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Abbreviations

ADC: apparent digestibility coefficients

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

TW: total wastes

VSI: viscerosomatic index

WG: Weight gain

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 liter 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 skin colour resulted to be affected by dietary inclusion of insect meal, especially at 50% of inclusion. 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 protein and ether extract digestibility, marketable indexes after 163 days feeding. On the contrary, when *Tenebrio molitor* larvae meal was included at 50%, nutrient digestibility, dressed yield, VSI and skin colour were penalized.

Key words: gilthead sea bream; *Tenebrio molitor* larvae meal; growth performance; nutrient digestibility; slaughter traits.

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 aquacultured 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; Sànchez-Muros et al., 2014; Henry et al., 2015; Lock et al., 2015). Insects are part of fish natural diet (Howe et al., 2014; Whitley and Bollens, 2014), show a high sustainability (Oonincx and de Boer, 2012; Van Huis, 2013) and have a high protein and lipid content (van Huis, 2013; Barroso et al., 2014) even if the hygiene and safety aspects of their production as well as the consumer perception must be further investigated (ESFA Scientific Committee, 2015). *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 broiler (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), and European sea bass (Gasco et al., 2016), black bullhead (Roncarati et al., 2015) with encouraging results.

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 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. After an adaptation period (15 days), fish were randomly allocated in 9 fiberglass 220 liter tanks (17 fish per tank). 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 fish were fed 3 isoenergetic and isoproteic diets and 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). These 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) have been slightly modified. Since the used TM contained high fat levels, also the fish oil content was reduced by 32% with increasing the percentage of TM inclusion. Chemical characteristics of the insect meal (Table 1) were determined and utilised to formulate the correspondent diets. Diets were formulated to meet nutrient requirements of gilthead sea bream, with particular attention to aminoacid profile of proteins. The full aminoacid composition of TM used for diets aminoacid calculation was supplied by the manufacturer and was reported in two previous studies in which the same insect meal was used (Bovera et al., 2015 and 2016) and this information was integrated by data available in literature for TM (Makkar et al.,2014) and for all the other ingredients (Monforte-Braga et al., 2006). 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 used. 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 in order to be accessible simultaneously for all the fish. During the trial, lasted 163 days, 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 individually weighed. The following growth performance indexes were calculated:

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^{-1}) = 100 × [(feed intake (g)/mean weight (g))/days]

Specific growth rate (SGR, % day−1) = [(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

A separate trial was conducted 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 were randomly distributed to 3 digestibility tank-units (24 fish per unit). The digestibility system, developed by the University of Guelph (Guelph CYAQ-2; Cho, 1992), consisted of 3 units, each unit composed by 3 tanks fitted with a common drain pipe connected to a settling column for collecting faecal material. 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 (average IBW: 86.97±2.3 g; biomass per unit: 3.9 kg); each diet was assigned to 1 unit and each diet was then tested in triplicate units. During the trial, temperature was kept at 22±1 °C and salinity at 30±1 g/l.

The diets apparent digestibility coefficients (ADC) 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 diets apparent digestibility coefficients (ADC) of dry matter (DM), crude protein (CP) and ether extract (EE) were calculated according to Maynard and Loosely (1969).

2.3 Somatic indexes and slaughter 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:

Dressed Yield = 100 × [eviscerated fish weight (g) / body weight (g)]

IL/FTL = intestinal length (cm) / fish total length (cm)

CF = 100 × [body weight (g)/total length $(cm)^3$]

HSI = 100 × [liver weight (g)/body weight (g)]

VSI = 100 × [visceral weight (g)/body weight (g)].

2.4. Morphometric and marketable traits

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 analyses.

