Animal Feed Science and Technology 226 (2017) 12–20

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

# 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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## ARTICLE INFO

*Keywords:*
Gilthead sea bream
*Tenebrio molitor* larvae meal
Growth performance
Nutrients digestibility
Slaughter traits

## 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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http://dx.doi.org/10.1016/j.anifeedsci.2017.02.007
Received 8 August 2016; Received in revised form 3 February 2017; Accepted 6 February 2017

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 | |
| *Tenebrio molitor* 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 | |
| Carboximethylcellulose | | 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, $CaHPO_4$ + $2H_2O$, 1.50, $KH_2PO_4$, 5.00, NaCl, 0.04, MgO, 2.50, FeCO3, 0.70, KI, 0.04, ZnO, 0.11, MnO, 0.10, $CuSO_4$, 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.

limited detrimental effects on growth performance and fish quality.

The aim of this study was to evaluate the effects of the inclusion of full fat TM in practical diets for gilthead sea bream (*Sparus aurata*) on growth performance, nutrient digestibility, somatic indexes, and some slaughter traits.

## 2. Material and methods

The experimental protocol was designed according to the guidelines of the current European Directive (2010/63/EU) on the protection of animals used for scientific purposes.

### 2.1. Growth trial

#### 2.1.1. Fish and experimental conditions

The trial lasted 163 days and was carried out in an indoor water recirculating system at the Department of Veterinary Medicine and Animal Production of Federico II University (Napoli, Italy), using 153 gilthead sea bream (105.2 ± 0.17 g average initial body weight – IBW) obtained from a local farm. The system was provided with thermostatic control and regulation of water temperature, mechanical sand filter, biological filter and UV lamp apparatus. Fish were randomly allocated in 9 fiberglass 220 l tanks (17 fish per tank) and were fed 3 isoenergetic and isoproteic diets. Each diet was randomly assigned to 3 tanks: a control diet (TM0), in which fish meal was the main protein source; TM25 and TM50 diets in which 25% and 50% of a full fat TM (Gaobeidian Shannon Biology CO., Ltd., Shannong, China) was included into the diet, respectively (as fed basis). After an adaptation period of 15 days the trial started. A constant and optimal environment quality was ensured to gilthead sea bream (daily water renewal: 5%, artificial day length: 12 h, temperature 21.9 ± 1.6 °C, salinity: 30.0 ± 2.0 g/l, dissolved oxygen 6.4 ± 1.5 mg/l, pH 7.5 ± 0.5, total ammonia nitrogen < 0.15 mg/l, nitrite-nitrogen < 0.05 mg/l, nitrate-nitrogen < 40 mg/l). Water temperature, pH and dissolved oxygen were measured daily using mercury thermometer, Orion digital pH meter and oxygen meter (WTW, OXI 330, Weilheim, Germany), respectively. Total ammonia nitrogen ($N\text{-}NH_3$), nitrite nitrogen ($NO_2\text{-}N$) and nitrate nitrogen ($NO_3\text{-}N$) were determined bi-weekly by colorimetric methods, using commercial kits and a spectrophotometer (Hanna Instruments, C-203, Leighton Buzzard, UK).

#### 2.1.2. Fish diets

The tested TM inclusion rates corresponded to 30% and 60% of inclusion on protein basis and 35% and 71% of fish meal substitution on protein basis for TM25 and TM50 diets, respectively. In order to keep the diets isoproteic and isoenergetic, the quantities of the other ingredients used in the formulation (corn gluten meal and starch) were slightly modified (Table 1). Since the used TM contained high fat levels, also the fish oil content was reduced by 32% with increasing the TM diet content. Chemical characteristics of the insect meal (Table 1) were determined and utilised to formulate the corresponding diets. Diets were formulated to meet nutrient requirements of gilthead sea bream, with particular attention to aminoacid profile of proteins (Table 1) (Gomez-Requeni et al., 2004; Peres and Oliva-Teles, 2009). The aminoacid composition of TM and raw materials was based on manufacturer data (Bovera et al., 2015, 2016) and literature data (Monforte-Braga et al., 2006; Makkar et al., 2014). The ingredients and proximate composition of the experimental diets are reported in Table 1. The diets were manufactured at the facilities of the Department of Veterinary Medicine and Animal Production, Napoli Federico II University (Naples, Italy). Before the final mixing, all ingredients were ground through a 0.5 mm sieve, then dry pelleted through a 3.5 mm dye. The feeds were stored at 4 °C until use. Each diet was administered twice a day (09:00 h and 16:00 h) to visual satiety (*i.e.* until the first feed item was refused), 7 days per week. The exact amount of feed distributed to each tank (feed intake) was recorded. Feeds were administered over the whole water surface in the tanks to be accessible simultaneously for all the fish. During the trial the tanks were inspected daily to check mortality.

