carp diet on growth performance and feed utilization.

Materials & Methods

Growth trial in 2 stages:

Stage 1: 12 tanks of 140l (1 RAS). 37 Common carp juveniles stocked per tank (ABW = 42.1±0.5g). Trial duration 9 weeks. At the end of stage 1, fish were transferred to larger tanks.

Stage 2: 3 RAS, each comprising 4 tanks of 1.2m³, 1 tank of each treatment per RAS. Trial duration 15 weeks.

Four diets tested in triplicate: positive control (PC) containing 6% fishmeal, negative control (NC) without fishmeal (replaced by corn gluten) and 2 enzyme treatments (NSPase 1 & 2 developed by Aveve Biochem; different concentrations of xylanase, β-glucanase and pectinase). The PC and NC were formulated to contain 24.9% of digestible protein and 2 375kcal.kg⁻¹ of digestible energy.

Feeding ad libitum using automatic feeders. Uneaten pellets were quantified and feed intake was corrected. At 3, 6, 9, 12, 16, 20 and finally 24 weeks all fish were individually weighed and measured.

After the growth trial, faeces were collected and analyzed to quantify the ADC of dry matter, protein, fat, carbohydrates and energy.

Results

The PC outperformed the NC in terms of ABW, SGR and FCR. Supplementing either NSPase significantly improves ABW and SGR while no statistical difference was found for FCR compared to NC (table 1).

No significant differences were found for ADC of dry matter, protein, fat, carbohydrates and energy (table 2).

Conclusion

Substituting all fishmeal by plant based ingredients affected growth performance and feed utilisation. Supplementation with either NSPase alleviated the negative effects on growth. Feed utilization did not improve, ongoing data analysis might explain these results.

Using NSPase is a promising technique for the development of more sustainable carp feeds.



FISHMEAL REPLACEMENT BY FEATHER MEAL AND FEATHER MEAL HYDROLYSATE IN RAINBOW TROUT (O. MYKISS).

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Introduction

Since the ban on the use of animal byproducts in fish feed has been lifted in the EU, interest from industry has increased. Empro Europe NV produces feather meal (EM'PAQ) and feather meal hydrolysate (PEP'SOL) from purified chicken feathers.

Objective: to investigate the potential of both ingredients as fishmeal replacers in rainbow trout.

Materials & Methods

The growth trial was performed in 18 tanks of 140 l (1 RAS, 6 treatments in triplicate). Each tank was stocked with 35 juvenile rainbow trout, individually tagged, with an ABW of 31.2±5.9g. Trial duration was 12 weeks, photoperiod 15L/9D, temperature during the trial was 18.0±0.3°C.

Six isonitrogenous and isoenergetic experimental diets were formulated to contain 42% CP, 22% CF and 5200kcal.kg⁻¹. The control diet contained 28% of fishmeal, 50% was replaced by different ratios of both test ingredients (table 1).

Table 1

Fish were daily fed 2,75% of their bodyweight in 3 meals. Individual bodyweight and bodylength were determined at week 0, 3, 6, 9 and 12. After the growth trial, faeces were collected and analyzed to quantify the ADC of protein, fat, carbohydrates, energy, phosphorus and ash.

Results

There was no significant difference in survival, SGR or FCR between treatments (table 2).

Table 2

No significant difference was found for ADC of protein, fat, carbohydrates, energy or ash. A significant difference was found for the ADC of phosphorus ($p = 0.032$) with FM and PEP'SOL having equal and higher ADC for phosphorus compared to the other treatments.

Conclusion

Growth performance and feed utilization was not affected when substituting 50% of dietary fishmeal by each one of the test ingredient ratios. The reduced phosphorus digestibility with diets containing EM'PAQ was not reflected in performance results. EM'PAQ and PEP'SOL are promising fishmeal replacers for the development of more sustainable trout feeds.