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. Afterwards fish head, fins and frame were removed from each and weighed. From the weight measurements, the marketable characteristics as fillet yield (FY) (with and without skin), frame, fins, head and total wastes (TW) incidences were calculated as follows:

FY with skin (%) = 100 × [right fillet weight (g) + left fillet weight (g) / body weight (g)]

FY without skin (%) = 100 × [(right fillet weight (g) + left fillet weight (g)) – (right skin weight (g) + left skin weight (g) / body weight (g)]

Frame (%) = 100 × [frame weight (g) / body weight (g)]

Fins (%) = 100 × [fins weight (g) / body weight (g)]

Head (%) = 100 × [head weight (g) / body weight (g)]

TW (%) = 100 × [frame + fins + head + viscera weight (g) / body weight (g)].

2.5. Skin colour

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). In this system, lightness (L*) is expressed on a 0 – 100 scale from black to white; redness index (a*) ranges from red (+60) to green (-60) and yellowness index (b*) ranges from yellow (+60) to blue (-60).

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 specimens, skin colour was measured on three dorsal and ventral spots of 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.

The total colour differences, i.e. $\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta Lb^*)^2}$, according to the formula for Euclidean distance between two points in the CIE – L*a*b* space were also calculated. According to Mokrzycki and Tatol (2011), a standard observer can see the difference in color as follows:

$0 < \Delta E < 1$: the difference is not noticed by an observer;

$1 < \Delta E < 2$: the difference can be noticed only by an experienced observer;

$2 < \Delta E < 3.5$: the difference is also noticed by an inexperienced observer;

$3.5 < \Delta E < 5$: a clear difference in colour is noticed;

$5 < \Delta E$: two different colours are noticed by an observer.

2.6 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).

2.7 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 protein source (i = TM0, TM25 or TM50 diet), and e the error.

Comparison between means was performed by Tukey's test (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. The final body weight was the highest ($P < 0.05$) in fish fed TM25 diet, while no differences were observed between TM0 and TM50 groups. Consequently, WG % resulted higher in TM25 group if compared to the other groups. No differences were observed as regards to DIR whist, for the other criteria reported in the Table, TM0 and TM50 groups showed no different values, while TM25 group showed the best value for FCR ($P < 0.05$) and the most favourable SGR and PER ($P < 0.05$).

3.2 Digestibility trial

The estimated ADC of DM, CP, and EE of the diets in the three groups are reported in Table 3. TM50 group had lower ($P < 0.001$) values for all the coefficients in comparison to TM0 and TM25 groups, which did not differ between them.

3.3 Somatic indexes and slaughter traits

The slaughter traits at the end of the trial are reported in Table 4. The dressed yield was the lowest for TM50 group and no differences were observed between TM0 and TM25 groups. Concerning intestinal length and IL/FTL ratio, the groups fed diets containing insect meal showed higher ($P < 0.001$) value than the TM0 group. This last group had the lowest ($P < 0.05$) CF and no differences were observed between TM25 and TM50 group for this parameter. As somatic indexes are concerned, the HSI showed a progressive increase ($P < 0.001$) from TM0 to TM50 groups, and VSI was the highest ($P < 0.001$) in TM50 group, while no differences were observed between TM0 and TM25 for this parameter.

3.4 Morphometric and marketable traits

In the Table 4, the effect of dietary mealworm inclusion on morphometric and marketable

characteristics of gilthead sea bream is shown. The body, dressed and left fillet weight resulted higher in TM25 specimens compared to TM0 and TM50 specimens. Concerning right fillet and skin, and head weight, fish fed TM25 diet showed higher ($p < 0.05$) value, following by TM0, than TM50 group, as a consequence of the higher final total body weight. No differences were observed groups for viscera weight. The different diets used in the trial did not affect the same morphometric and commodity-related parameters. Concerning fillet yields (with and without skin), frame and head incidences, despite the absence of significant differences among groups, a similar trend was observed. When fish meal was gradually replaced with insect meal, the value of these parameters decreased. Instead, dressed yield was the lowest ($p < 0.01$) for TM50 group than TM0 and TM25 and, consequently, VSI had the highest value in TM50 group, whilst and no differences were observed between TM0 and TM25 groups. Finally, TM0 group had a higher ($p < 0.05$) value in total wastes percentage, following by TM25, than TM50 group.