#### 2.1.3. Growth performance

At the end of the trial, fish were starved for 1 day, lightly anesthetised (tricaine methanesulfonate-MS222, Sigma Aldrich, St. Louis, MO, USA, 50 ppm) and group (tank) weighed. The following growth performance indexes and protein efficiency ratio (PER) were calculated according to the following formulas:

Weight gain (WG%) = 100 × [(FBW, final body weight (g) − IBW, initial body weight (g))/initial live weight (g)]

Daily intake rate (DIR, %/day) = 100 × [(feed intake (g)/mean weight (g))/days]

Specific growth rate (SGR, %/day) = [(lnFBW − lnIBW)/number of feeding days] × 100

Feed conversion ratio (FCR) = [total feed supplied (g)/weight gain (g)]

Protein efficiency ratio (PER) = [weight gain (g)/total protein fed (g)].

### 2.2. Digestibility trial

To measure dry matter, crude protein and ether extract digestibility of the 3 diets used in the growth trial, a total of 72 gilthead sea bream (average IBW: 86.97 ± 2.3 g) were randomly distributed to 3 digestibility units (24 fish per unit), each composed by three

tanks (60 l) fitted with a common drain pipe connected to a settling column for collecting faecal material (Guelph CYAQ-2; Cho, 1992). The tank apparatus was connected with the indoor partially-recirculating water system. Each 60 l tank within each unit was stocked with 8 gilthead sea bream (biomass per unit: 3.9 kg); each diet was assigned to 1 unit and each diet was then tested in triplicate. During the trial, temperature was kept at 22 ± 1 °C and salinity at 30 ± 1 g/l.

The coefficients of total tract apparent digestibility (CTTAD) of the diets were measured using the indirect method proposed by Cho and Kaushik (1990) and acid-insoluble ashes (AIA) were used as indigestible marker, incorporated in the diets as Celite® (Sigma-Aldrich, St. Louis, MO, USA) at 1%, before the final mixing of the ingredients. Fish were fed two meals a day (09:00 h and 16:00 h) to visual satiety and adapted over 3 weeks to the diets prior to faeces collection. After each meal, the tanks and settling columns were cleaned to avoid faeces contamination by uneaten pellets. Faeces were collected daily from the settling column and immediately separated from the surrounding water by centrifugation (10,000 × *g*; 20 min; 5 °C). Faeces were collected over 16 days, *i.e.* as long as a suitable amount of material (130–150 g fresh weight) was obtained for the subsequent analyses. During the trial, the faeces were stored at −20 °C until the end of the collection period, when the daily amounts of each unit (diet) were pooled and freeze-dried before the analyses. The CTTAD of dry matter (DM), crude protein (CP) and ether extract (EE) were calculated according to Maynard and Loosly (1969).

