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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