Partial replacement of fishmeal protein by poultry by-product meal protein and soybean meal protein in diets for juvenile hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂)

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Two consecutive growth trials were undertaken to study the partial replacement of fishmeal (FM) protein by poultry by-product meal (PBM) protein and together soybean meal (SBM) protein in diets of juvenile hybrid grouper. In the first trial (trial 1), eight isoenergetic and isoproteic (53.5% of dry matter) experimental diets were formulated to replace 0%, 10%, 20%, 30%, 40%, 50%, 60% and 70% FM protein with PBM protein, being abbreviated as FMP, PBM10, PBM20, PBM30, PBM40, PBM50, PBM60 and PBM70, respectively. Each diet was given to triplicate groups (initial average body weight, 6.0 ± 0.05 g/fish) for 8 weeks. Results showed that weight gain% (WG%), feed intake (FI), feed efficiency (FE), protein efficiency ratio (PER) and protein productive value (PPV) were not significantly influenced by different FM protein replacements with PBM protein. The lipid contents in whole body, muscle as well as liver were significantly increased as the inclusion levels of dietary PBM increased, and hepatic steatosis was observed in liver histology of fish fed high levels of PBM. In trial 2, another six isoenergetic, isonitrogenous (53.5% of dry matter) and isolipidic (7% of dry matter) experimental diets including a FM control diet and five experimental diets in which 0%, 7%, 14%, 21% and 28% PBM protein were replaced with SBM protein. Based on the results of trial 1, the PBM70 diet of trial 1 was used as the reference diet of trial 2 except for the FM control diet. Each diet was given to triplicate groups (initial average body weight, 6.83 ± 0.08 g/fish) for 8 weeks. Results showed that compared to the FM control group, growth performance and feed utilization of fish were not significantly affected by the PBM protein replacements with SBM protein from 0%-28%. There were no significant differences in lipid contents of whole body and muscle among all treatments. Gut morphology and liver histology did not show obvious variations among all groups. In conclusion, 70% FM protein would be replaced by 50% PBM protein and together 20% SBM protein without negatively affecting fish growth and health.



Apparent nutrient digestibility of PAPs from poultry rendering and insect meals in rainbow trout (*O. mykiss*) and European seabass (*D. labrax*)

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Poultry by-product meals (PBM) and insect meals deserve increasing attention as sustainable protein-rich ingredients in fish diets. Little information is available on the nutritive value of PBMs originating from different avian slaughterhouse raw materials and on insect meals in general. The present study was aimed at measuring in trout (RT) and seabass (ESB), the apparent digestibility of two PBMs differing in the proportion of chicken and turkey slaughterhouse scraps and two commercial *Hermetia illucens* meals (HMs). From a basal diet mash, including acid insoluble ash (1.5%) as an inert marker, four test diets were obtained by extruding into 3 mm pellets a mix of basal diet and test ingredients at a 70:30 w:w ratio. The apparent digestibility coefficients (ADCs) of dry matter (DM) and crude protein (CP) of the diets were measured in triplicate in RT (46.9 ± 2 g) and ESB (36.9 ± 1 g) kept at $13.5 \pm 1.22^\circ\text{C}$ (RT) and $21.2 \pm 0.7^\circ\text{C}$ (ESB) in tank units fitted with settling columns for faecal collection (Guelph layout). Each diet was offered to satiety in two daily meals. The ADCs of nutrients were calculated by difference relative to those of the reference diet.

The ADCs of the test ingredients were different and significantly affected by the fish species. The DM and CP digestibility of the two PBMs were similar within species. The former was higher in RT than in ESB (93 vs. 80%, $P < 0.05$) while no difference was noted in CP digestibility (97 vs 95%, $P > 0.05$). The two HMs resulted in different ADCs ($P < 0.05$) which were lower than those of PBMs, particularly in ESB ($P < 0.05$), ranging from 86.5 and 71% for DM and 95 and 88.5% for CP.

The results of this study confirms that PAPs are suitable alternatives to conventional protein sources in fish diets. Different avian slaughterhouse raw materials seem to have little impact on digestibility of the resulting PBMs. Differences in the digestibility of commercial HMs deserve further investigation to ascertain the possible roles of substrate or processing procedures in affecting their nutritive value.