### 2.3. Somatic indexes, slaughter traits and marketable traits

At the end of the growth trial all fish were euthanatized with tricaine methanesulfonate (MS222, 250 ppm), and utilised to collect data on body weight (BW, g), fish total length (FTL, cm), liver, mesenteric fat and visceral weights (g), and intestinal length (IL, cm) from pylorus to anus. These data were utilised to calculate the dressed yield, IL/FTL ratio and condition factor (CF), as well as hepatosomatic index (HSI) and viscerosomatic index (VSI), according to the following formulas:

$$\text{Dressed Yield} = 100 \times [\text{eviscerated fish weight (g)}/\text{body weight (g)}]$$

$$\text{IL/FTL} = \text{intestinal length (cm)}/\text{fish total length (cm)}$$

$$\text{CF} = 100 \times [\text{body weight (g)}/\text{total length (cm)}^3]$$

$$\text{HSI} = 100 \times [\text{liver weight (g)}/\text{body weight (g)}]$$

$$\text{VSI} = 100 \times [\text{visceral weight (g)}/\text{body weight (g)}].$$

For a more detailed analysis of the marketable characteristics, at the end of the trial a subsample of 31 fish (10 fish from TM0 group, 10 fish from TM25 group, and 11 fish from TM50 group) were randomly sampled and transported, in dry ice, to the Laboratories of the Department of Agri-Food Production and Environmental Sciences (DISPAA), University of Florence (Florence, Italy), where marketable traits and colour of fish skin were analysed. Immediately after the arrival, the fish were stored at −80 °C until the dissection since the analyses were not carried out immediately.

The day before the analyses, the fish were thawed, then they were weighed and, subsequently the filleting, the right and left fillets and the right and left skins obtained from each fish were weighed.

Colour measurements were performed by a Spectro-color®116 colorimeter (Bell Technology Ltd, Auckland, New Zealand), using the Spectral qc 3.6 software, according to the CIELab system (CIE, 1976), for measurements of lightness (L*), redness index (a*) and yellowness index (b*).

In addition, the values of Chroma = $(a^{*2} + b^{*2})^{1/2}$, as a measure of colour saturation, and of Hue = arctan(b*/a*) were calculated.

For each specimen, skin colour was measured on three dorsal and ventral spots of the left lateral side, in cranial, medial, and caudal locations. Finally, the colour parameters were expressed as mean of the values measured in the three sites of the dorsal and ventral regions.

### 2.4. Chemical analyses of TM meal, experimental diets and faeces

The following analyses were performed on TM, experimental diets and faeces, according to AOAC (2004): dry matter, ash, crude protein, ether extract, and acid detergent fibre (ADF) (procedure numbers 934.01, 942.05, 954.01, 920.39, and 973.18, respectively). Gross energy of the diets was measured with an adiabatic calorimeter bomb (IKA C7000, Staufen, Germany).

Only for the insect meal, the amount of protein linked to acid detergent fibre (ADF) was determined (AOAC, 2004, method number 990.03) and it was used to estimate the amount of chitin, according to Marono et al. (2015): chitin (%) = ash free ADF (%) − ADF-linked protein (%).

### 2.5. Statistical analysis

All the data were analysed by one way ANOVA, using the GLM procedure of SAS (2000), according to the model:

$$Yij = m + Di + eij$$

where Y is the single observation, m the general mean, D the effect of the diet (i = TM0, TM25 or TM50 diet), and e the error.

For growth and digestibility trials the experimental unit was the tank, while for somatic indexes, slaughter traits, marketable traits and skin colour the experimental unit was the individual fish.

In addition, to assess the probability of the linear and quadratic component and to compare treatments, the means were compared using orthogonal single degree of freedom contrast (Steel and Torrie, 1980; SAS, 2000).

## 3. Results

### 3.1. Growth performance

Experimental diets were well accepted by the fish and all feeds were consumed without loss. No mortality was observed during the trial. The growth performance of fish measured during the trial are reported in Table 2.

No differences were observed between control diet and TM based diets. Nevertheless, quadratic component of variance resulted significant for final body weight ($P < 0.01$), WG%, SGR and PER ($P < 0.05$), indicating that these parameters increased in TM25 group while in TM50 group returned at levels comparable to those of control diet.

### 3.2. Digestibility trial

The estimated CTTAD of DM, CP, and EE of the diets in the three groups are reported in Table 3. Control diet showed higher ($P < 0.001$) CTTAD of DM, CP and EE compared to TM based diets as evidenced by the contrast analysis. Furthermore, linear component indicated a gradual reduction of digestibility going from the control to TM50 ($P < 0.001$). Nevertheless, TM25 CTTADs resulted like those of control group but significantly higher than those showed by TM50 group as indicated by the quadratic component ($P < 0.001$).

