

respectively. In terms of immune functions, respiratory burst and complement activities, total serum protein, peroxidase activity and catalase activity, one phosphate supplement could not prove significant relative advantage over others. However, fish fed dietary group MSP showed significantly higher lysozyme activity and it was not significantly different with MKP and MAP diet groups. MCP diet showed significantly lower lysozyme activity. The results of different immune gene expressions will be discussed in the conference.

Keywords: Monophosphates, Post-prandial absorption, macro minerals, immunity, rainbow trout

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O-095.

Searching integrated strategies for the evaluation of the physiological status in fish fed functional diets: The example of SDPP in gilthead sea bream (*Sparus aurata*)

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Abstract

The sustained development and intensification of production systems may negatively affect the health of fish, compromising the industry productivity. One of the most used strategies for the sustained improvement in production efficiency is the generation of functional diets. In this study, we evaluated the effect of spray-dried porcine plasma (SDPP) as dietary supplement and its impact upon the physiological and defense status in the skin-associated lymphoid tissue (SALT) of gilthead sea bream. To do it, fish were fed a high-content fish meal diet supplemented with 3% SDPP. After 95 days of feeding, the SDPP-supplemented diet showed a higher somatic growth performance compared to those fish fed basal diet. A higher viscerosomatic index and lower perivisceral fat index were also registered in SDPPfed fish. Fillet proximate composition showed no changes in the fatty acid profile neither the lipid peroxidation nor the activity of oxidative stress enzymes. The skin transcriptomics showed differential expression of 194 genes (DEGs) involved in different processes including cell metabolism, gene expression, protein transport, and protein localization to membrane. The skin mucus proteome identified 35 differential synthesized proteins associated to different processes including epidermis & skin development, epidermal cell differentiation, and metabolism. Importantly, when an integrated multi-omics analysis was carried out, other biological processes were identified, including innate immune response and response to stimuli (organic substances, external stimuli). Taken together, our results suggest that (1) the SDPP is a promising feed additive for sea bream giving a beneficial impact on its growth performance; and (2) the multi-omics analysis is an interesting initial strategy for aquatic organisms that greatly helps to understand the biological processes in an integrated physiological context.

Keywords: Animal nutrition, dietary supplements, mucosal-associated lymphoid tissue (MALT), innate immunity.

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O-096.

Feeding stress due to soy bean meal as a model for the development of molecular immune markers in rainbow trout

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Abstract

The increasing intensification of aquaculture practices has led to public debate about the welfare status of cultured fish.

In order to verify the immune status of trout in context of husbandry stress and to investigate the relationship between chronic stress, immunosuppression, husbandry and feeding we established molecular stress markers using a feeding stress experiment with soybean meal. The welfare status of trout was verified by investigation of mRNA expression of different potential stress regulated genes in whole blood to establish a minimal invasive method. A 56-day feeding experiment was carried out. The triplicate fish groups were fed isoenergetic and isonitrogenic feed mixtures in which the fish meal (50 % of the total diet) was replaced by 0 %, 33 %, 66 % and 100 % soybean meal. EDTA blood was collected from the caudal vein of immobilized trout. In total 88 different genes were tested for their suitability in stress detection using a Fluidigm Biomark HD and a Light Cycler System. Specific primers were designed. Regulated genes belonging to the superior signal transduction pathways such as SERPIN G superfamily, intracellular PI3K/actin, Toll-like receptor, NF-κB, MAP kinase and JAK-STAT signal transduction or intracellular pathogen recognition receptors were tested. The mRNA expression of blood cells was tested for different pro- and anti-inflammatory cytokines, chemokines, substances involved in the acute phase reaction, complement cascade or inflammatory reactions, and heat shock proteins. Finally, different marker genes for specific cell populations were investigated. The housekeeping genes β-Actin, EF1 and RPS5 served as internal standards.

Different genes (e.g. SAA, MPO, NOS2, UCP2) emerged as suitable stress and immune markers and therefore as welfare indicators on a molecular level, while some genes (e.g. IL10, IFN, HSP47) revealed no correlation to feeding stress.

The results represent an important basis for a better assessment of animal welfare in trout farming. They are an important first step towards making well-founded, early assessments of chronic feeding stress of trout in the future, which are minimally invasive. So far, the parameters have often been based on observations such as behavior, color changes and such aspects that are difficult to standardize. These results provide a basis for the development of practical detection systems - comparable to a diabetes test.

Keywords: Stress, inflammation, soy bean meal, feeding stress, molecular marker

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O-097.