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Dietary impacts of sulphuric acid extracted fish bone compounds on tissue astaxanthin deposition and astaxanthin utilization in Atlantic salmon (*Salmo salar*)

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A fish bone hydrolysate (FBH) demonstrated useful as alternative phosphorus source in feed for Atlantic salmon (*Salmo salar*) smolt (Albrektsen et al., 2017), at the same time also increased plasma, liver and whole body Ax concentration significantly (unpublished data). In a follow-up study with large salmon, the aim was to evaluate how acid extracted soluble compounds in fish bones may improve muscle astaxanthin (Ax) deposition in salmon. Atlantic salmon (1.7 kg) were reared in 12 net-pens and fed with one of four practical formulated diets for 78 days. All diets (D) were balanced to meet dietary phosphorus (P) requirement (8 g kg⁻¹ P): the control diet (D1) and diet D4 were added CaPO₄ as P source, diets D2 and D3 were added a P rich ingredient extracted from fish bones at two levels (2 and 4 % FBH), and D2 was also balanced with CaPO₄. Diet D4 was added K₂SO₄ to study potential impacts of sulfate in sulfuric acid used for mineral extraction. An in vitro cell culture with hepatocytes was performed to study the effects of FBH on Ax uptake and transport. The soluble fish bone compounds significantly increased the Ax retention in muscle by 35 % (P < 0.05), explained by improved Ax digestibility followed by increased circulating Ax and tissue Ax deposition (ns). Reduced metabolic turnover of Ax was indicated, with about 10 % more of absorbed Ax retained in the flesh of fish fed 4 % FBH (D3). The in vitro study showed no impacts of the bone ingredients on the hepatic Ax uptake. In conclusion, overall results in the smolt trial and in the study with large salmon indicate that acid extracted fish bone compounds may improve Ax utilization in Atlantic salmon, most probably by increasing the Ax digestibility.



Evaluation of the Effects of Tuna Fish Hydrolysate inclusion in diets for Pacific White Shrimp (*Litopenaeus vannamei*) reared in floating cages under commercial conditions

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Introduction

Shrimp farming is the most important sector of the aquaculture industry worldwide in terms of economic value and it represents an important source of employment and income in developing countries. Two major challenges that the industry faces these days are: susceptibility to disease outbreaks and the ecological impact of using marine raw materials to produce shrimp feed.

Objective

The present study was carried out with the objective of assessing the effect of Tuna Fish Hydrolysate (TFH) inclusion in practical diets for *L. vannamei* reared in floating cages located in Mexico under commercial conditions.

Methodology

The trial was composed of three treatments, a control group and two treatments with 2% and 4% level of TFH inclusion. All diets contained 25% of crude protein. During the six-week experimental, biometrics were carried out every two weeks and the effects of TFH on growth, survival and FCR were evaluated. Additionally, at the end of the trial a thermal stress test was performed. Also, examinations of feed for peptides profile and gut microbiota was carried out at the Molecular Laboratory of Plymouth University using diverse molecular techniques as part of presenter's PhD work.

Results:

The peptide profile showed an increase of the soluble fraction of the protein with TFH, particularly in the 2% TFH diet with 1-5 KDa range. There were no significant differences in survival and FCR when compared to the control treatment.

The stress test demonstrated that shrimp of the 2% TFH diet had significantly more tolerant to thermal stress compared to the other treatments.

Molecular analysis showed that bacteria communities from gut displayed significant differences among groups. Finally, the economic analysis showed an improved return of investment by +16% on cost benefit.

Conclusions

The use of TFH in shrimp diets has a positive effect on growth performance and contributes to gut microbiota modulation. Due to its characteristics, TFH increases the number of peptides and boost shrimp immune response. Further analysis of gene expression is suggested to know more about the peptide assimilation and modulation of gastrointestinal function and feed efficacy under intensive shrimp production.



Effect of partial wild derived fish meal replacement on bile acid production and liver structure in Yellowtail Kingfish, *Seriola lalandi*

