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Does dietary insect meal affect the fish immune system? The case of mealworm, *Tenebrio molitor* on European sea bass, *Dicentrarchus labrax*

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ABSTRACT

Feeding small European sea bass, *Dicentrarchus labrax*, for 6 weeks with *Tenebrio molitor* larval meal showed significant anti-inflammatory responses (ceruloplasmin, myeloperoxidase and nitric oxide). Serum bacteriolytic activity against a Gram negative bacterium was not significantly affected by dietary *Tenebrio*, while both lysozyme antibacterial activity and serum trypsin inhibition usually linked to the anti-parasite activity of the fish, were significantly enhanced. The latter may be due to the similarities in the composition of the exoskeleton of parasites and insects that may therefore act as an immunostimulant potentially increasing the anti-parasitic activity. The addition of exogenous proteases significantly decreased both trypsin-inhibition and serum bacteriolytic activity probably through direct inhibition of the proteins responsible for these immune functions. Further investigation involving bacterial or parasitic challenges will be necessary to assess if the effects of dietary mealworm meal on the immune system observed in the present study are translated into an improved resistance to diseases.

1. Introduction

Yellow mealworm, *Tenebrio molitor* (TM), is a beetle that is considered a pest of grain and flour. Their larvae are easily raised on low-nutritive plant waste products. They are commercially produced to be used as pet food (birds and reptiles) or fishing baits. The recent EU commission regulation (2017/893-24/05/2017) to authorize the use of 7 insects (2 flies, 2 mealworms, 7 cricket species) in aquafeeds will further motivate the intensification of their production. They are rich in proteins (47–60%; up to 70% in defatted meal) and lipids (31–43%) and their amino acis and fatty acids profiles are suitable for inclusion in animal feeds (Makkar et al., 2014). Their use as a partial replacer of fishmeal (FM, 10–25% dietary inclusion) has been studied in poultry (Giannone, 2003; Klasing et al., 2000; Ramos-Elorduy et al., 2002; Wang et al., 1996) and more recently in fish: rainbow trout (*Oncorhynchus mykiss*) (Belforti et al., 2015; Gasco et al., 2014), African catfish (*Clarias gariepinus*) (Ng et al., 2001), common catfish (*Ameiurus melas*) (Roncarati et al., 2014), tilapia (*Oreochromis nilotica*) (de Haro et al., 2011), Gilthead seabream (*Sparus aurata*) (Piccolo et al., 2014) and European sea bass (*Dicentrarchus labrax*) (Gasco et al., 2016). At high dietary levels (25–50%), TM reduced fish growth and n-3 HUFA in fish fillets but at low dietary inclusion level (12.5–38%), fish growth was not affected for most of the fish species tested (Henry et al., 2015a) and 9% of dietary inclusion even improved the growth of African catfish (Ng et al., 2001). TM is therefore considered to be a good alternative for partial replacement of fishmeal in the diet of many fish species.

Some components of insects such as silkose or dipterose have been shown to have immunostimulating activity in mammals (Ohta et al., 2014, 2016). In fish, low dietary levels of crustacean chitin have been shown to immunostimulate and to increase the disease resistance of Gilthead seabream and common carp, *Cyprinus carpio* (Esteban et al., 2001; Gopalakannan and Arul, 2006).

Abbreviations: APA, anti-protease activity corresponds to the trypsin inhibition activity of the fish serum; Cerul, ceruloplasmin activity; HIS, Hepato-Somatic Index; NO, nitric oxide; MPO, myeloperoxidase; VSI, Viscera-Somatic Index.

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Insects, including *Tenebrio molitor*, also contain varying amounts of chitin (Finke, 2007, 2013). Although insect chitin composition is slightly different from that of crustacean chitin (Henry et al., 2015a), it is reasonable to hypothesize that low levels of insect chitin may also have an immunostimulating activity in fish. However, very few studies have studied the effects of dietary insects on the immune system of fish. Two studies investigated the effect of low dietary levels of housefly pupae on the immune system and disease resistance of the red sea bream (*Pagrus major*) (Ido et al., 2015) and the black carp (*Mylopharyngodon piceus*) (Ming et al., 2013). The effects of higher dietary levels of insects used to replace large parts of the dietary fishmeal were only investigated in the case of silkworm pupae in Jian carp (*Cyprinus carpio* var. Jian) (Ji et al., 2013).

Therefore, the present study aimed to assess the effect of the dietary inclusion of 25% of *Tenebrio molitor* larvae meal (corresponding to 36% of FM replacement) in absence or presence of exogenous enzymes on the immune system of European sea bass. This investigation was performed at the end of the digestibility trial described in a previous publication (Gasco et al., 2016).

