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THE EFFECT OF FISHMEAL REPLACEMENT BY *Zophobas morio* LARVAE MEAL ON PROXIMATE COMPOSITION OF GILTHEAD SEABREAM (*Sparus aurata*)

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Abstract
The present study was conducted in order to evaluate the effects of dietary fishmeal replacement by full- and low-fat *Zophobas morio* meal on whole body and muscle tissue proximate composition of *Sparus aurata* juveniles. Results indicate that dietary FM protein can be replaced up to 10% by full-fat or 30% by low-fat *Z. morio* meal without any major effect on body and muscle tissue composition of *S. aurata*.
Keywords: *Zophobas morio, insect, proximate, nutritional value, fishmeal*
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1.Introduction

Aquaculture is still on the search for suitable and sustainable alternative protein sources to replace fishmeal in aquafeeds. After their recent approval in the European aquafeed chain, insect proteins are considered as suitable alternatives to be used in aquafeeds since they are rich in essential amino acids (Henry *et al.* 2015). The use of superworm, *Zophobas morio*, as protein source in fish diets has not been adequately studied so far, despite its high nutritive value (Rumbos & Athanassiou 2021). In our growth trials with *Sparus aurata*, both full fat and defatted *Z. morio* larvae meal proved to be valuable in replacing dietary fishmeal protein without retarding growth (Asimaki *et al.* 2020; Asimaki *et al.* 2021). In order to better assess the efficiency of *Z. morio* protein in *S. aurata* nutrition we studied its effects on whole body and muscle tissue proximate composition of fish.

2. Materials and Methods

A total number of 540 *S. aurata* juveniles of 3.4g initial mean weight were allocated into 18 glass tanks (125L) within a closed recirculation seawater system and after an acclimatization period of 10 days divided into 6 dietary groups in triplicate tanks, each feeding on a different diet. Six isonitrogenous (52%) and isoenergetic (20 Mj/Kg) diets were formulated at which the fishmeal protein of the control diet (FM) was replaced by full-fat *Z. morio* larvae meal at 5% (ZFF5) and 10% (ZFF10) and by low-fat *Z. morio* meal at 10% (ZLF10), 20% (ZLF20) and 30% (ZLF30). After 100 days of feeding, four fish per tank were randomly selected for whole body proximate composition and four fish per tank for muscle tissue proximate composition according to AOAC methods. Specifically, moisture content was calculated by drying the samples in an oven at 105°C and ash content was determined with incineration at 600°C.

Crude protein content of the samples was determined by Kjeldahl analyses. To measure crude lipid content, lipids were extracted from the samples with petroleum ether in a Soxhlet extractor. The energy of the samples was measured with adiabatic bomb calorimetry. Comparison of means was performed by subjecting the data to one-way analysis of variance at a significance level of 0.05 using the IBM SPSS Statistics 24 statistical package. The significant differences between treatments were determined using Tukey's multiple comparison test.

3. Results and Discussion

Generally speaking, the replacement of dietary fishmeal by *Z. morio* did not alter extensively the body and tissue nutrient composition of *S. aurata*. The muscle tissue proximate composition of fish was unaffected ($P>0.05$) by the diet with the exception of the muscle energy content of fish fed the ZFF10 diet, which was significantly higher compared to that of the FM group, mainly due to its increased lipid content. This is an indication that the full-fat *Z. morio* meal may cause an increased lipid accumulation in the muscle tissue, although this did not occur in the whole

body of these fish. Regarding the whole body composition, some significant differences were detected among the dietary groups. The body moisture of fish fed the diet ZLF10 was significantly decreased due to its increased body lipid content in these fish. However, body lipid contents did not differentiate among the groups indicating a similar lipid metabolism. ZLF10 fish had also a lower body protein content, but there was not any significant trend on body proteins with higher fishmeal replacement.

The fishmeal replacement by low-fat *Z. morio* at 30% led to increased body ash in fish that may indicate a negative effect of high inclusion levels of *Z. morio* on the mineral metabolism and deposition in fish. Literature on the use of *Z. morio* is extremely limited. Jabir *et al.* (2012) reported an insignificant effect of *Z. morio* as 25% fishmeal replacer on body composition of *Oreochromis niloticus*, but Alves *et al.* (2021) observed an increased body lipid and moisture content and a decreased body protein and ash content when the same species was fed on diets with 30% inclusion level of full-fat *Z. morio*. A decreased body protein and ash content with incremental dietary *Z. morio* was also observed in *Oncorhynchus mykiss* (Shekarabi *et al.* 2021). The present results indicate that dietary FM protein can be replaced up to 10% by full-fat or 30% by low-fat *Z. morio* meal without any major effect on body and muscle tissue composition of *S. aurata*.

Table 1. Whole body and muscle proximate composition (% of dry matter) and energy content (MJ kg^{-1}) of *S. aurata* fed with the experimental diets.

	FM	ZFF5	ZFF10	ZLF10	ZLF20	ZLF30
Whole body composition						
Moisture	67.6±0.7^{ab}	69.1±0.5^{a}	68.0±0.7^{a}	65.7±0.6^{b}	67.9±0.7^{a}	67.4±1.1^{ab}
Crude protein	53.4±1.4^{ab}	56.2± 2.1^{a}	53.8±1.5^{ab}	51.4±1.3^{b}	54.0±2.5^{ab}	53.0±1.4^{ab}
Crude lipid	36.7±1.7	33.8±2.9	35.6±1.1	37.5±1.3	35.5±1.2	35.0±7.3
Ash	9.7±0.2^{a}	9.9±0.4^{ab}	9.9±0.3^{ab}	9.9±0.4^{ab}	9.7±0.2^{ab}	10.6±0.5^{b}
Gross energy	26.1±0.1	25.6±0.4	25.8±0.2	25.9±0.3	26.0±0.1	25.5±0.1
Muscle composition						
Moisture	75.4±0.3	75.0±0.9	74.8±0.9	74.9±0.5	74.0±0.5	74.9±0.3
Crude protein	83.3±1.4	82.3±1.9	81.4±0.6	82.2±0.9	81.3±2.0	83.3±1.2
Crude lipid	8.4±0.6	9.4±1.9	10.3±1.4	9.8±0.7	10.7±1.7	8.4±0.5
Ash	6.7±0.3	6.6±0.2	6.4±0.2	6.6±0.4	6.4±0.2	6.7±0.2
Gross energy	23.1±0.3^{a}	23.3±0.3^{ab}	23.8±0.2^{b}	23.6±0.1^{ab}	23.7±0.2^{ab}	23.2±0.1^{ab}

Values represent means ± st. deviation (n=3). Values within each row not sharing a common superscript letter are significantly different (P<0.05).

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