



Dietary use of mannan oligosaccharides in greater amberjack juveniles: effects on growth performance, immune gene expression and disease resistance against NEOBENEDENIA GIRELLAE

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The greater amberjack *Seriola dumerili* is a marine pelagic species of high interest for aquaculture due to its rapid growth and high commercial value. Nowadays, the production of this fish species needs to solve a major health bottleneck caused by infection with monogenean ectoparasites (Hirayama et al., 2009). For that reason, new strategies for increasing greater amberjack immune resistance must be performed. The effects of dietary mannan oligosaccharides (MOS) inclusion in fish production have been well studied in other fish species (Torrecillas et al., 2014; Guerreiro et al., 2017). MOS dietary supplementation improves the Mucosal Associated Lymphoid Tissue (MALT) function, and shows promising results against endoparasites and ectoparasites infections (Buentello et al., 2010). For these reasons, the aim of the present work is to determine the effect of two commercial MOS on the greater amberjack immune system and the impact on resistance to an experimental infection against the ectoparasite *Neobenedenia girellae*.

Two hundred and sixteen fish of 331 g were distributed in twelve 1000 L tanks (18 fish/tank) and fed to apparent satiety 3 times per day for 90 days. The experimental diets used were: greater amberjack base control diet (C), supplemented with MOS, supplemented with cMOS and a combination of both prebiotics (MOS+cMOS). Feed intake was monitored daily while growth performance and feed efficacy were recorded monthly. At the end of the experimental period, samples of posterior gut, gills, skin, head kidney and spleen were placed in RNA later for gene expression analyses by qPCR and a parasite challenge was conducted by cohabitation with *N. girellae* infected fish for 10 days.

No significant differences were observed for growth performance, feed efficiency or feed intake ($P>0.05$). Dietary cMOS reduced ($P<0.05$) parasite incidence compared to fish fed control diet (C). Dietary MOS and cMOS up-regulated of several genes related with the MALT response, such as $TNF\alpha$, $IFN\gamma$, $IL1\beta$, $MUC2$, IgM and IgT . Results of the gene expression analysis are discussed in relation to the functional diet supplemented and different tissues, showing cMOS an effect more focused in mucosal tissues and MOS in systemic lymphoid tissues.



Effects of α -lipoic acid on growth performance, body composition, antioxidant status and lipid catabolism of juvenile Chinese mitten crab *Eriocheir sinensis* fed two lipid levels

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The effects of α -lipoic acid supplementation in the diet of the Chinese mitten crab *Eriocheir sinensis* on growth performance, body composition, antioxidative status and hepatopancreas lipid catabolism at two levels (7% and 13%) of dietary lipid were evaluated. A total of six isonitrogenous diets were formulated with 0, 700 and 1400 mg/kg α -lipoic acid (α -LA) supplementation to each lipid diet, and fed to *E. sinensis* juveniles for eight weeks. Weight gain and specific growth rate of crabs fed the diet with α -LA supplementation were significantly higher than the control group regardless of lipid levels. α -LA significantly increased lipid accumulation in the whole body and hepatopancreas in a dose-dependent manner. Crabs fed 13% lipid showed a significantly higher hepatosomatic index than those fed 7% lipid. Triacylglycerol lipase and intracellular lipase mRNA expressions increased with the increase of α -LA supplementation in crabs fed 7% lipid. No significant difference was found in CPT-1 mRNA expressions among all treatments. α -LA supplementation at 1400 mg/kg significantly improved oxidative stress due to lipid accumulation in the hepatopancreas of crabs fed the 7% lipid diet as indicated by high superoxide dismutase and glutathione peroxidase activities, but low malondialdehyde. The diet with 13% lipid increased the lipid content in the hepatopancreas and suppressed antioxidative enzyme activities including low glutathione peroxidase and high malondialdehyde. Meanwhile, total antioxidant capacity increased with the increase of α -LA supplementation in crabs fed 13% lipid. This study indicates that α -LA supplementation can improve growth performance and accelerate lipid accumulation in the hepatopancreas by increasing lipid utilization efficiency. Furthermore, α -LA can relieve hepatopancreas oxidative damage induced by lipid accumulation and improve the health status of *E. sinensis*.



