18th International Symposium on Fish Nutrition and Feeding
40 Years of Research in Fish Nutrition
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Variability across and within fish species in energy utilization efficiency

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Determination of the optimal dietary digestible energy (DE) level in fish diets requires information on energy requirements for maintenance (DEm) and utilisation for growth (kgDE). This study assessed 1) variability in DEm and kgDE across fish species; 2) how digestible protein (dPro), fat (dFat) and carbohydrates (dCarb) affect kgDE and 3) if these effects differ between fish species. We reviewed literature data from studies relating retained energy (RE) to DE, under optimal nutritional and environmental conditions. Across fish species, DEm ranged from 16 to 110 kJ/kg0.8/d and kgDE from 0.31 to 0.82. Within and across species, variability in kgDE correlated with the trophic level of fish, macronutrient composition of diets and composition of growth. For quantifying the impact of digestible nutrient composition on kgDE, RE was related to dPro, dFat and dCarb using multi-variate analysis. For Nile tilapia and rainbow trout, this was done by systematic analysis across studies undertaken at Wageningen University and INRA, respectively. For common carp and Asian seabass (barramundi), this relationship was estimated within one experiment in which contrasts in dPro, dFat and dCarb were created by varying dietary macronutrient content (in 4 diets) and applying 3 to 5 feeding levels, respectively. For Nile tilapia and common carp, all digestible nutrient intakes were linearly related to RE and the energy efficiency was lowest for dPro and highest for dFat. The relationship between dCarb and RE was curvilinear; for trout significantly and for barramundi with a strong tendency. The estimated relationships between RE and dPro, dFat and dCarb can be used to predict the net energy (NE) value of diets, but also for ingredients if the macronutrient digestibility is known. In conclusion, kgDE is dependent on the digestible nutrient composition, but the extent of these influences is species dependent.

Adipocyte response to nutrient deprivation in Atlantic salmon is influenced by its endogenous lipid composition

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The present study aims to elucidate how Atlantic salmon adipose tissue enriched with different levels of lipids responds to an early stage of fasting. Thus, in vitro differentiated adipocytes pre-enriched with palmitic (16:0, PA), oleic (18:1n-9, OA), or eicosapentaenoic (20:5n-3, EPA) acid for 72h were incubated in nutrient deprived media containing glucagon to mimic a fasting condition.

The amount of intracellular lipid droplets was higher in adipocytes pre-enriched with OA than in adipocytes pre-enriched with PA or EPA. Radioactive PA (10 µM 1-14C PA) was added to the cells in all experimental groups to study their lipogenic capacities. Adipocytes pre-enriched with PA had a significantly higher Δ9-desaturation activity than adipocytes pre-enriched with OA or EPA, indicating a higher lipogenic capacity in PA-enriched adipocytes. Furthermore, adipocytes pre-enriched with EPA had lower intracellular leptin levels and lower secretion of leptin than the cells pre-enriched with PA or OA. After mimicking a fasting situation, all adipocyte groups showed a transcriptional downregulation of leptin. Likewise, the amount of leptin secreted to the culture media was significantly reduced in all treatments already after 3h of glucagon stimulation and nutrient deprivation, and these levels were maintained after 18h. Although adipocytes pre-enriched with PA had an intermediate intracellular leptin level prior to fasting, fasting triggered the highest secretion of leptin compared to the other groups. The mimicking of a fasting situation also stimulated adipocyte lipolysis, where the lipids were primarily secreted as esterified lipids, even though a significant proportion of free fatty acids and glycerol was also found. The intracellular mitochondria reacted rapidly (3h) to the fasting conditions by increasing their area in all experimental groups. However, after 18h of glucagon stimulation and nutrient deprivation, the mitochondria area was back to the value observed in adipocytes prior to the glucagon stimulation.

In summary, our results demonstrated that mimicking a fasting situation induced preferential mobilization of lipids from adipocytes, reduced leptin secretion and increased mitochondria area, indicating a huge plasticity of adipose tissue. The response was to some extent influenced by the cellular fatty acid composition.