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Alterations to the enterohepatic system, the storage of lipids and the consequent effects on liver structure need to be considered when replacing wild derived fish meal (wd-FM) with alternative ingredients in commercial Yellowtail Kingfish, *Seriola lalandi*, diets. Wild derived-FM is known to contain high levels of cholesterol and taurine relative to many other ingredients. Cholesterol and taurine are important precursors in bile acids synthesis. The replacement of wd-FM with other alternative ingredients is likely to alter the available level of de novo cholesterol and taurine for bile acid production, its availability for absorption of dietary lipids and fat-soluble vitamins, and excretion. In this 252 day study, Yellowtail Kingfish (2.52 ± 0.25 kg; 546 ± 20 mm [fork length; mean \pm standard deviation]) were fed five different diets formulated on a digestible basis that contained highly palatable and digestible ingredients at realistic commercial inclusion levels. A 30% wd-FM diet served as a control. Dietary wd-FM inclusions were reduced to 20% and 10%, and replaced with FM by-product meal. In addition, dietary wd-FM levels were reduced to 20% and replaced with poultry meal or soy protein concentrate in two other separate diets. The effect of wd-FM replacement on liver, digestive tract and faecal total bile acid concentrations, histological liver structure and lipid storage will be presented.



Graded levels of fish protein hydrolysates affect growth, free amino acid concentrations, and protein metabolism related gene expression in juvenile turbot (*Scophthalmus maximus* L.)

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This study aimed to investigate the effects of fish protein hydrolysates (FPH) on fish growth, postprandial free amino acid concentrations in serum and muscle, peptide and amino acid transporters in different intestinal sections and kidney, and protein metabolism-related gene expression in muscle of juvenile turbot (initial weight 11.77 ± 0.03 g). 0%, 4.5% and 18% of FPH (FF, FL and FH diets, respectively) were added to experimental diets containing 20% of fish meal. Results indicated that fish fed FH diet showed significantly higher specific growth rate compared with fish fed FL and FF diets ($P < 0.05$). At 2, 6, 12 and 24h after feeding, the concentration of muscle free EAA increased with the increasing levels of FPH, and the largest number of EAA in serum and muscle was found at 2h postprandial. The expression of PepT1 in proximal and distal intestine was lower in fish fed FH diet compared with FL and FF diets ($P < 0.05$), and there were no significant differences in PepT2 mRNA levels of kidney among different treatments ($P > 0.05$). BOAT1, CAT1, LAT2 and PAT1 in proximal intestine were significantly lower in FH group than that of FL and FF groups ($P < 0.05$). In kidney, the highest expression of BOAT1 was observed in FF treatment and the highest expression of CAT1 were observed in FH group ($P < 0.05$). The expression of muscle mTOR in FL group was the highest in all groups, and muscle 4E-BP1 gene expression was up-regulated with an increase of FPH levels. FPH also up-regulated the gene expression of FoxO1 in muscle. In conclusion, the growth of turbot increased with the increasing levels of FPH. In serum and muscle, the contents of most free EAA at 2h after the last meal were significantly higher in those fed FPH diets than in those fed non-FPH diet. 4.5% of FPH may up-regulate the gene expression of peptide and amino acid transporters, while 18% of FPH may reduce the transport ability of peptide and amino acids. Dietary FPH may increase protein synthesis and breakdown through TOR and FoxO signal pathway.



FISH MEAL QUALITY AND RAPID DIGESTIBILITY ASSESSMENT METHODS: TOWARDS USING LESS, BUT USING IT BETTER.

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Over the past several decades, the global aquafeed industry has progressively decreased the proportion of fish meal (FM) used in commercial diets via the increased utilisation of terrestrial alternatives. However, given its high palatability, optimal nutritional profile and superior digestibility compared to most alternative ingredients, FM remains an important protein source for many cultured species, including Atlantic salmon. But not all FM is created equal, and it can vary widely in its palatability, apparent digestibility, and nutritional profile – important factors that affect nutrient assimilation and therefore growth performance in fish. Indeed, Atlantic salmon fed low-quality FM diets have demonstrated reduced growth and feed efficiency but higher feed consumption compared to high-quality FM diets. This exacerbates the associated environmental and production costs since reduced growth and feed conversion ratios necessitate more FM per kilogram of salmon produced. Therefore, conservative use of high quality FM is increasingly being recognised as a pertinent consideration in diet formulations.

FM quality is affected by a great and disparate number of factors. In particular, the geographic origin of the FM is suggested to greatly influence its nutritional characteristics since this largely determines processing and manufacturing conditions, as well as its species composition. In order to examine this, this study compared eight different FM samples originating from various geographic locations for their performance and digestibility in juvenile Atlantic salmon over an eight week period. In vitro and in vivo techniques were also compared for their efficacy in measuring the protein digestibility of each FM sample. A detailed account of experimental results will be presented and discussed. Findings will provide valuable insights into differences in FM quality emanating from their origin, as well as the potential of rapid assessment methods for raw material digestibility.



