



Nutritional programming in gilthead sea bream (*Sparus aurata*): Improvements towards better utilisation of low n-3 LC-PUFA diets

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There is an increasing number of evidence regarding how dietary interventions during the early stages of development can have long-term effects on the metabolism of different organisms, including fish. In the recent years, in commercially important fish species, it has been demonstrated the possibility of routing the metabolism for better usage of more sustainable formulated diets. The present study summarises the effects of early dietary interventions in gilthead seabream aiming to improve the utilisation of low fishmeal and fish oil feeds on growth, n-3 LC-PUFA utilisation and synthesis. The studies showed that offspring utilisation of low n-3 LC-PUFA diets improved if nutritional clues were supplied during the spawning period, not during early or late larval stages in gilthead sea bream. Obtained offspring from brood fish fed different diets showed different expression of lipid metabolism-related genes such as *fads2* at the larval stage even when larvae were fed the same commercial protocol. Moreover, these metabolic adaptations led to better utilisation of low n-3 LC-PUFA diets in the offspring at 6 and 18 months. Thus, nutritionally programmed fish showed better growth and improved feed utilisation, particularly if fish also were nutritionally challenged with a low fish meal and fish oil diet in later life stages. In following studies, offspring growth was also improved by selection of the broodstock with high *fads2* expression, and particularly when offspring juvenile were fed low n-3 LC-HUFA diets. Thus, broodstock selection can also contribute to better utilisation of the low fishmeal and fish oil diets by offspring juveniles. Nutritional programming together with broodstock selection showed promising results to improve low fishmeal and fish oil diet utilisation. Further studies are being conducted to better define protocols for selective breeding and nutritional programming in gilthead seabream.



Dietary influence of omega-3 fatty acids on performance and lipid metabolism in three Atlantic salmon genetic groups selected by divergent Δ -6 desaturase capacity

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Fish oil (FO) and fishmeal are the major sources of omega-3 long chain polyunsaturated fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in Atlantic salmon diets. Due to expanding production and limited availability of marine feed sources, the use of alternative raw materials such as plant ingredients are essential for the sustainable development of aquaculture. Nevertheless, this switch from marine to plant ingredients is associated with changes in fatty acid profile in different tissues, which may have an important impact on fish robustness and its nutritional value for human consumption. This study investigates the consequences of replacing dietary FO with a plant oil from *Camelina sativa* (CO) on three pre-smolt Atlantic salmon groups either non-selected (NS) or selected for high and low Δ -6 desaturase capacity; evaluating the potential nutritional imprinting in freshwater prior to seawater transfer by both genetic background and diets. The experimental fish groups were transferred to diets with different ratios of α -linolenic acid (ALA), EPA, DHA, and EPA+DHA in the post-smolt phase. Fish fed the CO diet showed higher growth rates in the pre-smolt phase, especially in the high Δ -6 desaturase group. However, whole body DHA and EPA levels decreased compared to fish fed the FO diet, with no impact on mortality after seawater transfer. Fish fed the CO diet showed higher total lipids in the liver of the low and NS groups compared to the high Δ -6 desaturase group. The improved growth induced by the CO diet in freshwater levelled off in the seawater period with no differences between the genetic groups. Instead, higher dietary levels of DHA in the post-smolt phase increased growth rates of all three genetic groups indicating the importance of a FO-based diet during the seawater phase. Additional metabolic and transcriptomic analyses from different tissues and isolated hepatocytes are being performed to further elucidate the regulation and efficiency of the omega-3 fatty acid metabolic pathway in the groups. These results provide a better understanding of how to improve EPA and DHA metabolic capacities by nutritional programming in freshwater prior to seawater transfer using plant oil-based diets and by selective breeding



Selected strain of gibel carp shows better utilization on dietary carbohydrate

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Three strains of gibel carp (*Carassius auratus gibelio*) (F strain, A strain and D strain) were fed with three isonitrogenous and isolipid (400 g crude protein and 84 g crude lipid kg⁻¹ dry matter) diets containing increased levels of corn starch (0, 300, 450 g kg⁻¹ dry matter) to investigate the different response to dietary carbohydrate. Triplicates of 20 fish (body weight 3.0± 0.5 g) were fed to apparent satiation by hand two meals a day. After a 56-day growth trial, the results showed that F strain and A strain had a better capability to use dietary carbohydrate than D strain. Feeding rate (FR), specific growth rate (SGR) of F strain were significantly higher than D strain and A strain (P<0.05). F strain and A strain had a greater metabolic ability to clear a glucose load by converting excess glucose into triglyceride and storing more glycogen in liver and muscle (P<0.05). Amylase activity of A strain was significantly higher (P<0.05), compared to D and F line. Moreover, mRNA levels of hepatic glycolytic enzymes (GK, PFK and PK) were different between three strains. GK was expressed in significantly higher quantities in F strain and A strain than in D strain; The expression quantity of hepatic gluconeogenic enzymes PEPCK and FBP were concurrently higher in D strain; There were no significant differences in gene expression of hepatic enzymes (ACLY, FAS) involved in lipogenesis; However, D strain showed a higher expression of GLUT2, which was the major glucose transporter of liver. In conclusion, the glucose metabolism between three strains was not strongly affected by dietary carbohydrate levels. Selected F strain showed better utilization of dietary carbohydrate by improving the glycolysis and depressing gluconeogenesis.



