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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 in rivers. Particularly, the change of dietary lipid composition from around 20% to 10% drastically impacts fry physiology. Indeed, lipids play a key role in fry development and some polyunsaturated fatty acids (PUFA) such as eicosapentanoid acid (EPA) are involved in immune defenses. In addition, the use of dry "germ-limited" food in a controlled environment probably reduces the exposure of fry to environmental bacteria known to shape the immune system and that could later lead to weak immune responses to fight pathogens present in the environment.

In this study, we aimed to optimize the diets of fry to improve their immune defenses and thus their ability to survive in the wild. For this, fry were fed six experimental or commercial diets: experimental diets with 12.7% lipids enriched either with EPA (1) or ALA (2), experimental diets with 20% lipids enriched either with EPA (3) or ALA (4), commercial diet (5), commercial diet (70% of energy supply) supplemented by living chironomids (30% of energy supply) containing complex microbiota (6). After 6 weeks of nutritional conditioning, fry were challenged with the pathogen *Aeromonas salmonicida salmonicida* by bathing. The body part of fry was sampled before and 24h after infection and was used for immune gene expression analyses involved in innate immune responses (*mpo, mcsfra, lysozyme*). Before bacterial infection, the expression of *mpo* and *igm* genes was lower in fry fed chironomids (F) diets compared to all other diets. This suggested that the supplementation of chironomids in diet could differentially modulate the immune system compared to dry diet. The results of immune gene expression after bacterial challenge, still under analysis, should provide information on how this living preys influence immune defense when fry are exposed to a pathogen. In addition, despite no differences were observed before infection between fry fed EPA- or ALA-enriched diets at different lipid percentage, we could expect some differences in immune responses after bacterial challenge as EPA is a direct precursor of leukotriene and prostaglandins production.

Keywords: Living prey diet; PUFA; bacterial challenge; restocking program; *Salmo salar*

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O-100.

Meta analyses of transcriptome responses to infections and stress in Atlantic salmon

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Abstract

Transcriptomics provides comprehensive information on host responses to pathogens, PAMP, antigens and treatments affecting the immune system. The main goal and challenge in data analyses is finding of consistent trends. Identification of transcription signatures (TS) – gene sets with reproducible expression profiles – is of high value. TS are instrumental for functional annotations of genes, interpretation and classification of transcriptome profiles. We have accumulated a large volume of data for Atlantic salmon produced with DNA microarrays, studies included multiple challenges with viruses, bacteria and parasites and various treatments stimulating immune and stress responses, totally 125 experiments and 4464 microarrays. Meta analyses started with selection of representative controlled experiments with large scale transcriptome responses, finding and ranking of genes with expression changes in at least two related experimental series followed with analyses of expression profiles in the entire database. Three large functional groups with several TS in each are presented, gene numbers and five genes with top ranks are indicated. **Virus responsive genes** – **VRG** (123 genes, *receptor transporting protein 3, viperin, isg15, ifit5, sacsin*) were identified in multiple trials with four viruses (IPNV, PRV, SAV and salmon poxvirus). These genes are equally activated with viruses and PAMPs (poly-IC, CpG, gardiquimod and bacterial DNA) *in vitro* and *in vivo*. In addition to specialized immune genes, VRG include many members with versatile or unknown functions and genes that most likely have changed their roles in higher vertebrates. While viral infection and exposure to PAMP induce the entire group, VRG fall into subgroups under different conditions suggesting a complex regulatory network. Systemic suppression of VRG was observed in fish infected with *Moritella viscosa* and sea lice. **Markers of inflammation** (105 genes, *C-C motif chemokine 4, serum amyloid, mmp 9, neutrophil cytosolic factor 1 and cathelicidin*) were selected by responses to PAMP and wounds. Multiple functional groups and pathways represent different aspects of immune responses, while several transcription signatures correspond to different scenarios of inflammation. **Stress markers** (31 genes, *mmp 9*, *immediate early response 2, junb, c/ebp-b* and *natterin*) were identified in studies of wound healing and exhausting physical load. These genes respond to various stressors in different tissues of Atlantic salmon. Stress component is manifested in various diseases with different magnitude.

Keywords: Atlantic salmon, transcriptome, antiviral response, inflammation, stress

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O-101.

Genomic and biological characterization of inhibitors and activators of the NF-kB pathway in rainbow trout (*Oncorhynchus mykiss*)

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Abstract

Bacterial infection on aquaculture facilities are a major problem for productivity and quality of fish production. One of the most common and important pathogens is Aeromonas salmonicida. This Gram-negative bacterium is the causative agent of the disease furunculosis, which had a devastating impact on salmonid aquaculture up until the late 1980's. An efficient vaccination against this pathogen would represent a decisive improvement of healthcare concepts in aquaculture systems. The NF-kB pathway is considered an important target of vaccinations as it is the responsible for the expression of cytokines and other pro-inflammatory molecules. We profiled the expression of several genes involved in the NF-kB pathway and NF-kB-dependent effector genes in rainbow trout after immunization. using multiplex RT-qPCR. NKIRAS1/NKIRAS2, RelA/NFKB1 and PIAS1 were selected as interesting indicator genes. Using CHSE-214 cell line derived from a Chinook salmon (*Oncorhynchus tshawytscha*) embryo, as in vitro model, we transfected these three factors in order to analyze the subcellular localization

of these proteins. Additional luciferase reporter assays showed that NKIRAS1 inhibits the activation of the NFkappa-β pathway following stimulation with different PAMPs. Surprisingly, NKIRAS2 showed the opposite effect as reported in literature for mammals. On trout cells it rather acts as pro-inflammatory molecule which drives the overexpression of cytokines. Furthermore, a panel of genes, related to the immune response, was selected to determine the modulation of gene expression during the immunization and transfections and analyzed with

Fluidigm technology. Results will contribute to better understanding of the role of inhibitors during inflammatory stimuli.

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