



Health and flesh quality of Atlantic salmon fed a modern low fishmeal diet supplemented with Antarctic krill, *Euphausia superba*

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Atlantic salmon (*Salmo salar* L.) were fed a commercially relevant diet with 15% fishmeal (Control group) or the same diet substituted by 12% Antarctic krill meal (Krill group). The isoprotein (35%) and isolipid (35%) diets were fed to quadruplicate net pens (125m³; average seawater temperature 11°C) during a 10-week period, autumn 2017. The body weight increased from 2.2 to 3.9 kg during the experimental period, corresponding with a TGC of 3.88 and 3.92 for Control and Krill group, respectively. The average FCR was 1.1. The Krill group had a more voluminous body shape compared with the Control group (higher CF). The hepatosomatic and cardiosomatic indices were similar for the dietary groups, but the livers of the Krill group were darker and the amount of visible fat on the heart was significantly lower. Microarray analyses of liver tissue revealed that dietary treatment affected a large number of immune genes, and a panel of structural genes were upregulated in the livers of salmon fed the Krill diet, including cadherin and connexin (tight junction proteins). Gene enrichment analyses revealed that sugar metabolism was stimulated in fatty hearts and a significant downregulation was observed for complement genes and the retinoid metabolism. Additionally, downregulation of extracellular matrix proteins, including collagens and glycan's, indicated weaker tissue structure of the Control group. Fillets of salmon fed the Krill diet had firmer texture, more intense red coloration, and the problem with fillet gapping was lower (i.e. improved muscle integrity).

It is concluded that dietary inclusion of krill meal in modern low fishmeal diets has a positive effect on health and fillet quality of Atlantic salmon



A microalgal oil containing EPA+DHA can be an effective source of omega 3 for Atlantic salmon post-smolts.

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Being a major source of essential omega 3 (EPA and DHA) in human diet, fish consumption is today associated with numerous health benefits. Aquaculture, especially salmon farming, is expected to increasingly contribute with sustainable and affordable supply of EPA and DHA to human diets. Our major goal is to help the industry maintaining the high nutritional value of DHA and EPA in salmon fillet while reducing reliance on limited supplies of fish oil (FO), traditionally used as the omega 3 source in salmon diets.

With this purpose, we have conducted two studies to evaluate a microalgae oil rich in EPA+DHA (Veramaris) as an effective replacer of either fish oil (Study 1) or EPA+DHA of fish oil (Study 2) in diets of Atlantic salmon post-smolts. A diet with 10% FM and 10% FO was used as control in both studies. Results on growth, digestibility and fillet deposition of EPA and DHA of the control diet were compared with 5 other diets gradually replacing FO (25, 50, 75 or 100% -Study 1) or EPA+DHA in FO (25, 50 or 100% -Study 2).

Replacement of either FO or EPA+DHA of FO with the microalgae oil in Atlantic salmon diets did not affect growth of 140 g or 415g initial body weight salmon. Dietary EPA and DHA, originating from microalgae oil, were highly digestible. Furthermore, their deposition in salmon flesh reflected well the content in the diets, as it has been previously described in the literature.

Overall, these results show that a microalgae oil containing EPA+DHA can effectively replace FO or EPA+DHA of fish oil as an alternative source of omega 3 in diets of Atlantic salmon post-smolts.



Different physiological roles of insulin receptors in mediating nutrient metabolism in zebrafish

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Insulin, the most potent anabolic hormone, is critical for somatic growth and metabolism in vertebrates. Type 2 diabetes, which is the primary cause of hyperglycemia, results from an inability of insulin to signal glycolysis and gluconeogenesis. Our previous study showed that double knockout of insulin receptor a (*insra*) and b (*insrb*) caused β -cell hyperplasia and lethality from 5 dpf to 16 dpf. In this study, we characterized the physiological roles of *Insra* and *Insrb*, in somatic growth and fueling metabolism, respectively. A high-carbohydrate diet was provided for insulin receptor knockout zebrafish from 60 dpf to 120 dpf to investigate phenotype inducement and amplification. We observed hyperglycemia in both *insra*^{-/-} fish and *insrb*^{-/-} fish. Impaired growth hormone signaling, increased visceral adiposity, and fatty liver were detected in *insrb*^{-/-} fish, which are phenotypes similar to the lipodystrophy observed in mammals. More importantly, significantly diminished protein levels of P-PPAR α , P-STAT5 and IGF-1 were also observed in *insrb*^{-/-} fish. In *insra*^{-/-} fish, we observed increased protein content and decreased lipid content of the whole body. Taken together, although *Insra* and *Insrb* show overlapping roles in mediating glucose metabolism through the insulin signaling pathway, *Insrb* is more prone to promoting lipid catabolism and protein synthesis through activation of the GH signaling pathway, whereas *Insra* primarily acts to promote lipid synthesis via glucose utilization.



The Potential Applications of Stable Isotopes in Experimental Nutrition Studies

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Stable isotope techniques are widely used in ecological research to study the nutrient flow and trophic level in nature. However, it has been used very little in experimental nutrition studies where it could be useful method for estimation of nutrient assimilation from known feed components. When feeding the animal with a specific diet, the stable isotope ratio of the tissues gradually changes towards that present in their diet, and the tissues will isotopically represent the consumed diet with a tiny isotopic difference referred to as diet-tissue shift.