2. Materials and methods

2.1. Fish diet

A full fat *Tenebrio molitor* meal (TM) was used to prepare four isonitrogenous (53% CP) and isoenergetic (21.5 MJ.kg^{-1}DM) diets based on the control diet containing 70% fishmeal (CD). The three other diets contained 24.75% of *Tenebrio molitor* meal replacing 36% of FM without exogenous digestive enzymes (TMD), with proteases (Ronozyme ProAct) (TM-Prot) or with carbohydrases (xylanase and β-glucanases; Ronozyme MultiGrain) (TM-Carb) both obtained from DSM Animal Nutrition & Health (Heerlen, The Netherlands). The experimental feeds were prepared at the IMBBC laboratory using a pellet mill. The produced 1 mm-pellets were dried at 40 °C and stored at −20 °C. The feed formulation and proximate composition of these diets are available in our previous publication reporting the digestibility results (Gasco et al., 2016).

2.2. Experimental trial and sampling

European sea bass of 65.3 ± 5.7 g initial weight were distributed in 12 circular fiberglass tanks (15 fish per tank) of 270 L supplied with an open-circulation of borehole aerated seawater under natural photoperiod. Water conditions were maintained constant at 19.5 ± 0.5 °C, 36‰ salinity and 6 ± 0.7 mg/L dissolved oxygen. Fish were fed the 4 experimental diets *ad libitum* twice a day for 6 weeks.

At the end of the trial, 6 fish per tank were anaesthetized and bled by the caudal vein using a syringe without anti-coagulant. Blood samples were left to clot overnight at 4 °C and centrifuged the next day to collect the sera. Serum samples were frozen at −80 °C until immunological analyses were performed.

2.3. Immunological analyses

The myeloperoxidase activity, the nitric oxide concentration and the bacteriolytic (anti-Gram negative bacterium; *E.coli*) activities of the serum were measured as described before (Henry et al., 2015b). The ceruloplasmin and anti-protease (APA) activities (Henry and Fountoulaki, 2014) and the lysozyme antibacterial activity of the serum (Cotou et al., 2013) were assessed following the methods described before.

2.4. Statistical analysis

SPSS 13.0 software was used for all statistical analyses. Normality of data and homogeneity of variances were checked using Kolmogorov-Smirnov and Levene tests respectively. One-Way ANOVA or Kruskal-Wallis test were performed when appropriate. Pearson's parametric and Kendall's non-parametric bivariate correlations were assessed between all tested morphometric and immune parameters using corresponding values for each fish.

3. Results

As shown in our previous publication, the final fish weight was not affected significantly by the dietary treatment, while the digestibility of proteins was improved in TMD compared to the 3 other diets (Gasco et al., 2016). Hepato-Somatic Index was also improved by dietary TM supplemented or not by the exogenous enzymes (Gasco et al., 2016). Immunological parameters are presented in Figs. 1–4. The correlations between all morphometric and immunological parameters are presented in Table 1.

The serum ceruloplasmin activity (Fig. 1), the serum myeloperoxidase activity (Fig. 2A) and nitric oxide serum concentration (Fig. 2B) were significantly decreased in fish fed TM-based diets with or without added enzymes compared to fish fed the FM-based diet.

The antibacterial activity of serum against a Gram positive bacterium (*Micrococcus luteus*, lysozyme activity, Fig. 3A) did not show any significant difference between fish fed the different experimental diets, whereas the bacteriolytic activity against a Gram-negative bacterium (*E.coli*, bacteriolytic activity, Fig. 3B) was significantly lower in fish fed the insect diet supplemented with exogenous proteases compared to the 3 other diets (ANOVA, $P < 0.0001$). The anti-protease activity was increased in fish fed TMD and TM-carb compared to both control fish and fish fed TM-prot (Fig. 4). It was strongly positively correlated to the lysozyme and to the morphometric indexes (Table 1).

4. Discussion

The incorporation of insect larvae into the fish diet is a relatively recent research theme and to our knowledge, only few studies of their effect on the immune system and antioxidant enzymes of the fish have been performed so far: Black carp (*Mylopharyngodon piceus*) fed with low doses (2.5%) of maggot (*Musca domestica*) for

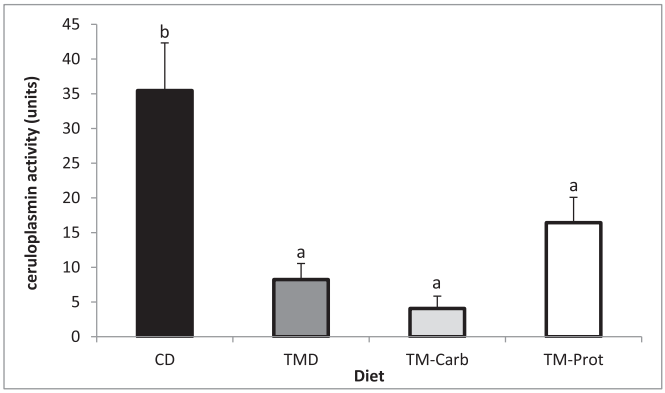

Fig. 1. Serum ceruloplasmin activity in European sea bass fed the 4 experimental diets. Different Latin letters show significant differences between dietary groups. (ANOVA, $P = 0.00003$). Bars represent mean ± S.E.M. n = 18. CD stands for control diet; TMD stands for *Tenebrio molitor* diet; TM-Carb and TM-Prot are the insect-containing diets enriched in carbohydrases and proteases respectively.