### 3.3. Somatic indexes, slaughter traits and marketable characteristics

The slaughter traits at the end of the trial are reported in Table 4. Intestinal length expressed both in cm and related to fish total length was higher in TM groups compared to control group ($P < 0.01$), with a progressive increase from control diet to TM 50 diet ($P < 0.01$). Furthermore, TM25 group showed higher intestinal length (cm) compared to the other groups ($P < 0.01$). CF resulted higher in TM containing diets compared to control diet ($P < 0.05$) and TM25 showed the highest value ($P < 0.01$). Dressed Yield was higher in control group compared to TM diets ($P < 0.01$) and linear component indicated a gradual reduction from control to TM50 ($P < 0.01$). HIS and VSI resulted higher in TM diets compared to control ($P < 0.01$) and a progressive increase of their values from control to TM50 diet was registered ($P < 0.01$). The body weight of fish from the subset used to calculate fillet yield resulted higher in TM25 specimens compared to TM0 and TM50 specimens ($P < 0.01$). Concerning fillet yields with and without skin, a lack of significant differences among groups was observed. The colour parameters values of the skin, at the dorsal and ventral regions, were found similar in fish fed the experimental diets (Table 5).

## 4. Discussion

Based on the analyses results, the amount of chitin in the insect meal used in this study was 46.2 g/kg, as fed, corresponding to 64.2% of ash free ADF, in line with the finding of Finke (2007), who indicated that the ADF fraction in insects contained an amount of protein from 9.3 to 32.7% and the amount of chitin ranged from 2.7 to 49.8 g/kg.

Our results showed that *Tenebrio molitor* larvae meal included in *Sparus aurata* diets at 25 and 50% in FM substitution, had no negative effects on growth performance of fish, and TM25 diet gave the best results in terms of final body weight, weight gain, FCR and PER while the coefficients of total tract apparent digestibility decreased as TM inclusion level increased. The comparison with other researches is difficult due to the lack of studies available in literature on the use of insect meals in *Sparus aurata* production. Our

**Table 2**
Growth performance of gilthead sea bream fed the experimental diets.

| Tanks per diet | TM0<br>3 | TM25<br>3 | TM50<br>3 | RMSE | TM0 *vs* TM<br>*P*-value | linear<br>*P*-value | quadratic<br>*P*-value |
|---|---|---|---|---|---|---|---|
| Live weight, g | | | | | | | |
| Initial body weight | 105.1 | 105.1 | 105.4 | 2.16 | 0.9277 | 0.1549 | 0.9038 |
| Final body weight | 239.6 | 294.6 | 238.9 | 18.9 | 0.0880 | 0.9648 | 0.0060 |
| Weight gain, % | 127.9 | 180.9 | 126.5 | 21.24 | 0.1365 | 0.9378 | 0.0117 |
| Daily intake rate | 6.30 | 5.86 | 6.06 | 0.41 | 0.2871 | 0.4978 | 0.3189 |
| Specific growth rate | 0.50 | 0.63 | 0.54 | 0.01 | 0.1374 | 0.9463 | 0.0121 |
| Feed conversion rate | 1.34 | 1.02 | 1.28 | 0.09 | 0.1969 | 0.7193 | 0.0655 |
| Protein efficiency ratio | 1.74 | 2.26 | 1.79 | 0.11 | 0.1778 | 0.8251 | 0.0379 |

Abbreviations: TM0: fish meal group; TM25 and TM50: *Tenebrio molitor* larvae meal at 25 and 50% inclusion level groups, respectively; RMSE: root mean square error.

