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Modeling the bioaccessibility of nutrients in the gut of the gilthead seabream (Sparus aurata) using the response surface methodology: constraints and possibilities

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A detailed knowledge of factors influencing the digestive bioaccessibility of nutrients within the fish gut would have practical applications in the selection of suitable ingredients, technologies for feed preparation, and feeding patterns. Nevertheless, the relevance of those factors is difficult to assess using in vivo experiments, since each factor cannot be easily isolated from other physiological influences. An alternative approach is the use of an in vitro simplification of the digestion process combined with a good experimental design. In the present work, we analyzed five key factors (stomach and intestinal pH, stomach and intestinal food residence time, and intestinal enzyme:substrate ratio) affecting potential bioavailability of both proteins and carbohydrates, after enzyme hydrolysis, in the gut of the gilthead seabream Sparus aurata. For this purpose, reference data for the indicated parameters were obtained from live fish, and in vitro assays, carried out using purified enzyme extracts, obtained from the stomach and intestine of this species, and pure hemoglobin and starch, as specific substrates. The experimental procedure involved: a) a preliminary assessment of the significance of the different factors using a factorial design, b) a further evaluation of the effect of the selected significant factors, within their physiological ranges, in order to construct a predictive model for the release of both amino acids and reducing sugars, using a Response Surface Design, and c) an evaluation of the best combination of values within each factor determining the highest values for the hydrolysis of both protein and carbohydrates, using the Composite Desirability function.

Results showed a not significant effect of the factors related to gastric hydrolysis, but significant of all those related to the intestinal hydrolysis. Besides, higher pH, small food rations, and longer residence times improved protein hydrolysis, while starch hydrolysis was mainly affected by pH. Optimal conditions to maximize both responses resulted in 52% hydrolysis of hemoglobin and 19% of starch. This result is in agreement with the observed differences in the ability to digest both substrates in live fish.

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Comparative study on the apparent availability of zinc, selenium and manganese as inorganic metal salts or chelated sources in plant-based feeds for Atlantic salmon (Salmo salar) in seawater

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The composition of salmon feeds has during recent years changed from the use of mainly marine feed ingredients, such as fish meal and fish oil, to an increased use of plant ingredients. The changed feed composition has an impact on the mineral content and bioavailability. Phytic acid found in plant-based ingredients has a high binding affinity for metal ions, with negative effects on mineral bioavailability. Minerals such as, zinc (Zn), selenium (Se) and manganese (Mn) are supplemented to feeds to cover the nutritional requirements. These minerals may be added in organic or inorganic forms. The main goal of this study was to compare the apparent availability coefficients (AAC) of Zn, Se and Mn from inorganic metal salts and their chelated forms in Atlantic salmon (Salmo salar) feeds in seawater. Sixteen experimental diets were prepared based on a two-level full factorial design for four factors, namely a 24 design. The tested factors were Zn source (A), Se source (B), Mn source (C) and phytic acid level (D) (Table 1). Yttrium was used as inert marker. These feeds were fed to Atlantic salmon (~ 300 g) for 11 days, following which faeces were collected by stripping. The total mineral and yttrium levels in feeds and faeces were determined using ICP-MS, and the AAC of Zn, Se and Mn were estimated. The AAC of Zn, Se and Mn were evaluated as responses. Preliminary results show that the level of phytic acid and the source of minerals affected the AAC of Zn, Se and Mn. For instance, higher AAC values were obtained when the level of phytic acid was low and Se was added as Se methionine. Interactions between the factors studied were also found to significantly ($p < 0.05$) affect the AAC. The results will be discussed in detail. This is the first study in Atlantic salmon with a full factorial design focussing on the chemical forms of the supplemented minerals as well as the interactions among factors.