THE EFFECT OF DIETARY LIPOIC ACID SUPPLEMENTATION ON GROWTH, SURVIVAL AND FEEDING EFFICIENCY OF *Chirostoma estor* LARVAE

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Alpha Lipoic Acid (ALA) has been used as an animal feed additive because of its positive effect on growth and the regulation of oxidative stress. An experiment was carried out in order to evaluate the effect of dietary ALA on growth, survival, and feed efficiency of *Chirostoma estor* larvae with the use of micro-aggregate diets. The performance of newly-hatched larvae was evaluated with five different microdiets supplemented with increasing levels of ALA (0, 20, 40, 80 y 160 mg ALA.kg diet⁻¹). Newly-hatched larvae were fed live rotifers until 7 day post hatch (dph) when a cofeeding with the different experimental diets occurred. From day 10 dph until the end of the trial at day 27 dph larvae were exclusively offered microdiets. According to the results, survival was higher in larvae fed with microdiets supplemented with ALA compared with those without the additive ($p \leq 0.05$). Growth, in terms of weight gain and specific growth rate, was not significantly different ($p > 0.05$) for larvae fed the experimental diets. However, larvae with ALA supplementation showed a lower degree of size dispersion, which is an important asset for larviculture in general as can help avoid cannibalism. Larvae fed with 20 mg ALA.kg diet⁻¹ presented the lower feed consumption and feed conversion rate (FCR) and the higher protein efficiency ratio (PER) ($p \leq 0.05$). It can be concluded that ALA supplementation significantly promotes survival but does not affect growth of *C. estor* larvae. Microdiets with 20 mg ALA.kg diet⁻¹ can be used to improve survival and feed utilization, in terms of FCR and PER, for *C. estor* larvae until 27 dph.



Benefits of spray-dried plasma (SDP) dietary inclusion on skin and epidermal mucus of a fish model marine species: histological, transcriptomic and proteomic approaches.

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Spray-dried blood and spray-dried plasma (SDP) proteins, as preparations from a terrestrial animal by-product, are an untapped safe source of animal protein available for the aquafeed industry, supplying high-quality feed ingredients for farmed animals. In gilthead sea bream, recent studies have indicated that the inclusion of SDP enhanced the intestinal and serum innate immune function, the activity of the intestinal antioxidative stress enzymes and promoted somatic growth. The aim of this study was to evaluate the potential dietary stimulation at the skin and epidermal mucus level.

The trial was conducted at indoor facilities of the IRTA-San Carles de la Ràpita, Spain. Two groups, by quadruplicate, of sea bream juveniles (10 g, BWi) were fed with the same diet supplied with either 0% (control) or 3% of SDPP (AP820P; APC Europe SA) by quadruplicate. At the end of the growth period the following analyses were performed: 1) skin histology 2) skin transcriptome expression via microarray and 3) proteome maps of soluble epidermal mucus proteins obtained by 2D-electrophoresis. SDPP-diet significantly improved growth performance by a 10%. Skin in both experimental groups showed a normal histological organization, however, the thickness of the epidermis and the stratum spongiosum of the dermis was higher in the sea bream fed the SDP diet in comparison to the control group ($P < 0.05$). In addition, the density of epidermal mucous cells was higher in the SDP group than in fish fed the control diet ($P < 0.05$). Sustained supplementation with dietary SDPP resulted in the modulation of the transcriptomic response of genes mainly related to cell metabolism, transcription, protein processing, and proliferation, thus evidencing a higher epidermal activity. On the other hand, several defensive related-proteins and putative antimicrobial peptides were significantly augmented on SDP-fed fish epidermal mucus. Importantly, an association between transcriptomic and proteome response was established by an interactome thus showing the induced biological processes after the treatment.

Altogether, these results indicate that the SDPP-supplemented diet may improve gilthead sea bream health status by enhancing the skin activity and the mucus protective function, which might be linked to the high functional components of this feed ingredient.