**Table 3**
Coefficients of total tract apparent digestibility (CTTAD) of dry matter ($CTTAD_{DM}$), crude protein ($CTTAD_{CP}$), and ether extract ($CTTAD_{EE}$) of gilthead sea bream fed the experimental diets.

| Tanks per diet | TM0<br>3 | TM25<br>3 | TM50<br>3 | RMSE | TM0 *vs* TM<br>*P*-value | linear<br>*P*-value | quadratic<br>*P*-value |
|---|---|---|---|---|---|---|---|
| $CTTAD_{DM}$ | 87.02 | 87.44 | 78.46 | 1.24 | < 0.0001 | < 0.0001 | < 0.0001 |
| $CTTAD_{CP}$ | 89.97 | 87.26 | 79.19 | 1.47 | < 0.0001 | < 0.0001 | 0.0008 |
| $CTTAD_{EE}$ | 91.12 | 89.93 | 82.39 | 1.56 | < 0.0001 | < 0.0001 | 0.0007 |

Abbreviations: TM0: fish meal group; TM25 and TM50: *Tenebrio molitor* larvae meal at 25 and 50% inclusion level groups, respectively; RMSE: root mean square error.

results are in contrast with other studies in which growth performances generally decrease at higher inclusion of insects (Sánchez-Muros et al., 2014) but partially in agreement with the findings of Rapatsa and Moyo (2017) who observed an increase of SGR and PER and a significant decrease of protein digestibility with higher (up to 24%) mopane worm inclusion levels in *Oreochromis mossambicus* diets.

Ng et al. (2001), replacing 40 and 80% of fishmeal with mealworm in African catfish (*Clarias gariepinus*), observed similar growth performance and feed intake to the control group, suggesting a high palatability for this kind of insect meal by the considered species of fish. In a more recent paper, Belforti et al. (2015) reported that the inclusion of 25 or 50% of TM in rainbow trout diets did not affect the final fish weight and weight gain, but significantly ameliorated performances parameters as FCR, SGR and PER. Finally, in a study on European sea bass of 5.23 g initial body weight (Gasco et al., 2016), the 25% of fishmeal replacement with mealworm larvae meal had no adverse effects on all considered growth performance parameters in comparison to the control group, but at 50% of replacement the authors observed significant reductions in growth rate, specific growth rate and feeding rate, while no effects were observed on FCR and PER. In our study, PER ranged from 1.74 (TM0) to 2.26 (TM25). These values are in agreement with literature (de Francesco et al., 2007) and the highest value registered in fish fed TM25 confirms the findings for WG and FCR. Limited knowledge is available on TM digestibility. Some trials have been conducted *in vitro* (Marono et al., 2015; Sánchez-Muros et al., 2016; Yi et al., 2016) but, to our knowledge, no trial was performed to evaluate the TM *in vivo* digestibility in gilthead seabream.

The effect of insect meal on nutrient digestibility is affected by chitin that in our trial showed a dose-dependent effect. Chitin, a linear homopolymer of β(1–4)-linked *N*-acetylglucosamine units, is a major constituent of the insect cuticles (Lindsay et al., 1984) and is not digestible by monogastric animals. In cuticle, chitin is linked to protein, reducing the apparent and true digestibility of nitrogen. However, chitinase genes have been sequenced in several carnivorous marine teleost, confirming that some fish are able to produce chitinase and thus to degrade chitin (Kurokawa et al., 2004). Standing the amount of chitin estimated in insect meal (4.62% as feed) and considering the level of insect meal inclusion and the feed intake of fish, TM25 and TM50 group individuals ingested 0.14 g/d and 0.22 g/d of chitin respectively. The effect of chitin on protein digestibility is well established in literature (Schiavone et al., 2014) as chitin showed a higher protein binding capacity. Chitin also has high water-binding capacities and can form ionic bonds that bind to various ionic substances like lipid and bile that will escape hydrolysis by lipase and lower lipid absorption in mammals (Tharanathan and Kittur, 2003). It has also been suggested that feeding chitin leads to decreased bile acid levels in the pylorus, and thereby lipid digestibility as bile acid is essential for activation of lipase and efficient lipid absorption (Hansen et al., 2010). The improvement of growth performance of TM25 group could be ascribed to prebiotic activity of chitin at lower concentrations (Esteban et al., 2001; Sakai et al., 1992; Kono et al., 1987) that increases the butyrate production in the caeca (Khempaka et al., 2011; Bovera et al., 2016; Loponte et al., 2016) and this has a positive effect on intestinal development (Bovera et al., 2016). Butyric acid is considered the prime enterocytes energy source (Bovera et al., 2010) and it is also necessary for the suitable development of the gut-associated lymphoid tissue (Mroz, 2005). In confirmation, intestinal length of fish fed TM25 diet was higher than the control. Evidences that chitin via butyrate may lead to an increased intestinal length have been demonstrated in a recent study in broilers fed TM based diets (Bovera et al., 2016). However, is not easy to understand why the strongly reduction of