3.5 Skin colour

The colour parameters values of the skin, at the dorsal and ventral regions are presented in Table 5. No significant differences were found in skin colour of fish fed the experimental diets. Nevertheless L* and Hue*, both at dorsal and ventral locations, tended to gradually increase with the inclusion of TM in the feed. Whilst the other parameters as redness (a*), yellowness (b*) and Chroma* showed a progressive decrease from TH0 to TM50 groups. The colour differences measured by ΔE showed that the skin colour of fish fed TM0 diet can be perceived as different when compared to TM50 group colour (11.10 at dorsal level and 5.56 at ventral level), whilst ΔE values between TM25 and TM50 were 8.93 and 4.27, at dorsal and ventral level, respectively. Between TM0 and TM25 groups the ΔE showed lower values (3.20 and 1.33 at dorsal and ventral level, respectively).

4. DISCUSSION

In this trial we tested the inclusion of 25% and 50% of TM in diets for *Sparus aurata*, that corresponded to 30 and 60% of inclusion on protein basis, and to 35 and 71% of fish meal substitution on protein basis for TM25 and TM50 diets, respectively.

Currently, no studies are available in literature on the use of insect meals in *Sparus aurata* production. 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.

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.

Limited knowledge is available on TM digestibility. Some trials have been conducted *in vitro* (Marono et al., 2015; Sánchez-Muros et al., 2015; Yi et al., 2016) but to our knowledge, no trial was performed to evaluate the *in vivo* digestibility in gilthead seabream. It is very important to observe that, during the entire period of the trial, the TM50 group showed no differences in comparison to TM0 group regarding growth performance even if the crude protein and ether extract apparent digestibility coefficients are lower by –12.0 and -9.6 % than the TM0 group, respectively. 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 lower crude protein digestibility in TM50 diet can reflect the higher chitin content of the diet leading to a higher chitin-linked protein quota (Bellucco et al., 2013). 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.

Chitin, a linear homopolymer of β(1 - 4)-linked N-acetylglucosamine units, is a major constituent of the insect cuticles (Lyndsay 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. It also has to be considered that the insect meal protein is reported (Bovera et al., 2016) to have a low content in essential aminoacids (methionine, cystine, lysine, tryptophan) and that the cuticle proteins present an aminoacid composition different from that of the whole insects (Finke, 2007). Based on these considerations, and considering that dietary protein fraction is the most important factor affecting fish growth (Garcia De La Serrana et al., 2012), the hypothesis is that the amount of essential aminoacids available for digestion was sufficient to sustain fish growth, also in the case of the TM50 diet.

The current trend in fish feed production is to increase lipid content with the aim to reduce the use of protein as energy source and thus reduce the use of fishmeal (Valente et al., 2011). However, is well reported (Mongile et al., 2014) that the increase of lipid content in the diet (from 16 to 24 %) had no effects on specific growth rate and final body weight of gilthead sea bream. This could justify why, in our trial, the reduction of lipid digestibility in TM50 diet had no effects on fish growth performance when compared to TM0 diet.

Very interesting, in TM25 group the crude protein and ether extract digestibilities were not different from those of TM0 group, while growth performance was better than that of the other 2 groups. The lowering crude protein digestibility observed in fish fed TM50 diet could be ascribed to the chitin level. 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). So, it is possible that the amount of chitin in the TM25 diet can be partly degraded by endogenous chitinase of *Sparus aurata* and then protein of *Tenebrio molitor* meal can be available for digestion at the same way that fish meal. However, as the amount of chitin in the digestive system of fish increase as a consequence of the increased TM inclusion level, the chitinase level could be not

sufficient to break the increased amount of chitin, so that the crude protein digestibility in TM50 group resulted more than 10 percentage points lower compared to that of the control group. Also the ether extract fraction strong digestibility reduction in TM50 group in comparison to the control one can be ascribed to the increase of chitin level in daily feed intake. To explain that, two mechanisms can be assumed: 1) an amount of lipids are tied to insect cuticle where their function is to prevent desiccation (Klowden, 2013); 2) in general, lowering digestibility due to an increased chitin level in diet is responsible of an increased passage rate of feed through the digestive tract, lowering the apparent coefficient digestibility of all the nutrients.