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OPTIMUM SELENIUM, MANGANESE AND COPPER LEVELS IN DIETS HIGH IN PLANT BASED FEEDSTUFFS FOR GILTHEAD SEABREAM (Sparus aurata) FINGERLINGS

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Substitution of fishmeal and oil by plant sources alters selenium (Se), manganese (Mn) and copper (Cu) levels in feeds. These minerals prevent oxidative damage and can be potential toxicants at high levels. Gilthead seabream (GSB) is of major interest for Mediterranean aquaculture, however the requirements for Se, Mn and Cu are not determined. This study aimed to determine the effects of dietary Se, Mn and Cu levels in low FM-FO diets for GSB fingerlings.

Three independent trials were conducted using a common plant-based diet (FM 10% and FO 6%). Five diets per trial were supplemented with 0.0, 0.2, 0.4, 0.7 or 1.1 mg Se kg-1 diet, supplied as sodium selenite; 0, 10, 16, 23 and 56 mg Mn kg-1 as MnSO4; or 0, 1, 3, 7 and 29 mg Cu kg-1 diet as CuSO4. 450 GSB fingerlings (weight 12.6 ± 1.5 g (mean ± S.D.)) were distributed into 15 tanks per trial and fed until apparent satiation thrice daily over 42 days. Growth and productive parameters were monitored and samples for biochemical, mineral, histological, gene expression and X-ray studies were taken.

GSB almost tripled their body weight in all trials. In trial 1, increase in Se supplementation up to 0.7 mg Se kg-1 improved growth and up to 0.4 mg Se kg-1 increased lipid deposition in fish and reduced the expression of glutathione reductase (gr). However further elevation of Se up to 1.1 mg Se kg-1, significantly reduced growth, and increased the expression of both gr and glutathione peroxidase, suggesting a potential selenosis. In trial 2, increase in dietary Mn did not affect fish growth or survival, but raised body lipid contents and, elevation up to 16 mg Mn kg-1, increased expression of Mnsod and reduced that of catalase. Finally in trial 3, body weight and other performance parameters, such as FCR, SGR or TGC, were not significantly affected by dietary Cu levels, suggesting that the basal levels of 6 mg Cu kg-1 were enough to cover Cu requirements in GSB juveniles.

Overall the results pointed out the importance of determining optimum dietary levels of micro-minerals in plant based diets for gilthead seabream.

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Effects of different dietary selenium sources on antioxidant status and oxidative stress-related parameters in rainbow trout juveniles fed plant ingredients

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Selenium (Se) is an essential micronutrient required for normal growth, development and antioxidant defence. Oxidative stress induced by stressful conditions has been shown to be reduced by dietary Se supplementation. However, replacing fishmeal, an important source of Se, with ingredients of plant origin usually results in decreased Se content in aquafeeds. The objective of the study was hence to assess the impact of dietary Se supplementation by an inorganic or organic source on the antioxidant defence system of rainbow trout (Oncorhynchus mykiss) juveniles fed practical plant-based feeds under normal or hypoxic conditions. Fish (initial mean weight: 42 ± 2 g) were maintained under normal (25 fish/tank, dissolved oxygen: 7.9 mg/L) or hypoxic conditions (50 fish/tank, dissolved oxygen: 5.9 mg/L) for 12 weeks at 17°C. Rainbow trout juveniles were fed three plant-derived protein-based diets containing 0.5 mg Se/kg diet supplemented or not with 0.3 mg Se/kg diet supplied as sodium selenite or Se-enriched yeast Selsaf®.

Dietary Se supplementation had no significant impact on growth performance contrary to hypoxia that decreased final body weight and feed intake. The apparent digestibility coefficients (ADC) of dry matter, protein, ash and Se were improved by Se-enriched yeast supplementation while the ADC of Se was reduced by sodium selenite supplementation and the ADC of dry matter, starch, energy, ash and phosphorus were reduced by hypoxia. Total Se content of fish was increased by both dietary Se supplementations but to a larger extent with Se-enriched yeast. Fish fed Se-enriched yeast displayed increased hepatic selenomethionine content and decreased muscle anisidine value. Plasma glutathione content was reduced by hypoxia. Glutathione peroxidase (GPX) activity was decreased in non-Se supplemented fish liver while catalase activity was increased by hypoxia. Other antioxidant enzyme activities were not significantly affected. GPX1b2 and GPX4a2 transcript expression was decreased in the non-Se supplemented group similarly to GPX activity whereas hypoxia modulated antioxidant transcript expression differently to antioxidant enzyme activity.