The aim of this project is to investigate the potential applications of stable isotope analysis in fish nutrition. The idea was to prepare experimental feeds with distinctive C and N stable isotope ratios and trace the C and N assimilation from different protein sources.

At the first stage, we studied the potential effect of both dietary protein sources and type of tissues on the diet-tissue shift. Diet-tissue shift is the net result of a series of biochemical reactions involved in metabolism. A negative correlation was observed between the diet-tissue shift of nitrogen stable isotope ratio and growth. The protein sources with higher nutritional quality was assimilated effectively compared to those with lower quality, and led to a lower value of diet-tissue shift.

In the second experiment, we used a Bayesian mixing model FRUITS to estimate the relative contribution of corn gluten meal and potato protein towards the muscle and liver tissue of juvenile rainbow trout fed compound feed produced from relevant protein sources. The concentration-dependent mixing models were applied to estimate the relative contribution of dietary protein sources and original tissues over time, using both nitrogen and carbon stable isotope ratio. The contribution of protein sources increased over time although the growth differed among treatments.

In conclusion, stable isotope analysis is a potential method for nutritional research. However, further studies with advanced experimental design are needed in order to comprehensively unearth the biological mechanism behind the uncertainties associated with stable isotope mixing models.



Impact of dietary ingredient composition on fecal characteristics, nutrient availability and waste production in common carp reared in RAS.

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In aquaculture, feed is a common factor that affects fish production as well as the nutrient load of effluent waste. In feeds designed for recirculation aquaculture systems (RAS), it is of added importance to understand the impact of specific dietary components on the fish growth as well as the physical and chemical characteristics of the waste produced. Therefore, ingredients were chosen to assess the effect of (i) starch (field peas, PEA); (ii) protein (feather meal, FeM); (iii) insoluble non-starch polysaccharides, NSP (sunflower meal, SFM); and (iv) soluble NSP (wheat dried distillers grain with solubles, WDG) on waste characteristics in common carp.

Five diets, a control diet (CON) and four other diets (PEA, FeM, SFM and WDG) were prepared by replacing 30% of the basal mixture in CON with one of the test ingredient. All the diets had yttrium oxide as the inert marker. Common carp juveniles (initial weight, 94 ± 4 g) were stocked in 15 tanks (15 fish/tank) of a RAS system. The fish were randomly assigned to one of the five diets, in triplicate and fed restrictively for 6 weeks (at 23°C). During the last week, faeces were collected by settling tanks and stripping to quantify nutrient availability and fecal physical characteristics, respectively.

Growth was the highest for CON and WDG; and lowest for FeM and SFM groups ($P < 0.001$). Osmolality and viscosity of stripped faeces were unaffected by diet ($P > 0.10$); while dietary treatments affected fecal stability at day 29 ($P < 0.05$); but not thereafter. Apparent digestibility of dry matter, protein and apparent availability of phosphorus and other minerals were also differentially affected by dietary groups ($P < 0.001$). The recovery percentage of faecal waste was high for all groups (71 and 80%), but was still influenced by diet ($P < 0.05$); highest for CON and WDG, and the lowest for SFM diet. The amount of removed solids (faeces) was highest at both NSP-rich diets, SFM and WDG; however, SFM diet also led to the highest level of non-removable solids ($P < 0.001$). The present study showed that the quantity, consistency and composition of fecal waste were affected by dietary ingredient composition in common carp.



FATTY ACID METABOLISM AND PERFORMANCE IN ATLANTIC SALMON AS AFFECTED BY DIETARY OILS AND SEASONALITY: RESULTS FROM A LONG-TERM, ON-FARM GROWTH TRIAL

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Seasonal changes in water temperature affect the utilisation of dietary fatty acids in Atlantic salmon (*Salmo salar*). Furthermore, fatty acid profiles of dietary oils dictate their suitability in terms of the provision of metabolic energy and final product quality. In this study an on-farm growth trial was conducted in Tasmania, Australia over the final year of an Atlantic salmon grow-out cycle (323 days), consisting of a 'summer phase' and a 'winter phase'. Three commercially manufactured experimental extruded diets, containing either poultry by-product oil, canola oil or tallow at 80 % inclusion (and remaining 20% fish oil), were assessed for growth, fillet fatty acid composition and fatty acid metabolism. Under the sub-optimal conditions of the summer phase (11.7 – 18.2 oC), the apparent in vivo fatty acid metabolism results indicated that the tallow diet provided greater substrate for β -oxidation and produced significantly higher fillet n-3 LC PUFA concentrations. Additionally, due to very low levels of dietary omega-6 in the tallow diet, a superior n-6/n-3 ratio was achieved, despite overall growth and lipid digestibility being compromised. The following winter phase (9.7 – 13.1 oC) results indicated that all treatments equally, and adequately provided digestible energy, and fillet n-3 LC PUFA concentrations were comparable.

As a mechanism to overcome the shortfall in lipid digestibility - and maintain the nutritional and β -oxidation substrate advantages of dietary tallow inclusion - it is suggested that optimal seasonally tailored diets should be formulated to a pre-determined digestible lipid content, in the same manner that protein inclusion is commonly formulated in aquafeed. A further, unintended finding of this study was the need to appropriately interpret results pertaining to growth parameters. The vast majority of fish growth trials are conducted in triplicate replication. However, in the present study, power analysis demonstrates that statistically significant results can be difficult to detect due to such experimental design constraints. A reduction in statistical power observed when comparing quadruplicate vs triplicate replication highlights that studies with only three replicates are substantially "under-powered".