



Effect of *Tenebrio molitor* larvae meal on growth performance, in vivo nutrients digestibility, somatic and marketable indexes of gilthead sea bream (*Sparus aurata*)

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ABSTRACT

The aim of this study was to evaluate the effects of the inclusion of *Tenebrio molitor* larvae meal in practical diets for gilthead sea bream on growth performance, nutrients digestibility, somatic and marketable indexes. Two separate trials were carried out: in the first a total of 153 gilthead sea bream (105.2 ± 0.17 g average initial body weight) were randomly allocated in 9 fiberglass 220 l tanks (17 fish per tank) in an indoor water recirculating system. The fish were fed three isoenergetic and isoproteic diets formulated to contain increasing levels of TM meal inclusion and precisely: a control diet (TM0), in which fish meal was the main protein source; TM25 and TM50 diets, in which 25% and 50% of *Tenebrio molitor* larvae meal was added to the diet, respectively. These inclusion rates corresponded to 30% and 60% of inclusion on protein bases and 35% and 71% of fish meal substitution on protein bases for TM25 and TM50 diets, respectively. Each diet was randomly assigned to 3 tanks and the trial lasted 163 days. In the second trial, the apparent digestibility coefficients of the 3 diets were measured on 72 fish randomly distributed to 3 digestibility tank-units (24 fish per unit, average body weight: 86.97 ± 2.3 g) using an indirect method (acid insoluble ash). The group fed TM25 showed a higher ($P < 0.05$) final weight, specific growth rate, weight gain%, protein efficiency ratio, and a lower feed conversion ratio compared to the other 2 groups. The estimated apparent digestibility coefficients of crude protein and ether extract of the diets were lower ($P < 0.01$) in TM50 than in the other 2 groups. No significant differences have been found between TM0 and *Tenebrio molitor* larvae meal groups in morphometric and commodity-related characteristics, except for dressed yield and viscerosomatic index (VSI), that resulted the lowest and the highest, respectively, in TM50. The general evaluation of the results demonstrates that *Tenebrio molitor* larvae meal can replace fish meal up to 25% of inclusion in the diet for *Sparus aurata* without negative effects on weight gain, crude

Abbreviations: CTTAD, coefficient of total tract apparent digestibility; ADF, acid detergent fibre; AIA, acid-insoluble ashes; BW, body weight; CF, condition factor; CP, crude protein; DIR, daily intake rate; DM, dry matter; EE, ether extract; FCR, feed conversion ratio; FM, fish meal; FTL, fish total length; FY, fillet yield; HSI, hepatosomatic index; IBW, initial body weight; IL/FTL, intestinal length/fish total length; IL, intestinal length; PER, protein efficiency ratio; SGR, specific growth rate; TM, *Tenebrio molitor* larvae meal; TM0, control diet; TM25, 25% *Tenebrio molitor* larvae meal in the diet; TM50, 50% *Tenebrio molitor* larvae meal in the diet; VSI, viscerosomatic index; WG, weight gain

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protein and ether extract digestibility, marketable indexes after 163 days of feeding. On the contrary, when *Tenebrio molitor* larvae meal was included at 50%, nutrients digestibility and dressed yield were penalized.

1. Introduction

Fish meals (FM) have represented the largest protein source in farmed carnivorous teleost feeds. However, FM are a limited resource that cannot be produced in the future in sufficient amounts to sustain the growth trends of aquaculture production (FAO, 2014). Soya and other protein-rich plants have been used in farmed fish diets to replace FM (Espe et al., 2006; Gatlin et al., 2007). However, due to the presence of anti-nutritional factors (Ogunji, 2004; Collins, 2014), the potential digestive tract inflammation (Gai et al., 2012; Merrifield et al., 2011) or the feed palatability (Papatryphon and Soares, 2001) are of concern. Insect larvae meals can represent a valuable alternative (Makkar et al., 2014; Henry et al., 2015; Mancuso et al., 2016). Insects are part of fish natural diet (Howe et al., 2014; Whitley and Bollens, 2014), they have a high protein and lipid content (van Huis, 2013; Barroso et al., 2014; Sánchez-Muros et al., 2014) and their production is more sustainable, leading to lower greenhouse gas emissions and requiring less water and land compared to other animal and plant protein sources (Oonincx and de Boer, 2012; van Huis, 2013). Recently, European Member States Representatives endorsed a Commission proposal to amend Annex IV to Regulation (EC) No 999/2001 on processed animal proteins (Reference: Ares(2016)6396619). The definitive regulation and the authorization of the use of insect meals in fish feeds is expected by middle of 2017. *Tenebrio molitor* (yellow mealworm beetle) is a coleopter that can be found as unwanted guest in the food industry (flour, bran, pasta products). It is already raised on an industrial scale, but there are few data in literature on its use in animal feeding. *Tenebrio molitor* larvae meal (TM) has been used in broilers (Bovera et al., 2015; De Marco et al., 2015; Biasato et al., 2016) and laying hens (Giannone, 2003; Wang et al., 2005). In fish, TM was used in African catfish (Ng et al., 2001), rainbow trout (Belforti et al., 2015), black bullhead (Roncarati et al., 2015) and European sea bass (Gasco et al., 2016) with encouraging results in view of the possibility to supply with its use significant amounts of protein to fish in partial substitution of fish meal with