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Poultry meal in red claw crayfish (Cherax quadricarinatus) diets in comparison to fish meal and vegetable protein mix

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Substitution of fish meal (FM) with vegetable/animal protein sources has been reported to decline feed costs without detrimental effects on growth of crustaceans. Also, preliminary data from our institute show that red claw crayfish (Cherax quadricarinatus) can be successfully cultivated in recirculation system (RAS) in the Mediterranean. This study aimed to develop economic, healthy and sustainable diets, using low-cost local ingredients, for crayfish reared in RAS. A total of 300 crayfish fingerlings (13 g) were randomly distributed into 5 treatment groups into 500 L-1 fiberglass tanks (in three replicates) connected to a RAS, at 20 crayfish per tank. Each group was fed for 12 weeks one of five isocaloric/nitrogenous experimental diets as follows: Diet-1, FM only (48% of the diet; as control), Diet-2) 10% FM+52.5% poultry meal (PM), Diet-3) 10% FM+27.5% soybean meal (SBM)+27.5% corn gluten meal (CGM), Diet-4) 34.5% SBM+34.5% CGM, and Diet-5) 34.5% PM+32.4 SBM/CGM mix. The results indicated that growth performance, feed utilization efficiency, survival and exuviation cycle were similar among diets. The protein content was lowest with Diet-2 (176 g kg-1) and highest with Diet-3 (212 g kg-1), but lipid content was similar among diets. The sum of SFA was highest with Diet-2 (24%) and lowest with Diet-1 (22.4 mole%) but was similar among the other 3 diets. The sum of MUFA showed no significant difference among diets. Linoleic acid and n-6 PUFA was highest with Diet-5 (16.8 and 19.4 mole%) and lowest with Diet-1 (8.72 and 10.8 mole%), but were similar among the other diets. n-3 LC PUFA and n-3 PUFA were greater with Diet-1 (38.6 and 39.1 mole%, respectively). EPA was highest with Diet-1 (18.3 mole%) and lowest with Diet-2 (15.3 mole%) and Diet-5 (14.3 mole%). DHA was higher with Diet-3 (7.94 mole%) compared to other four treatments, which ranged between 5.81 and 6.63 mole%. In conclusion, crayfish can perform well with FM-free vegetable diets and PM-based diets. Also, PM can be safely used as a suitable replacer for FM in crayfish diets.

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The effect of poultry protein concentrate and phosphorus supplementation on growth, digestibility and nutrient retention efficiency in barramundi Lates calcarifer.

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Two experiments were conducted on juvenile barramundi testing the replacement of regular poultry by-product meal (PBM) with a premium grade poultry protein concentrate (PPC). In the first experiment, a series of five iso-nitrogenous and iso-calorific dietary treatments composed of incrementally increasing levels of PPC from 0% to 20%, substituting POM were formulated (PPC0, PPC5, PPC10, PPC15, PPC20). The diets were restrictively pair-fed to 63g barramundi for 56 days. In the second experiment, a series of four iso-nitrogenous, iso-calorific and iso-phosphoric dietary treatments composed of incrementally increasing levels of PPC from 0% to 20% substituting POM were formulated (PPC0+P, PPC6.7+P, PPC13.3+P, PPC20+P). An additional dietary treatment was formulated without any phosphorous supplementation (PPC20-P). The diets were restrictively pair-fed to 69g barramundi and the PPC20+P and PPC20-P were also tested when fed to satiation for 42 days. Experiment 1 demonstrated a clear linear trend towards poorer growth performance and feed utilisation when increasing the level of PPC in the diet. Significant differences were noted for final weight, weight gain, SGR and FCR ($P<0.05$) in the fish fed diets PPC15 and PPC20 compared to the PPC0. Protein retention efficiency and the apparent biological value of protein and energy was lower ($P<0.05$) in the PPC20 compared to the PPC0 fed fish. The apparent digestibility of crude protein, gross energy, phosphorous and several amino acids all increased with increasing level of PPC in the diet. In experiment 2, using iso-phosphoric diets, the reverse trend was demonstrated whereby increasing PPC improved the performance of barramundi. Significant differences were noted for the final weight, weight gain, SGR and FCR ($P<0.05$). When comparing the PPC20+P and the PPC20-P fed fish, there was a clear negative impact of not supplementing phosphorus, which was further compounded in fish fed to satiation (interaction, $P<0.05$). The results of these two experiments demonstrate that the effective replacement of regular PBM with high quality PPC is achievable and when the diets are supplemented with phosphorus to an inclusion >1.5%, promotes faster growth, for fish fed restrictedly and to satiation. The results indicate a pressing need to redefine the phosphorus requirement of barramundi juveniles.

