



**The activation of farnesoid X receptor inhibits inflammation via antagonizing NF- $\kappa$ B in large yellow croaker (*Larimichthys crocea*)**

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Farnesoid X receptor (FXR) is a nuclear receptor and highly expressed in liver, kidney and intestine of large yellow croaker (*Larimichthys crocea*). In this study, the effect of FXR activation on the inflammation of large yellow croaker was investigated in vivo and in vitro. Four groups of fish were fed fish oil diet, soybean oil diet and soybean oil diet with the supplementation of 300mg/kg or 900mg/kg chenodeoxycholic acid (CDCA, a natural FXR ligand), respectively. The results showed that the high-level replacement of fish oil by soybean oil significantly active the NF- $\kappa$ B pathway and increased the expression of TNF $\alpha$ , COX-2, IL-6 and IL-1 $\beta$  which were reversed by the supplementation of CDCA ( $P < 0.05$ ). In vitro study showed that CDCA and GW4064 (a synthetic FXR ligand) treatment significantly decreased the linoleic acid (LA) and Lipopolysaccharides (LPS)-induced expression of proinflammatory genes in PCK cells of large yellow croaker ( $P < 0.05$ ). Meanwhile, dual luciferase reporter assay system analysis showed that co-transfection of FXR inhibited the P65-induced NF- $\kappa$ B reporter activity. The results demonstrated that the activation of FXR may inhibit the expression of proinflammatory genes of large yellow croaker via suppressing the NF- $\kappa$ B pathway.



## **The effect of Mycotoxin contaminated feed on performance and health status of *Litopenaeus vannamei***

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This abstract summarizes different studies to evaluate the effect of mycotoxins on juveniles of *Litopenaeus vannamei*.

Trials were conducted during 42 days, contaminating shrimps with either 650 ppb of aflatoxin (AFLA), 1.5 ppm fumonisin (FUM), or 1 ppm T2 toxin with or without a mycotoxin eliminator (Elitox®) at 2.5 kg/T, to be compared with an uncontaminated control group. Each treatment consisted of 4 replicates of 40l aquariums containing 10 shrimps each and equipped with individual biological filters.

At the end of the experiment all animals were weighed and measured to determine survival (%), final total length (cm), final weight (g), biomass gain (g) and specific growth rate (%). Feed conversion ratio was calculated based on feed intake per aquarium. Hepatopancreas, antennal gland tissue and lymphoid organs of 16 animals per treatment were collected to perform histopathological evaluation. Haemolymph was sampled to measure activity of detoxifying enzymes such as phenoloxidase and alkaline phosphatase. Immune response was quantified based on total haemocyte counts, hyaline cell counts and granular cells.

All mycotoxins resulted in reduced length and weight after 42d and decreased survival. T2 contamination at 1 ppm even led to an increase in mortality up to 43%. Feed intake and growth was suppressed in the contaminated group, leading to a significant increased FCR. After contamination the amounts of phenoloxidase and alkaline phosphatase was increased. These detoxifying enzymes give a good indication of the toxic status of the shrimps and hence are good biomarkers for mycotoxicosis. Immune status was also affected as concluded from the decrease in total haemocyte counts and hyaline cells. Damage by all tested mycotoxins to hepatopancreas, antennal gland tissue and lymphoid organs was clearly demonstrated by the histopathological evaluation.

In conclusion, little or no research is done on aquatic species to evaluate the harmful effect of mycotoxins in diets. This research clearly indicates that mycotoxins are as harmful for shrimps and lead to huge economic losses.



**Non-integrated nutrition promotes hepatic inflammation and apoptosis involving MAPK signaling pathway in largemouth bass (*Micropterus salmoides*) , and the clinical function of Bile acids**

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Anorexia and nutritional hepatitis (NH) is a common phenotype in largemouth bass fed artificial diet (AD). Firstly, we investigated the inducers and hepatitis phenotype related to bile acids synthesis, hepatic inflammation, apoptosis, and the regulation of MAPK signaling pathway in largemouth bass (*Micropterus salmoides*) fed with chilled fish (CF, 1.51% carbohydrate) or AD (17.5% carbohydrate). Serum and liver tissues were taken when fish fed AD experienced severe anorexia. Hepatitis phenotypes were assessed by hematological, histological and immunofluorescence assays, and related genes mRNA levels were determined by qPCR. The role of MAPK signaling pathway in NH was evaluated using western blotting. Fish in AD group had higher VSI, HSI and hepatic lipid content. Although CF was much more oxidized than AD, and serum MDA and 4-HNE in CF group were much higher than those fed AD, fish in this group showed higher growth performance, normal liver histology and low inflammation and apoptosis responses. Whereas fish fed AD suffered liver tissue damage including phenotypes of vacuoles, accumulation of glycogen and fibrosis, suggesting that oxidative stress was not the main occasion for liver disease developed in this study. Biomarkers for cholestasis, AKP and TBA, increased significantly in AD group, and the two key enzymes in bile acid synthesis CYP7A1 and CYP8B1 were down-regulated sharply, indicating that cholestasis was induced by fed AD. Striking inflammatory responses and apoptosis were observed in AD group. ERK1/2 and JNK were significantly activated, but p38MAPK phosphorylation was inhibited in liver of fish fed AD. Thereafter, we designed a clinical experiment countering to the cholestasis of large-mouth bass by fed various level of bile acid (BA). 300mg/kg BA inclusion will increase the growth performance but showed phenotypic adaptation status with severe pro- and anti-inflammatory response. 1500mg/kg dietary BA significantly improved hepatic function and alleviated cholestasis by effectively activated p38 MAPK phosphorylation at Thr180. However the ERK1/2 and JNK activation were not affected. The results identified that p38MAPK is the main regulator in MAPK family for NH development of large-mouth bass.