These results suggest the necessity to supplement plant-based diets with Se for rainbow trout juveniles and highlight the superiority of organic form of Se to fulfil the dietary Se requirement and sustain the antioxidant status of fish.

Assessment of twelve dietary macro and trace minerals on growth and tissue composition of black tiger prawns, Penaeus monodon, using a Plackett-Burman screening design

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Minerals cannot be synthesised by animals and need to be supplied exogenously to serve a myriad of biological functions. Unlike terrestrial animals, determining dietary mineral requirements of some fish and crustaceans is complicated by their often undetermined capacity to uptake minerals from seawater. Consequently, there is limited research assessing mineral requirements, compared to other nutrient groups. Furthermore mineral studies in prawns have varied in their scope in terms of species, dietary formulations, environmental conditions and parameters assessed, and limited research has been done on P. monodon. Here we employ a novel experimental method, the Plackett Burman (PB) screening design to determine the essentiality of twelve macro and trace minerals in P. monodon. A PB screening design was used to determine the response to dietary mineral supplementation in isoenergetic and isonitrogenous purified diets in terms of survival, feed intake, growth, and tissue mineral concentrations. The inorganic minerals investigated were boron, calcium, cobalt, copper, magnesium, manganese, phosphorus, potassium, selenium, sodium, strontium and zinc where calcium and phosphorus were considered together at a 1:1 ratio. The array of minerals were investigated as a two-level factor (included or excluded from diets) where their assignment to treatments was determined by the PB assembly as shown in Table 1. Minerals were included at twice the requirement levels as reported by the NRC or at twice the analysed concentration of whole prawns, relative to dietary concentrations. A negative control diet was included to compare the effects of no mineral addition. Juvenile prawns were fed five times a day to satiation for six weeks. PB rankings were determined on preliminary interim growth data for each mineral and indicated a set of minerals that positively influenced weight gains while the inclusion of other minerals tended to reduce weight gain. Overall the inclusion of some minerals in diets generated a 17% increase in weight gain (189.0% vs. 161.1% initial body weight) by week 3. The final study results will be discussed in the context of essential mineral requirements for prawns at the conference.

Using an in vitro model of the fish liver to study the role of phosphorus availability on cell proliferation, cell metabolism, and intracellular trace metal homeostasis.

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Phosphorus (P) is a key nutrient in fish diets. It is for instance, a structural component of nucleic acids and ribosomes, and is therefore supplemented in fish food. However, only a fraction of the phosphorus present in current fish food is actually absorbed into the fish, which results in a high excretion of P into the environment. This is wasteful and can have detrimental consequences for ecosystem health. Moreover, the link between P availability and other essential trace metals important for fish health and growth is largely unknown. Understanding the absorption and interactions of essential nutrients requires a model system that allows detailed analysis of the reactions occurring at the interface between the cell and the extracellular medium. In this study we used an in vitro model of the fish liver, the RTL-W1 cell line, from rainbow trout (Oncorhynchus mykiss), and medium of well-defined composition, to understand the effects of limiting P availability on cell proliferation and cell metabolism. We modified the commercial cell culture medium, Leibovitz's L-15, by lowering P concentration by 50, 75, 90 and 100%, for a total of five P treatments. Identical numbers of RTL-W1 cells were seeded in multiwell plates and incubated in medium of different P content. Cell proliferation was quantified over 7 days using an automated cell imager which counts stained nuclei. Cell metabolism was measured using the Alamar blue assay that measures mitochondrial, microsomal and cytosolic oxidoreductases. While cell proliferation was reduced only in cells cultured in P free medium, we found that cells cultured in low P conditions (90% less than L-15) proliferated at similar rates as the full P (L-15) treatments. However, low P cells exhibited significantly increased metabolism. Future studies will determine the ionomic profile, comprising of 20 different essential elements of RTL-W1 cells exposed to P limiting and P normal conditions. This in vitro approach has the potential to be used as a tool to improve fish diets in a system that is more ethical, less expensive and provides detailed mechanistic information linking nutrient availability and ratios to cell health and proliferation.