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Tailoring of insects as aquafeed ingredients

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Insects are a promising source of protein and lipid for animal feeds. Black soldier fly (BSF, Hermetia illucens) larvae are now being increasingly produced on an industrial scale in Europe and could be a potential feed ingredient also in aquafeeds. The nutritional composition of the BSF larvae depends partly on the composition of the feeding medium and the current trial therefore aimed to look at the effects of rearing BSF larvae on the brown seaweed, Ascophyllum nodosum. A broader understanding of the effect of the composition of the feeding media on the larvae composition can help to tailor BSF larvae into a nutrient profile especially suited for carnivorous marine fish species.

A plant-based feeding medium from industrial side-streams was used as a control and up to 100% of this was replaced by ground seaweed during the 6 day growth period. When the feeding media consisted of more than 50% seaweed, the larvae experienced poorer growth, lower nutrient retention and lower lipid levels, compared to when grown on pure plant-based feeding medium. Eicosapentaenoic acid (EPA, 20:5n-3), iodine and vitamin E concentrations increased in the larvae when more seaweed was included in the diet. However, also undesirable substances like cadmium, lead, mercury and arsenic were transferred from the seaweed to the BSF larvae. Concentrations of these elements in the larvae increased when more seaweed was added to the feeding media. When seaweed inclusion exceeded 20 % of the media, this resulted in larval concentrations of cadmium and total arsenic above the current European Union maximum levels for these elements in complete feed.

In conclusion, BSF larvae have a beneficial nutrient composition for use in aquafeeds and it is also possible to tailor their composition by using marine raw material to increase their content of marine nutrients.

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The oil fraction and partially defatted meal of black soldier fly larvae (Hermetia illucens) affect differently growth performance, feed efficiency, nutrient deposition, blood glucose and lipid digestibility of rainbow trout (Oncorhynchus mykiss)

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The effects of black soldier fly larvae oil (BSFLO) and partially defatted meal (BSFLM) on growth, feed utilization, nutrient digestibility, body composition, gut histology and blood biochemistry of rainbow trout were evaluated over a three-month study. Seven experimental diets containing 0 (Diet A), 6.6 (Diet B), 13.2 (Diet C) and 26.4% (Diet D) BSFLM, and 2.5 (Diet E), 5.0 (Diet F) and 10% (Diet G) BSFLO were randomly allocated to 21 tanks at 30 trout (45.7 ± 1.4 g) per tank. The thermal-unit growth coefficients (TGC) were high with values ≥ 0.260, regardless of BSFLM or BSFLO inclusion. Feed conversion ratios (FCR) were ≤0.91. However, TGC was significantly lower with Diet D (26.4% BSFLM) ($P<0.020$) and FCR values increased significantly at each increment level of BSFLM ($P<0.05$). There was a negative relationship between relative body protein content and dietary inclusion of BSFLM, but not BSFLO ($P<0.000$). Hydroxyproline was the sole amino acid that increased in whole-body of trout fed BSFLM or BSFLO. The inclusion of BSFLM and BSFLO had no impact on the histology of the posterior intestine, but villi in the anterior intestine of trout fed Diet D (26.4% BSFLM) were significantly shorter relative to the Control (1035 ± 146 µm vs. 1176 ± 160 µm). There was a negative relationship between blood glucose and BSFLO inclusion level, which suggested BSFLO had some antihyperglycemic effect in fasted rainbow trout. The ADCs of protein and amino acid in BSFLM varied between 87 and 93%, but the digestibility of lipid and dry matter in BSFLM was relatively low at 73 and 75%, respectively. The digestibility of hydroxyproline was significantly superior in diets containing BSFLM or BSFLO than in the Reference diet ($P=0.014$). Based on these results, the maximum inclusion of BSFLM recommended in rainbow trout diets is 13%. The maximum inclusion of BSFLO could potentially be superior to 10%, but this hypothesis needs further study. The enhanced digestibility of hydroxyproline in diets containing BSFL products has potential benefits for fish fed low fishmeal diets.