**Table 4**
Somatic indexes, slaughter traits, morphometric and marketable traits of gilthead sea bream fed the experimental diets.

| Number of fish | TM0<br>51 | TM25<br>51 | TM50<br>51 | RMSE | TM0 *vs* TM<br>*P*-value | linear<br>*P*-value | quadratic<br>*P*-value |
|---|---|---|---|---|---|---|---|
| Intestinal length, cm | 12.30 | 16.79 | 16.13 | 3.13 | < 0.0001 | < 0.0001 | 0.0028 |
| Intestinal length/Fish total length | 0.49 | 0.63 | 0.65 | 0.013 | < 0.0001 | < 0.0001 | 0.0879 |
| Condition Factor | 1.51 | 1.60 | 1.58 | 0.14 | 0.0142 | 0.2218 | 0.0044 |
| Dressed yield, % | 93.88 | 93.31 | 92.12 | 1.06 | < 0.0001 | < 0.0001 | 0.2770 |
| Hepatosomatic Index, % | 1.22 | 1.64 | 2.16 | 0.43 | < 0.0001 | < 0.0001 | 0.6697 |
| Viscerosomatic Index, % | 5.09 | 5.52 | 6.77 | 0.96 | < 0.0001 | < 0.0001 | 0.1075 |
| Number of fish | 10 | 10 | 11 | | | | |
| Total body, g | 247.00 | 292.50 | 226.91 | 44.92 | 0.4679 | 0.3147 | 0.0033 |
| Fillet with skin yield, g/g | 0.45 | 0.45 | 0.45 | 0.03 | 0.8863 | 0.8661 | 0.9988 |
| Fillet without skin yield, g/g | 0.35 | 0.31 | 0.33 | 0.05 | 0.1676 | 0.4158 | 0.1652 |

Abbreviations: TM0: fish meal group; TM25 and TM50: *Tenebrio molitor* larvae meal at 25 and 50% inclusion level groups, respectively; RMSE: root mean square error.

**Table 5**
Skin colour parameters of gilthead sea bream fed the experimental diets.

| Number of fish | TM0<br>10 | TM25<br>10 | TM50<br>11 | RMSE | TM0 *vs* TM<br>*P*-value | linear<br>*P*-value | quadratic<br>*P*-value |
|---|---|---|---|---|---|---|---|
| Dorsal region | | | | | | | |
| L* | 35.93 | 39.58 | 46.35 | 17.058 | 0.2921 | 0.1728 | 0.8136 |
| a* | 3.67 | 4.18 | −0.03 | 7.580 | 0.5893 | 0.2734 | 0.4232 |
| b* | −8.65 | −8.38 | −3.60 | 10.203 | 0.5038 | 0.2670 | 0.5686 |
| Chroma | 10.90 | 10.69 | 4.39 | 11.837 | 0.4669 | 0.2189 | 0.5087 |
| Hue | 219.58 | 206.03 | 219.74 | 27.822 | 0.6024 | 0.9906 | 0.2937 |
| Ventral region | | | | | | | |
| L* | 66.23 | 67.63 | 71.28 | 9.309 | 0.3749 | 0.2248 | 0.7564 |
| a* | 1.57 | 1.19 | −0.64 | 4.048 | 0.4115 | 0.2218 | 0.6468 |
| b* | −4.64 | −4.00 | −1.92 | 6.290 | 0.4940 | 0.3324 | 0.7694 |
| Chroma | 7.08 | 6.05 | 3.17 | 6.180 | 0.3070 | 0.1586 | 0.7004 |
| Hue | 176.72 | 182.36 | 191.36 | 35.393 | 0.5354 | 0.4216 | 0.9181 |