Potential of Aquaculture By-products for Fish Meal and Fish Oil Production in Turkey

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Fishmeal (FM) and fish oil (FO) are two major dietary ingredients used in aquafeed. The estimate of FM use for aquaculture nutrition varies from 46 to 56 percent and of FO use is over 80 percent of total production. Many fish species such as anchovy, horse mackerel, sardines, sprat, haddock, and herring are used in the production of FM and FO. FM and FO production is mostly linked to fish caught from nature and their prices differ according to the change in the amount of fish caught. But the sustainability of aquaculture requires guarantees of sustainability of feed raw materials used in fish feed. Aquaculture by-products used in fish feeds as substitutes for FM and FO produced from wild pelagic fish is becoming more important for sustainability. The purpose of this study is to describe the potential of aquaculture by-products usage for FM and FO production in Turkey.

Fillet yield and by-products ratios (backbone with head, liver, and guts) of sea bream, sea bass and trout were measured in the fish processing plant. The productivity of FM and FO from by-products were calculated from using of by-products and obtained FM and FO from it. Proximate compositions (protein, lipid, ash, moisture, pepsin digestibility) of FM were analyzed in Fisheries Faculty and Essential Fatty acids (tuna by-products oil and aquaculture by-products) and phospholipid analyses of by-products oil were measured in Akvatek Hatchery Feed Analyses Lab.

Study results were given in Table 1, Table 2, Table 3, Table 4. Turkey has a rapidly growing aquaculture, as well as an aquafeed and fish processing industry. Fish processing industry has nearly 100 plants in Turkey and total discharge of aquaculture by-products was calculated as 25.000-30.000 tons in 2017. This amount is enough to produce 5.400 tons of fish meal and 4.200 tons of fish oil and 27.000 tons fish feed (with using 20% fish meal in diet) and 42.000 tons fish feed (with using 10% fish oil in diet). Using aquaculture by-products in the fish feed will be more important when the fish processing capacities increase.



Evaluation of defatted krill meal as partial and total fishmeal replacement in diets for gilthead seabream (*Sparus aurata*) juveniles

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In order to support marine aquaculture growth, it is necessary to find alternative protein sources to fishmeal (FM) that are more sustainable and cost-effective. Antarctic krill (*Euphausia superba*) has been regarded as a valuable alternative marine resource, both as protein and lipid source. Krill oil, in particular, is of high interest for human nutrition and as a source of essential fatty acids for marine fish. The residue leftover from oil extraction is a protein-rich product with promising properties to be included in fish feeds at a competitive price. Therefore, the aim of this study was to evaluate defatted krill meal (KM) as FM replacement in diets for gilthead seabream juveniles. Four extruded diets (44% protein; 21% lipids) were formulated: a control diet (KM0), containing 57% FM, and three diets where FM protein was replaced by KM at 33% (KM33), 66% (KM66), and 100% (KM100). Triplicate groups of 45 fish (initial body weight of 24 g) were fed the experimental diets for 12 weeks in a thermo-regulated (22.5 °C) seawater system. Growth performance, nutrient and amino acid retention, and digestive enzymes activity were evaluated. Growth performance linearly decreased with the increase of dietary KM level. Feed intake tended to decrease with KM inclusion, being significantly lower at the 100% substitution level, but feed conversion ratio and digestive enzymes activity were not affected by diet composition. Whole-body dry matter, protein, amino acids, and lipid content were not affected by diet composition, but energy content was lower in fish fed the diet KM33. Similarly, protein, essential amino acid (EAA), and lipid retention (% intake) were similar among diets, while energy retention (% intake) was lower in fish fed diet KM33. The EAA ratio (diet/fish) was similar for the majority of EAA, except arginine and histidine, which were lower in diets KM66 and KM100, respectively. Overall, results of this study indicate that palatability of KM may be an issue at high replacement levels and that FM replacement by KM may have compromised energy digestibility and, therefore, growth performance.