## **Plant sterols and cholesterol in the diet of Atlantic salmon (*Salmo salar* L.)**

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The increasing use of plant-based ingredients in aquafeeds for Atlantic salmon has led to increasing feed concentrations of plant sterols (phytosterols) combined with decreasing concentrations of cholesterol. Questions have been raised regarding how this may affect performance, health and lipid metabolism in a carnivorous fish species adapted to a high cholesterol diet in nature, and whether phytosterols could be involved in the liver lipid accumulation commonly observed in salmon fed diets with a high content of vegetable oils. The current trial is the first to feed salmon graded levels of phytosterols, cholesterol and their ratio without changing the dietary fatty acid composition, and thereby isolating the effects of dietary sterols.

A feeding trial with 9 diet groups with varying additions of a phytosterol mix and cholesterol was conducted at two temperatures (6 and 12°C), as well as three cell culture studies on hepatocytes and adipocytes to investigate mechanisms and individual phytosterols. The main hypothesis regarding phytosterols or phytosterol : cholesterol ratio affecting liver lipid accumulation appeared to be false, supported both by the feeding trial and the cell culture studies. However, dietary cholesterol did increase muscle pigmentation at both temperatures, while fillet dry matter also increased at 12°C. Dietary cholesterol also had some minor effects on the fatty acid composition of liver polar lipids, probably mediated by LXR. In general, more effects were seen from variations in dietary cholesterol compared to phytosterols. Cholesterol retention values were clearly dependent on dietary cholesterol, and showed that fish fed cholesterol levels <1000 mg kg<sup>-1</sup> feed produced considerable quantities of cholesterol de novo. Despite this production, cholesterol content increased with increasing dietary cholesterol in a range of fish tissues. Campesterol and brassicasterol appeared to be the phytosterols with the highest intestinal absorption in Atlantic salmon. There was a high biliary excretion of campesterol, but not of brassicasterol, which accumulated in tissues and particularly in adipose tissue. Other phytosterols had very low retention, and the data showed clear differences in the absorption, biliary excretion, retention, tissue storage and distribution of cholesterol and individual phytosterols in Atlantic salmon.



**Dietary tryptophan deficiency and supplementation compromises European seabass immune status, inflammatory mechanisms and disease resistance**

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Tryptophan participates in an array of physiological mechanisms of the neuroendocrine-immune network. For instance, tryptophan catabolism plays a critical role in the functions of both macrophages and lymphocytes. However, a potential benefit of tryptophan supplementation for animal health management is not fully demonstrated, and its immunomodulatory potential in fish must be further investigated. Therefore, the present study aimed to evaluate the effects of dietary tryptophan deficiency and supplementation on the European seabass immune status, inflammatory response and disease resistance.

European seabass juveniles ( $8.5 \pm 0.7$  g) were fed four experimental diets in triplicate tanks: control diet (CTRL) formulated to meet the European seabass amino acid requirements; the CTRL diet supplemented with 15 and 30 % above L-Tryptophan requirement (TRP15 and TRP30, respectively) and a negative control diet (NTRP), deficient in tryptophan. Twelve fish per tank were sampled after 2 and 4 weeks of feeding in order to assess their immune status. The remaining fish were intraperitoneally (i.p.) injected with *Photobacterium damsela* piscicida (Phdp;  $5 \times 10^3$  cfu / fish) and four fish per tank were transferred to a similar system following i.p. injection and sampled after 4, 24, 48 and 72 h. The mortality of the remaining twenty fish was recorded during 21 days. Fish haematological profile, peripheral and peritoneal cell counts as well as plasma cortisol and humoral immune parameters were assessed.

No dietary effect was observed regarding immune parameters during the feeding trial. In contrast, fish fed TRP30 showed higher plasma cortisol concentration than fish fed CTRL at 4 weeks, possibly due to the stimulation of a neuroendocrine response.

In response to the Phdp insult, fish fed NTRP presented the highest cumulative mortality and led to lower levels of peritoneal leucocytes and monocytes while displaying increased levels of plasma cortisol compared to CTRL. Tryptophan supplementation led to similar results, with a reduction of peripheral lymphocytes and monocytes and peritoneal macrophages. Accordingly, fish fed TRP30 presented higher mortality than fish fed TRP15 and CTRL, both with a similar disease resistance. These results suggest that both dietary tryptophan deficiency and supplementation may compromise the inflammatory mechanisms and disease resistance.



**Evaluation of the optimum dietary  $\gamma$ -aminobutyric acid (GABA) level in juvenile Nile tilapia, *Oreochromis niloticus***

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The present experiment was conducted to evaluate the effects of optimum dietary GABA level in Nile tilapia. Twenty four groups of fish were randomly distributed in rectangular 40 L volume tanks. Triplicates of 20 fish averaging  $3.03 \pm 0.07$ g (mean $\pm$ SD) were fed one of the eight experimental diets; a basal diet without GABA was used as the control (CON), and the other seven diets were prepared by adding 75 mg GABA (GABA75), 75 mg GABA + 50 ppm killed vaccine (GABA75V), 150 mg GABA (GABA150), 150 mg GABA + 50 ppm killed vaccine (GABA150V), 300 mg GABA (GABA300), 600 mg GABA (GABA600), and 900 mg GABA (GABA900) per kg of diet. Parameters including growth performance, non-specific immune responses, and disease resistance would be measured after 8 weeks of the feeding trial, and the results will be reported.