Transcriptomic and epigenetic effects of high dietary arachidonic acid in the next generation

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The increased replacement of marine feed ingredients with plant-based raw materials in fish feed has changed the fatty acid profiles towards omega-6 and away from omega-3 PUFAs. Diets enriched in omega-6 polyunsaturated fatty acids (PUFAs) and deficient in omega-3 PUFAs, have been associated with the promotion of pathogenesis of many diseases in humans. Increased levels of arachidonic acid (ARA), an omega-6 PUFA, have been shown to impact bone development, growth and reproduction in fish. Nutrients can also influence epigenetic key players, such as DNA methylation, which can regulate gene expression.

In a zebrafish trial, we investigated the impact of increased dietary ARA intake on the next generation. We compared two groups that were fed a diet either high or low in ARA in the first generation, while the progeny of both groups were fed the low ARA diet. Changes in gene expression and DNA methylation profiles in adult male livers from both generations were investigated using RNA-sequencing and reduced representation bisulfite sequencing (RRBS).

In the parents' generation, we observed a general shift in lipid profiles, signs of lipid peroxidation and increased oxidized and pro-inflammatory environment in response to high dietary ARA. The parental diet was associated with changes in liver DNA methylation and gene expression profiles in both parents and the adult progeny. Interestingly, we observed more differentially expressed genes in the progeny than in the parents, showing changes in the expression of genes involved in the methionine cycle, transsulfuration pathway, estrogen signaling, and lipid and retinoid metabolism. Looking at upstream regulators of differentially methylated genes identified effects on de novo lipogenesis, possibly affecting energy metabolism and estrogen signaling.

The present study demonstrates that the level of ARA in parental diets can affect gene expression and DNA methylation pattern in both parents and adult progeny, possibly influencing lipid metabolism. Several links were found between the previously reported metabolic profiles of the parents and both DNA methylation and gene expression in adult progeny. These results suggest an impact of parental diet on adult progeny, which may be initiated during early embryonic development.



**Molecular and functional characterisation of two elovl4 elongases involved
in the biosynthesis of very long-chain (> C24) polyunsaturated fatty acids in
black seabream *Acanthopagrus schlegelii***

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Elongation of very long-chain fatty acid (Elovl) 4 proteins are important fatty acyl elongases that participate in the biosynthesis of long-chain (C20–24) and very long-chain (>C24) polyunsaturated fatty acids (LC-PUFA and VLC-PUFA, respectively) in teleost fish, especially in marine species. Moreover, knowledge of Elovl4 and other

elongases such as Elovl2 has contributed to an advanced understanding of the LC-PUFA biosynthetic pathway in marine fish. In the present study, elovl4a and elovl4b were cloned from black seabream *Acanthopagrus schlegelii* and functionally characterised using recombinant expression in yeast. The elovl4a and elovl4b cDNA sequences included open reading frames (ORF) of 969 and 918 base pairs (bp), encoding proteins of 322 and 315 amino acids (aa), respectively. The functional characterisation of *A. schlegelii* Elovl4 proteins showed they were able to utilise all assayed C18–22 PUFA substrates except 22:6n–3. Moreover, it was particularly noteworthy that both *A. schlegelii* Elovl4a and Elovl4b proteins had the ability to elongate 20:5n–3 and 22:5n–3 to 24:5n–3, which can be potentially desaturated and β -oxidised to 22:6n–3. Tissue transcript abundance analysis showed the highest expression of elovl4a and elovl4b in brain and eye, respectively, suggesting these tissues were major sites for VLC-PUFA biosynthesis in black seabream. The functions of the *A. schlegelii* Elovl4-like elongases, Elovl4a and Elovl4b, characterised in the present study, along with those of the Elovl5 and fatty acyl desaturase (Fads2) proteins of *A. schlegelii* characterised previously, provided evidence of the biosynthetic pathways of LC-PUFA and VLC-PUFA in this teleost species.



Vitamin E stimulates the secretion of gonadotropin hormone of broodstock tongue sole (*Cynoglossus semilaevis*): Evidences from in vitro and in vivo studies