In vitro protein digestion of hydrolyzed and fermented soy protein concentrates with specie-specific digestive enzymes of whiteleg shrimp

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The objective of this trial was to estimate the protein digestibility of different sources of soy protein concentrate (SPC) submitted to micronization, hydrolysis and fermentation processes. Trial was carried out in the facilities of Aquaculture Laboratory (LAM), Oceanographic Institute University of São Paulo, Brazil. Six samples of soy protein concentrate were evaluated for in vitro protein digestion with specie-specific digestive enzymes of whiteleg shrimp (*Litopenaeus vannamei*), described as: Conventional Soy protein concentrate (C-SPC), micronized SPC (M-SPC), hydrolyzed SPC with protease (H-SPC), fermented SPC (F-SPC), fermented and hydrolyzed with cellulase SPC (FC-SPC), fermented and dephytinized SPC (FD-SPC). These samples had the crude protein content of 60.5%, 61.2%, 59.0%, 62.1%, 64.3%, 62.3%, in as is basis respectively. Standardized digestive enzymes were recovered from shrimp hepatopancreas at 10g average weight inds, pond farmed at practical conditions. The degree of protein hydrolysis (DH) of 80mg sample were incubated with shrimp enzyme extracts is determined in a pH-stat reaction at alkaline medium (pH = 8.0) in sterilized marine water (35‰ salinity). The pH-stat reaction simulates enzymatic protein digestion in shrimp stomach and hepatopancreas. Degree of hydrolysis measurement has a good correlation with apparent crude protein digestibility in juveniles shrimp ($r^2 = 0.86$) Lemos et al. (2009). Samples were tested in four replicates and mean values were statistically evaluated by one-way ANOVA at 5% of probability (Table 1). DH results of the conventional SPCs in the present sample set were in agreement with previous studies values. Although conventional SPC has high DH values, micronization, fermentation and enzymatic treatments appear to improve protein digestion by shrimp. A significant difference was found in the degree of hydrolysis (DH) of test ingredients. Though not significant, a trend of decreasing SPC particle size upon DH was found beneficial (Conventional versus micronized SPC). Highest DH was found for H-SPC, suggesting a potential for improved use in shrimp diets. Aquafeed industry seeks for sustainable and highly digestible ingredients that result in little residue in shrimp farming. In this context, the application of technologies such as fermentation and enzymatic treatment are important tools to improve the digestibility of soy protein concentrate.



In vitro bacterial and viral response in head kidney leukocytes of Atlantic salmon (*Salmo salar*) fed dietary insects meal

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With the fast growth of today's aquaculture industry, the demand for aquafeeds is expanding dramatically. Insects, which are part of the natural diet of salmonids, may represent a sustainable ingredient for aquaculture feed. The aim of the current study was to test how a partial or total replacement of dietary fishmeal by insect meal affect gene responses involved in inflammation and signalling pathways in Atlantic salmon head kidney leukocytes after exposure to bacterial or viral mimics.

Insect meal (IM) was produced from black soldier fly (BSF, *Hermetia illucens*) larvae. Seawater Atlantic salmon were fed three different diets; a control diet (Ctl), protein from fishmeal and plant based ingredients (25:75) and lipid from fish oil and vegetable oil (33:66); IM-66 and IM-100 diets, where 66 and 100% of fishmeal protein was replaced with IM, respectively. Leukocytes were isolated from the head kidney of fish from the three dietary groups (2 fish per cage, 3 cages per treatment). Isolated leukocytes were seeded into culture wells and added bacterial mimic lipopolysaccharide (LPS) or viral mimic polyinosinic acid: polycytidylic acid (poly I: C) to induce inflammatory response. Controls without LPS and poly I: C were included.

Interleukin 1 β (IL-1 β), IL-8 and IL-10 transcription were elevated in LPS treated leukocytes isolated from the three dietary groups, which confirmed the suitability of the in vitro model in this experiment. Gene expression of the fish specific Toll-like receptor (TLR22) and the transcription factor T CCAAT/enhancer-binding protein β (C/EBP- β) was significantly affected by the diets and decreased in LPS and poly I: C treated leukocytes isolated from IM-100 compared to the control diet. Salmon fed insect-based diets showed as well a lower expression of stress factors, such as superoxide dismutase (SOD) in untreated and treated leukocytes, when compared to the control group. Prostaglandin D and E synthase (ptgds, ptges) and arachidonate 5-lipoxygenase (5-LOX) gene expression were not affected by the dietary treatments. These results suggest that replacement of fishmeal with IM in the diets of Atlantic salmon have normal transcription of pro-inflammatory genes in the head kidney of salmon and might affect the stress response in the fish.