Abbreviations: TM0: fish meal group; TM25 and TM50: *Tenebrio molitor* larvae meal at 25 and 50% inclusion level groups, respectively; RMSE: root mean square error.

nutrient digestibility in TM50 group had no effects on growth performance. Also Bovera et al. (2015), using *T. molitor* larvae meal as complete replacement of soybean meal in broiler diets, showed a decrease in crude protein, dry matter and organic matter ileal digestibility without effects on growth performance in comparison to the control group. The same effect was observed by Belforti et al. (2015) which reported a decrease in crude protein digestibility in fish fed diet containing an inclusion of 50% of TM without effects on weight gain. The lower nutrient digestibility observed in TM50 group can justify the higher intestinal length and the higher VSI and, as a consequence, the lower yield of TM50 fish. There are several evidences in literature that diets with low digestibility increased the relative intestinal length (German and Horn, 2006; Kramer and Bryant, 1995; Odedeyi et al., 2014), according to a compensatory mechanism by which the organism try to increase the amount of nutrient absorption (Borin et al., 2006).

HSI is an index normally utilized to investigate the effects of feeding on the liver functionality which is a key organ for metabolism (Dernekbaşı, 2012). Values of the HSI higher than the standard values (between 1 and 2%) show that feeding or the feed cause some troubles in fish, especially in the carbohydrate and fat metabolism, the existence of oxidized feed in the diet, and extra carbohydrate and vitamin deficiency (Munshi and Dutta, 1996). In our trial, TM50 group had a HSI slightly higher than 2% and this aspect needs further investigation as could indicate a metabolic trouble in fish. In the other hand, for TM25 group the HSI fall in the physiological range even if higher than that of the TM0 group. Opposite results were obtained for HSI in rainbow trout with a decrease in this index value at the increase of TM levels in the diets (Belforti et al., 2015).

For what concerns CF, both TM25 and TM50 groups showed higher values of this parameter compared to TM0 group, indicating that fish of the 2 first groups attained a better general condition (Nehemia et al., 2012).

No significant differences have been found between control and *Tenebrio molitor* larvae meal groups in fillet yield, while for dressed yield and VSI, TM groups resulted in worse values compared to control with TM50 group presenting the worst values. Tibaldi et al. (2015) in a study on European sea bass (*Dicentrarchus labrax* L.) found that the use of freeze-dried biomass of *Isochrysis* sp. (clone T-ISO) as a partial substitute of fish derivatives not lead changes on biometry traits and slaughter yield. Based on the available literature, there is no ready explanation for the different results to diets including different levels of insect meal as replacement of conventional protein source on marketable traits. However, the present outcomes confirm that the final commodity-related features were not detrimental affected by 25% of dietary inclusion of mealworm larvae meal in diet of gilthead sea bream.

About other marketable traits, it is well known that the colour is the one of the main quality parameters to evaluate finfish products and seems to influence consumer choices and acceptance. In fish, the skin colour can be affected by the diet characteristics, as found by García-Romero et al. (2014) on red porgy (*Pagrus pagrus*) and by Tibaldi et al. (2015) on European sea bass. The presence of various pigments in the diet ingredients can result in enhanced pigmentation of fish skin (Belay et al., 1996; Walker and Berlinsky, 2011; Tulli et al., 2012). Probably, the number of specimens examined and the known high variability showed by colour parameters, being analysed by punctual measurements, did not allow to express as significant the numerical differences observed.

## 5. Conclusions

*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 protein and ether extract digestibility, and some *post mortem* traits, after 163 days feeding. In addition, at this level of inclusion, feed conversion ratio and protein efficiency ratio were improved compared to the control group. At higher level of inclusion (TM50 group) gilthead sea bream's nutrient digestibility was penalized but this did not lead to negative effects on growth performance in comparison to the control group, whilst some negative effects resulted in slaughter traits, such as a lower dressed yield.

## Conflict of interest

The authors declare that there are no conflicts of interest.

## Acknowledgment

Ateneo Project (ex-60%) funded by the University of Florence.

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