



Effects of glycinin and β -conglycinin on growth performance, digestion and intestinal morphology in juvenile Chinese mitten crabs (*Eriocheir sinensis*)

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This study was performed to investigate the effects of dietary antinutritional factors, glycinin and β -conglycinin on the growth, digestive ability, immunity and intestinal morphology of juvenile Chinese mitten crabs (*Eriocheir sinensis*). Five isonitrogenous and isolipidic diets were formulated to contain 0 (control group), 70 or 140g/kg β -conglycinin, and 80 or 160g/kg glycinin, which was fed to *E. sinensis* juveniles for seven weeks. Dietary inclusion of either glycinin or β -conglycinin had a significantly negative effect on the survival rate and weight gain of crabs. The catalase activities in intestine of crabs fed diets containing antinutritional factors were significantly lower than the control group, while malondialdehyde concentrations in hepatopancreas and intestine were significantly higher than those in control group. In addition, activities of trypsin and amylase in intestine were suppressed by dietary β -conglycinin and glycinin, but the activity of lipase was not affected. Dietary glycinin or β -conglycinin impaired the immunity and morphological structure of intestine, especially the peritrophic membrane. Consistent with this, the mRNA expression of lipopolysaccharide-induced TNF- α factor (LITAF) and interleukin-2 enhancerbinding factor 2 (ILF2) increased, which are constitutive and inducible immune response gene involved in Chinese mitten crab innate immunity. Moreover, the mRNA expression of peritrophin-like gene (EsPT) and peritrophic2 (PM2), the main factors related with the structural integrity peritrophic membrane, significantly decreased. The results indicated that both dietary β -conglycinin and glycinin could induce inflammation and oxidation damage in intestine, and cause dysfunction of intestinal digestion in crabs, which was the reason why the growth was impaired.



Intestinal health and function of Atlantic salmon fed feed ingredients of insect origin

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In salmon aquaculture, the limited availability of sustainable feed ingredients is a major obstacle. Insects, being part of the natural diet of salmonids, may act as a sustainable resource to expand the raw material repertoire. In the AquaFly project, the potential of black soldier fly (*Hermetia illucens*) grown on low-quality organic matter as a source of sustainable feed ingredients for Atlantic salmon was assessed in one fresh- and one seawater feeding trial. Herein, we summarize data from the 8-week freshwater trial with salmon pre-smolts fed either a test diet in which insect meal (IM) provided 85% protein, or a practical reference diet with a combination of fish meal and vegetable meals as protein source. Results from the seawater trial wherein fish meal was fully replaced by the IM are currently being generated and will also be presented and discussed.

No differences between diet groups were recorded for feed intake, feed conversion ratio, body weight gain or protein productive value. The hepatosomatic and viscerosomatic indices were higher in the IM fed group. IM inclusion did not cause any differences in hemoglobin value or in plasma clinical chemistry, but increased plasma cholesterol levels. Histological examination of the pyloric caeca showed clear signs of lipid droplet accumulation in fish fed the reference diet, whereas this was less pronounced in the IM fed fish. Expression profiling of genes relevant to lipid metabolism reflected the histological findings. Immune gene expression profiles and genes related to barrier function were generally not affected by diet. The fish fed IM showed increased expression of certain genes indicative of stress response and detoxification. The gut microbiota of fish fed IM showed a distinctive community structure and functional adaptations to the diet composition, as illustrated by the divergent metabolic capacities regarding fermentation, cellulolysis and chitinolysis.

In conclusion, results from the freshwater trial suggest that the growth and gut health of salmon were not affected by IM inclusion, whereas the gut mucosa lipid deposition and gut microbial community were clearly affected.



Environmental concentrations of antibiotics impair zebrafish gut health

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Antibiotics have been widely used in human and veterinary medicine to both treat and prevent disease. Due to their high water solubility and low bioavailability, many antibiotic residues have been found in aquatic environments. Fish are an indispensable link between the environmental pollution and human health. However, the chronic effects of environmental concentrations of antibiotics in fish have not been thoroughly investigated. Sulfamethoxazole (SMX) and oxytetracycline (OTC) are frequently detected in aquatic environments. In this study, zebrafish were exposed to SMX (260 ng/L) and OTC (420 ng/L) for a six-week period. Results indicated that exposure to antibiotics did not influence weight gain of fish but increased the metabolic rate and caused higher mortality when treated fish were challenged with *Aeromonas hydrophila*. Furthermore, exposure to antibiotics in water resulted in a significant decrease in intestinal goblet cell numbers, alkaline phosphatase (AKP), acid phosphatase (ACP) activities, and the anti-oxidant response while there was a significant increase in expression of inflammatory factors. Antibiotic exposure also disturbed the intestinal microbiota in the OTC-exposed group. Our results indicated that environmental antibiotic concentrations can impair the gut health of zebrafish. The potential health risk of antibiotic residues in water should be evaluated in the future.



Modelling responses to virus and functional amino acids in immune cells from diploid and triploid Atlantic salmon reared at different temperature

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Environmental climate change implies that farmed fish may be exposed to more frequent periods of sub-optimal water temperatures, which results in several physiological and fitness consequences for the fish. Fish exposed to temperatures above the optimum for growth alter the metabolism, resulting in changes in nutritional status, membrane lipid composition, tissue antioxidants and redox potential. This may influence the integrity and function of the immune cells, as well as the ability to mount an optimal immune response. Viral diseases cause major losses in aquaculture and interactions between sub-optimal temperatures and reduced nutritional status on the immune response is therefore important to elucidate. Triploid salmon are considered for commercial aquaculture due to environmental and production purposes. Triploids are functionally sterile, which eliminate genetic interactions between escaped farmed and wild fish and mitigate pre-harvest maturation in the production. However, triploids are more sensitive to sub-optimal environmental conditions and differences are seen in the immune response after viral challenges compared to diploids, and were therefore included in the experimental model.