Invertebrate meals as a sustainable aquafeed component

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Invertebrate-meals (e.g. polychaetes and insects) present novel and sustainable high quality nutrient sources for use in fish feed formulations. To test this innovative source, an eleven-week feeding trial was conducted evaluating the effects of replacing the fishmeal (FM) component as an example of a superior protein source (FM CTRL) with ragworm meal (RW, *Nereis virens*), or/and silkworm pupae (SWP, *Bombyx mori*) in mirror carp (*Cyprinus carpio*) diets. Three experimental diets with partial replacement of FM (diets: RW+FM, SWP+FM, and RW+SWP+FM) were formulated. All diets were formulated to be iso-nitrogenous, iso-lipidic, and iso-energetic. Growth performance and feed utilisation indices were assessed, and the feeding trial concluded with the analysis of haematological parameters to provide an indication of carp physiological and health status. Mean weight gain was greatest in mirror carp fed RW+FM (60.83 g fish⁻¹ day⁻¹; P<0.05 vs all other diets) followed by SWP+FM (40.62 g fish⁻¹ day⁻¹; P<0.05 vs all other diets). The least weight gain was achieved in fish fed FM+SWP+RW+ and FM CTRL (34.34 g fish⁻¹ day⁻¹ and 33.96 g fish⁻¹ day⁻¹, respectively; not significantly different from each other). Fish fed on RW+FM diet had significantly lower plasma ammonia concentrations than any other dietary groups (P=0.04). Mirror carp fed on SWP+FM diet (111.52 units mL⁻¹) were observed to have a marked enhancement in alternative complement activity than FM CTRL (79.21 units mL⁻¹, P=0.041). Both ragworm and silkworm pupae meal present attractive sustainable functional feed component in carp diets, with benefits on enhancing growth performance and specific physiological parameters.



Feeding Tenebrio meal during larval stage of Nile Tilapia improved fish productive performance and feed utilization

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Tenebrio meal (TM) became a promising alternative protein source in animal feed as substitute of fish meal and soybean meal, however, the content of chitin may negatively affect fish productive performance. Even some fish species have expressed chitinase activity it may be restricted to a unique development phase. Thus, to evaluate the effects of feeding TM during larval stage on productive performance of Nile Tilapia juveniles, triplicate groups of larvae (0.28 ± 0.09 grams initial mean weight) were distributed in six tanks (500 L), at density of 200 larvae tank⁻¹ and fed isoproteic (370g kg⁻¹ digestible protein) and isoenergetic (3700 kcal kg⁻¹ digestible energy) diets with or without TM inclusion (250 g kg⁻¹) for 60 days. Then, three isoproteic (260g kg⁻¹ digestible protein) and isoenergetic (3300 kcal kg⁻¹ digestible energy) diets were formulated to contain 0, 150 and 300 g kg⁻¹ of TM inclusion replacing dietary soybean meal (up to total). Quadruplicate groups of fish, from trial described above, were reallocated in 24 tanks (100 L), at density of 12 fish tank⁻¹ in factorial design 2x3, larval feeding type (with or without TM) and experimental diets (0, 150 and 300 g kg⁻¹ TM). Fish were fed with experimental diets for 60 days. Feeding fish with TM during larval stage increased the weight with which fish started the next phase (represented as initial weight) in almost 15%. Dietary inclusion of increasing levels of TM as soybean meal replacement improved fish productive performance depending on the type of feeding during larval stage. The inclusion of 300 g kg⁻¹ of TM improved final body weight, weight gain, feed intake, feed conversion ratio and carcass weight of fish fed TM during larval stage. The same was not true for fish not previously fed with TM during larval stage, where the level of 150 g kg⁻¹ promoted better results. Thus, feeding 300g kg⁻¹ of Tenebrio meal during Nile tilapia larval stage upgrade fish feed utilization and productive performance, allowing higher dietary inclusion of Tenebrio meal in fish diets.