Table 1

Ingredients, chemical composition and estimated aminoacid profile of experimental diets and *Tenebrio molitor* larvae meal (TM), along with data on ideal IAA profile for gilthead sea bream.

	TM	TM0	TM25	TM50	Ideal IAA profile ^f
Ingredients (g kg⁻¹)					
Fish meal		500	333	130	
Corn gluten meal		150	125	130	
<i>Tenebrio molitor</i> larvae meal ^a		–	250	500	
Gelatinized starch		180	170	150	
Fish oil		140	95	60	
Mineral mix ^b		10	10	10	
Vitamin mix ^c		10	10	10	
Carboxymethylcellulose		10	10	10	
Chemical composition^d					
DM (g kg ⁻¹)	939	951	952	952	
Ash (g kg ⁻¹ , as fed)	47	89	71	50	
CP (g kg ⁻¹ , as fed)	519	438	435	430	
EE (g kg ⁻¹ , as fed)	236	193	190	194	
ADF (g kg ⁻¹ , as fed)	72	8	25	44	
Arg, % CP ^e	3.61	5.7	5.4	5.0	5.4
Phe, % CP	4.0	5.1	4.8	4.7	2.9
Ile, % CP	2.6	4.4	4.3	4.3	2.6
His, % CP	2.1	2.2	2.6	2.9	1.7
Leu, % CP	4.5	9.3	9.1	9.2	4.5
Lys, % CP	1.7	6.5	6.0	5.7	5.0
Met, % CP	1.6	4.1	3.7	3.5	2.4
Thr, % CP	2.7	3.8	3.8	3.8	2.8
Trp, % CP	0.8	0.9	0.8	0.7	0.6
Val, % CP	3.7	4.4	4.9	5.1	3.0
Gross Energy (MJ kg ⁻¹ , as fed)	24.4	21.81	21.25	21.10	

Abbreviations: DM, dry matter; CP, crude protein; EE, ether extract; ADF, acid detergent fibre.

^a *Tenebrio molitor* larvae meal purchased from Gaobeidian Shannong Biology CO. LTD (Shannong, China).

^b Supplying g/kg diet, CaHPO₄ + 2H₂O, 1.50, KH₂PO₄, 5.00, NaCl, 0.04, MgO, 2.50, FeCO₃, 0.70, KI, 0.04, ZnO, 0.11, MnO, 0.10, CuSO₄, 0.01, Na Selenite, 0.0004.

^c Supplying mg or IU/kg diet: vit. A, as retinyl palmitate 5000 IU; vit. D3, 2400 IU; α-tocopheryl acetate, 350; menadione, 50; thiamin HCl, 40; riboflavin, 50; pyridoxine HCl, 40; Ca-pantothenate 50; vit. B12, 0.01; niacin, 300; biotin, 3.0; folic acid, 5.0; choline 3750, myo-inositol, 500; vit. C as ascorbate Mg-phosphate, 200.

^d Values are reported as mean of duplicate analyses.

^e The amount of diets AAs were calculated using TM AAs profile (Bovera et al., 2015 and 2016) and integrated by data available in literature for TM larvae meal (Makkar et al., 2014) and for all the other ingredients (Monforte-Braga et al., 2006).

^f Gomez-Requeni et al. (2004); Met = Met + Cys; Phe = Phe + Tyr.

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