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Gonadotropin Hormone (GtH), as the intermediate signal molecule in the hypothalamus – pituitary – gonadal endocrine system, plays an important role in the reproductive development and endocrine regulation in fish. The mechanism between vitamin E and GtH is not clear, especially in marine aquaculture broodstock. Therefore, this project was conducted to investigate the relationship between vitamin E and GtH by given vitamin E or not through the primary pituitary cells in vitro. The cells were cultured in L-15 medium supplemented with 5% fetal bovine serum, 100 U/mL penicillin, 100 µg/mL streptomycin, and were survived as long as 7 days in the incubator of 24°C. The cells can secrete the GtH. Adding vitamin E (0, 18 and 54 µmol/mL) to the cells for 3 days, and then the supernatant and the cells were separated and collected to determine the indexes. The results showed that GtH levels in the supernatant were increased with the increasing vitamin E, the GtH mRNA level in cells were up-regulated, and the cellular immunohistochemistry showed that GtH secreting cells increased. A graded level of vitamin E (vitamin E acetate: 0, 200, 400, 800 and 1600 mg/kg basal diet) feeding trail were conducted to confirm the previous results with 3-year-old female broodstock tongue sole for 8 weeks. The results showed that GtH content and its mRNA level in the pituitary were both significantly increased with the increasing dietary vitamin E. These data demonstrated that Vitamin E can stimulate the secretion of gonadotropin hormone in the pituitary by in vitro and in vivo approaches. The cultured cells in this study can be applied in the mechanism study on fish nutrition and reproduction at the cellular level, also, this provides a new way of doing the relative study with this convenient experimental materials.



Effect of rearing temperature on the digestive function in Cobia fry

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Water temperature is a key factor affecting metabolic rates and therefore fish feeding physiology and behavior. These effects have a special significance in the scenario of the global warming. In this study we have examined effects of an increased water temperature on the daily pattern of gastrointestinal pH, enzymatic proteolytic digestive activity and the food transit time in early juveniles of cobia (*Rachycentron canadum*), a candidate species for aquaculture in tropical and subtropical waters.

Juveniles (3.7 ± 0.4 g wet BW) were randomly distributed to 6 experimental 200-L tanks (60 fish tank⁻¹) and reared at two temperatures (30 and 34 °C) in recirculation systems for six weeks. Fish were fed twice a day (8:00 and 16:00h) with an experimental diet containing 47% protein and 10% lipid. At the end of the experiment, fish were sampled every 4 hours to obtain daily patterns of processes related to digestive activity.

Cobia reared at 30°C grew faster and with higher FCR than those at elevated temperature (34°C). The fish reared at high temperature had lower intestinal luminal pH but similar gastric pH. Overall the pepsin activity during the complete daily cycle, but it was significantly different from the fish maintained at 30°C only at 8 hours post-feeding. Similarly, the trypsin activity was not significantly affected by the water temperature but tended to be higher at 34°C during the first hours post-feeding. The transit rate was notably faster 34°C in the stomach but similar in the intestine.

These results indicate that the slight increase of proteolytic activity that was observed at 34°C cannot compensate for the faster gut transit rate. The reduced time the dietary proteins are available for hydrolysis can explain the lower growth observed at this temperature.

Acknowledgements: Project WISEFEED funded by the European Union's H2020 program (Marie Skłodowska-Curie grant No 691150)



Effect of dietary inorganic and organic selenium supplementation on reproduction and egg quality in rainbow trout (*Oncorhynchus mykiss*)

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A feeding trial was conducted to investigate the effect of dietary selenium (Se) supplementation in a plant ingredient-based diet on reproductive performance and egg quality of rainbow trout *Oncorhynchus mykiss*. Three plant ingredient-based diets were designed with either no supplementation (Se1, basal Se level 0.3 mg/kg diet) or supplemented with 0.3 mg/kg diet as sodium selenite (Se2) or hydroxy-selenomethionine (Se3). Each group consisted of 25 females and 15 males with a mean (\pm SD) weight of 1100 ± 199 and 861 ± 294 g, which were fed the test diets over a 6 month period prior to spawning. There was no significant difference in growth, but the total number of spawning females was significantly higher for the Se-supplemented groups Se2 and Se3 compared to Se1 (21 and 24 vs. 17). Females fed Se3 began to spawn earlier compared to females fed Se1 and Se2. However, no significant difference in other measures of reproductive performance, including gonadosomatic index (15 ± 3), relative fecundity (2459 ± 732), egg size (weight: 51.6 ± 10.0 mg and diameter: 4.0 ± 0.3 mm) and hatching rate (86.9 ± 14.8 %) was detected between groups.

Total Se content was significantly higher in muscle of females fed Se3, compared to the ones fed Se1 or Se2 (128 ± 26 vs. 91 ± 13 and 99 ± 14 $\mu\text{g}/\text{kg}$). The same tendency was noticed for female liver (969 ± 163 vs. 654 ± 180 and 932 ± 369 $\mu\text{g}/\text{kg}$). Total Se content in oocytes was significantly higher from females fed the hydroxy-selenomethionine supplemented diet Se3 and lower for non-supplemented diet Se1 compared to the Se2 group (505 ± 105 and 220 ± 37 vs. 334 ± 49 $\mu\text{g}/\text{kg}$), whereas for males no significant difference in total selenium concentration in milt between the groups was detected (25.3 ± 7.4 $\mu\text{g}/\text{kg}$). These first results highlight the importance of dietary selenium and its source in rainbow trout broodstock with increased spawning and increased parental transfer to oocytes. Further investigation is underway to evaluate the effects of Se supplementation in plant ingredient-based broodstock diets during further development of F1 generation.