Modulation of the immune condition in European seabass (*Dicentrarchus labrax*) juveniles through long-term methionine supplementation

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Abstract

Methionine is the first limiting AA in fish diets, particularly in those containing high levels of plant protein sources (e.g. soybean). It is also important to highlight that methionine presents a key role in the immune system of many vertebrate animal models, including fish. In this context, the present study aimed to assess, by means of a dose-response trial, the effects of dietary methionine deficiency or supplementation on the European seabass (*Dicentrarchus labrax*) immune status within the context of an alternative feed formulation (i.e. 0 % fish meal; FM). After acclimatization, European seabass juveniles with a mean body weight of 10.34 ± 0.19 g were randomly distributed in 1000L tanks. In a complete randomized design, five treatments were evaluated in triplicate groups: fish meal free diet with 0.65 % methionine in feed (MET0.65, below requirement), 0.85 % methionine in feed (MET0.85, at req.), 1.25 % methionine in feed (MET1.25, above req.) and 1.5 % methionine in feed (MET1.5, above req.) and a high fish meal diet with 1.18 % methionine in feed (FM, above req.). After two weeks of feeding fish fed a fish meal free diet with an increased methionine dietary content showed an enhanced expression of genes with direct relationship with methionine aminopropylation pathway and cell proliferation. The immune-enhancer role of methionine was more evident after 12 weeks of feeding with an increased percentage of the peripheral neutrophils and a decrease of apoptotic signals at the transcriptional level. This may indicate an enhancement of fish immune status fed a methionine-supplemented (MET1.25 and MET 1.5) diet compared to the MET0.65 and MET0.85 diets.

Even though MET0.85 and FM dietary treatments presented similar methionine contents, the dietary protein source seemed to also modulate the fish immune status. For instance, European seabass fed the MET0.85 diet presented a reduced expression of several immune-related genes compared to fish fed FM diet. The results suggested the possibility that in a practical plant protein based diet scenario the requirement level of methionine could increase, since fish fed both MET1.25 and MET1.5 dietary treatments as well as the FM diet presented a similar growth performance at the end, and higher than those fish fed MET0.65 or MET0.65 and CTRL, respectively.

Keywords: Amino acids, immunostimulation, fish, plant protein, fish-meal free

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O-098.

Administration of single versus combined herbal extracts enhances some immune parameters and protects striped catfish (*Pangasianodon hypophthalmus*) against *Edwardsiella ictaluri*

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Abstract

Psidium guajava and *Phyllanthus amarus* are plants well-known in Vietnamese traditional medicine. However, the capacity of these plants in improving the immune system of striped catfish (*Pangasianodon hypophthalmus*) has received less attention. This study aimed to investigate the effects of single versus combined (1: 1, v/v) ethanolic extracts of *P. guajava* and *P. amarus* on immune response and disease resistance of striped catfish against *Edwardsiella ictaluri*. Fish were fed diets with different concentrations of plant extracts including basal diet 0% [B0]; *P. guajava* 0.08% [Pg0.08], 0.2% [Pg0.2], 0.5% [Pg0.5]; *P. amarus* 0.08% [Pa0.08], 0.2% [Pa0.2], 0.5% [Pa0.5] and their mixture (v:v) at similar doses [Co0.08, Co0.2 and Co0.5] for 6 weeks. The growth was examined at week 6 (W6); the cellular immune response (reactive oxygen species-ROS and nitric oxide synthase-NOS) and humoral immune responses (lysozyme and complement activities, total immunoglobulin) were examined at W3, W6 post-feeding and after challenge test; challenge test was performed by injection with *E. ictaluri* at W6, and mortalities were recorded over 15 days post-infection. The extracts supplemented diets did not induce any significant growth difference compared to control. Levels of spleen ROS increased statistically in Pa0.2, Pa0.5 and Co0.5 groups at W6 compared to control. After challenge test, the spleen ROS activity was significantly higher in Pa and Co groups than in the control group ($p < 0.05$). However, only Pg0.2 group improved remarkably NOS activity in spleen at W3 and W6. Serum lysozyme activity started to increase significantly after 3 weeks of feeding. Moreover, fish fed Pg diets exhibited a remarkable increase in serum lysozyme levels at W6. Similarly, Pg0.2, Pa0.2 and Co0.2 groups markedly enhanced skin mucosal lysozyme level ($p < 0.05$). In addition, Pg0.2 group possessed the highest level of serum complement activity compared to control at W3, while Pa0.5 group showed a maximum ACH50 level at W6. Pa0.2, Pg0.5 and Pa0.5 also strongly enhanced the serum total immunoglobulin (Ig) level at W6 compared to control. After six weeks of feeding, the skin mucosal total Ig level increased remarkably in Pa0.08 and Pa0.2 groups compared to control. The dietary supplementation of single versus combined *P. guajava* and *P. amarus* extracts could significantly reduce the mortality and increase the disease resistance of striped catfish following challenge with *E. ictaluri* compared to control. These results suggest that *P. guajava* and *P. amarus* extracts have the potential to modulate the immune mechanisms and disease resistance of striped catfish, especially at the medium and high concentrations tested.

Keywords: Striped catfish, *Pangasianodon hypophthalmus*, immune response, disease resistance, plant extract, *Psidium guajava* and *Phyllanthus amarus*

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O-099.

Living prey and pufa-enriched diets to improve immune defenses of Atlantic salmon (*Salmo salar*) fry destined to restocking

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Abstract

Over the last 40 years, the population of Atlantic salmon (*Salmo salar*) in Europe has decreased by more than 80%, and completely disappeared in Belgium, necessitating restocking programs. To restore salmon populations, artificial reproduction from wild salmon breeders is widely used to produce fry or parr that will be released in river. However, the use of large-scale rearing methods derived from intensive fish farming production could reduce the fitness and health of salmon fry, with possible consequences on its adaptability to natural environment after restocking