An in vitro study was performed to investigate whether rearing temperature, with analyzed alterations in the metabolic and nutritional status, influenced the immune response to a viral mimic (Poly I:C) in diploid and triploid Atlantic salmon of the same genetic origin. In the same model, it was examined whether the immune response could be influenced by histidine and arginine, since these amino acids may influence both inflammation and oxidation processes in immune cells. Primary head kidney cells were isolated from diploid and triploid Atlantic salmon reared at 13 or 18°C for two months. The cells were exposed to Poly I:C, with or without surplus histidine and arginine in the medium for 24 hours. Results will be presented with focus on changes in the nutritional status of the fish, and immune response, signaling and oxidative stress in poly I:C stimulated cells from fish reared at different temperatures. The results from the present study may be used to improve robustness during challenging production periods such as periods with high temperatures and disease outbreaks.



Dietary amino acids inclusion in fishmeal-free diet for gilthead seabream (*Sparus aurata*) juveniles induces opposite effects depending on feeding time.

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The concept of maintaining animal health through the best possible nutrition is well-accepted in modern animal farming, and amino acids appear to be good candidates as functional additives to improve health. The present study aimed to explore short and long-term effects of dietary amino acids (AA) supplementation on the gilthead seabream growth performance and immune status, in the context of a challenging fishmeal-free formulation.

Triplicate groups of fish (13.3 ± 0.3 g) with a fishmeal diet background were either fed a control diet with an extreme formulation (0% fishmeal) but meeting the AA requirements (CTRL), or the CTRL diet with a 2% or a 9% surplus of histidine, threonine and tryptophan (HTW2 and HTW9, respectively). After 2 and 12 weeks of feeding, fish samples of head-kidney, liver and muscle were collected for gene expression, whereas plasma was suited for humoral immune parameters.

There were no differences in final body weight among treatments. However, there was a tendency for an impaired growth performance in fish fed HTW9 after 12 weeks, which may be linked to an increased FCR at early stages in this group. The expression of a panel of 29-32 genes specific for muscle, liver or head-kidney confirms an effect due to the treatments across time. A two-way ANOVA analysis revealed that 15-24 genes vary significantly depending on the tissue, and the multivariate analysis of all shows that around 83% of variation is explained by the two first principal components (PC). In this regard, PC1 (60.3%) justified the effect of individual variability within diet mostly due to HTW9 at early stages, whereas PC2 (22.8%) represents a clear time effect. Particularly in the case of HK, fish fed HTW9 displayed an immunostimulated state at 2 weeks. No major differences were observed in plasma humoral parameters, with only an increase in antiprotease and peroxidase activities after 12 weeks regardless of dietary treatment.

Overall, dietary AA supplementation might improve the seabream immune status over a short-term period. Hence, the use of nutraceutical feeds is especially relevant before/during/after exposure to stress, taking into account that these putative advantageous effects seem to disappear after a long-term feeding period.



GROWTH PERFORMANCE, SKIN STRENGTH AND CONSEQUENT INFESTATION OF SEA LICE *Caligus rogercresseyi* ON ATLANTIC SALMON *Salmo salar* FED DIETS CONTAINING AVAILA[®]ZN ZINC AMINO ACID COMPLEX

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The objective of this study was to evaluate experimental diets with differing zinc sources on growth performance, skin strength and effect against sea lice (*Caligus rogercresseyi*), after an infestation challenge in Atlantic salmon *Salmo salar*.

Two zinc sources were used in this experiment: an inorganic form as zinc sulfate and zinc amino acid complex (Availa[®]Zn, Zinpro Corporation, Eden Prairie, MN USA). A commercially relevant basal diet was supplemented with 1 of 3 Zn combinations to form treatments: 1) 120 mg kg⁻¹ of Zn from zinc sulfate; 2) 60 mg kg⁻¹ of Zn from zinc sulfate + 60 mg kg⁻¹ Zn from Availa-Zn; and 3) 60 mg kg⁻¹ of Zn from Availa-Zn. Two phases made up the experimental timeline, with days 0 to 60 evaluating growth performance, and days 61 to 81 being used for an infestation challenge with sea lice. In Phase 1, growth performance was acceptable for all dietary treatments, with final weight of fish fed solely Availa-Zn being greater ($P < 0.05$) than final weight of fish fed the combination of zinc sulfate and Availa-Zn. In Phase 2, fish fed with solely Availa-Zn had less ($P < 0.05$) sea lice adherence compared to fish consuming solely zinc sulfate. This indicated that fish fed 60 mg kg⁻¹ of Zn as Availa-Zn had significantly higher resistance to sea lice infestation, compared with fish consuming inorganic zinc. A skin damage score was used to evaluate skin integrity after sea lice infestation, which indicated that fish fed solely Availa-Zn had a more desirable level of skin integrity than fish fed either of the other two diets, though the difference was not significant ($P > 0.05$).

These data indicate that feeding diets containing 60 mg kg⁻¹ Zn as Availa[®]Zn zinc amino acid complex is suitable for improving growth performance and lessening the impact of sea lice in Atlantic Salmon.