Influence of insect-based diets on growth performance and body composition in fresh- and sea-water phase Atlantic salmon

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Insects are part of the natural diet of salmonids, so insect-derived products represent a potentially rich source of nutrients for cultured Atlantic salmon (*Salmo salar* L.). In the present study, we aimed to assess the effect of dietary insect meal (IM) on nutrient digestibility, growth performance, body composition and tissue fatty acid composition of fresh- and sea-water phase Atlantic salmon (*Salmo salar*). The IM used in both trials was produced from black soldier fly larvae (*Hermetia illucens*) grown on media partially containing seaweed (*Ascophyllum nodosum*).

Fresh-water Atlantic salmon were fed during 8 weeks either with a diet in which IM represented the bulk protein source (60 % in the diet), or a standard diet with a combination of fish meal (FM) and plant protein (50:50). For the trial with sea-water Atlantic salmon, FM was replaced with 33%, 66% or 100% of IM in the diets for 16 weeks.

No differences between dietary groups were recorded for final body weight, body length, feed conversion ratio, condition factor, feed intake, or protein- and lipid efficiency ratio in either the fresh- or the sea-water phase. The apparent digestibility coefficients (ADC) were significantly lower for protein, amino acids and for fatty acids in IM fed groups, though all these nutrients remained highly digestible. Whole-body protein and lipid contents were not affected by IM protein source. However, in the fresh-water phase, the whole body fatty acid composition was significantly different between the dietary treatments, with a high content of lauric acid in the IM dietary groups.

In general, these studies showed that protein meal from black soldier fly larvae holds a great potential as a source of nutrients for Atlantic salmon.



Free, dipeptide and tripeptide forms of lysine and leucine affected the growth, free amino acid concentrations, and protein metabolism-related gene expression of juvenile turbot (*Scophthalmus maximus* L.)

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A 73 day feeding trial was carried out to compare the effects of free, dipeptide and tripeptide forms of lysine and leucine on fish growth, postprandial free amino acid concentrations of serum and muscle, the expression of peptide and amino acid transporter in different intestinal sections and the kidney, and protein metabolism-related gene expression in muscle in juvenile turbot (initial weight 11.77 ± 0.03 g). Lysine/leucine, Lys-Leu and Lys-Leu-Lys/Leu-Lys-Leu (CAA, Di and Tri diets, respectively) was supplemented to low fish meal-based diets. Specific growth rate in Di treatment was significantly higher compared with CAA treatment ($P < 0.05$). The levels of free lysine and leucine was significant higher in CAA group than that of Di and Tri groups at 2, 6 and 24h postprandial in muscle and at 6h postprandial in liver ($P < 0.05$). In proximal intestine, PepT1 mRNA levels had an increasing tendency in Di and Tri treatments ($P = 0.106$), B0AT1 mRNA levels in Di group were significantly higher than that of Tri group ($P < 0.05$), and LAT2 mRNA levels in CAA treatment was significantly higher than that of Di and Tri treatments ($P < 0.05$). Fish fed Di and Tri diets showed higher the expression of Akt2 than that of CAA treatment ($P < 0.05$). The expression of TOR and S6k β 1 in Di group was the highest among all the diets ($P < 0.05$). MurF1 mRNA level of Tri group was the highest, followed by the Di group, the lowest in the CAA group ($P < 0.05$). MARbx/atrogin-1 mRNA level had a similar tendency with MurF1 ($P = 0.122$). In conclusion, Lys-Leu dipeptide had more beneficial effect on fish growth compared with free lysine/leucine and Lys-Leu-Lys/Leu-Lys-Leu tripeptide. Compared with free form of lysine and leucine, small peptide form of those decreased the levels of lysine and leucine at 6h postprandial in serum and 2, 6 and 24h postprandial in muscle. Exogenous small peptide may be transported in proximal intestine by PepT1, and exogenous crystalline amino acid may also be transported in proximal intestine by LAT2 and B0AT1. At the transcriptional level, lysine and leucine in the form of crystals, dipeptides and tripeptides may regulate synthesis and breakdown of muscle proteins via Atk/TOR and Atk/FoxO signaling