The positive effect registered with TM25 diet on fish growth performance could also be ascribed to chitin. In fact, recent studies on humans and mice (Neyrinck et al., 2011 a and b; Brownawell et al., 2012) have suggested that chitin may be useful in restore compositional balance of the intestine microbial community and in improving colonic function even when a high fat diet is consumed. Chitin is not degraded and absorbed in the intestine, therefore it can be fermented by the microbiota acting as a prebiotic. In addition, chitin seems to exhibit a bacteriostatic effect on the Gram negative bacteria *Escherichia coli*, *Vibrio cholerae*, *Shigella disenteriae* and *Bacteriodes fragile* (Vidanarachchi et al., 2010). Khoushab and Yamabhai (2010) also showed antifungal and antimicrobial proprieties of chitin. The knowledge on these activities of chitin have yet to be totally uncovered and several mechanisms have been suggested. One of them is the ability of chitin and its derivatives to activate defence mechanisms of the host organisms by inducing the accumulation of chitinases and other pathogenesis-related proteins (El Ghaouth et al., 1992). Another one is leakage in the cell wall of bacteria due to the interaction between positively charged chitin molecules and the negatively charged surface of the bacteria (Young et al., 1982). In *Sparus aurata*, Esteban et al. (2001) observed an increase in the activity of innate immune system when chitin was incorporated in the diet. Similar effects were observed also in rainbow trout (Sakai et al., 1992), while Kono et al. (1987) found an increase of growth rate and feed assimilation in some aquacultured fish due to chitin supplementation. Based on these considerations, our hypothesis is that chitin, acting as prebiotic, was

able to partly recover the negative effects of low nutrient availability, showing a positive effect on growth traits of fish fed TM25 diet.

All the slaughter trait parameters considered in this trial were higher in fish fed TM50 diet than in TM0 group, while the yield was lower. The lower nutrient digestibility observed in the first group can justify the higher intestinal length and the higher VSI and, as a consequence, the lower yield of TM50 fish. There are several evidence 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). In fish fed TM25 diet, VSI was not different from that of TM0, but the intestinal length was higher. This effect could be ascribed to the prebiotic effects of chitin that can increase the production of butyric volatile fatty acid in caeca (Khempaka et al., 2011; 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). It is documented that butyrate is the major intestinal energy source even when other fuel sources (glucose or glutamine) are available and could stimulate the growth of colorectal and ileal mucosal cells (Montagne et al., 2003; Topping and Clifton, 2001).

HSI is an index normally utilised 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 - 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. However, both TM25 and TM50 groups had a higher CF than that TM0 group, indicating that fish of the 2 first groups attained a better general condition (Nehemia et al., 2012). 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).

In this study, the inclusion of *Tenebrio molitor* larvae meal at 25% in feeding of gilthead sea bream affected positively the body weight of fish, at the end of the trial. In addition, the increase of body weight affected the linear and weight measures as dressed, right and left fillets, skin, head, frame, fins and viscera weight that tend to rise in fish fed TM25 diet. No significant differences have been found between control and *Tenebrio molitor* larvae meal groups in morphometric and commodity-related characteristics, excepted for dressed yield and VSI, which values confirmed a similar pattern for fish fed TM0 and TM25 diets, whilst the highest level of fish meal replacement negatively affected those parameters. 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 dietary inclusion of mealworm in diet of gilthead sea bream when a 25% of FM replacement is considered.

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 affect consumer choices and acceptance. In fish, the skin colour can be affected by the diet characteristics. García-Romero et al. (2014) found that skin colour of red porgy (*Pagrus pagrus*) improved by marine crab meal inclusion in the diet, while sea echinoderm meal promoted the yellowness of skin. Different levels of dried microalgae biomass in diets for European sea bass promoted differences in a*, b*, Chroma* and Hue* values of dorsal and ventral regions of the skin (Tibaldi et al., 2015). It is well investigated that the presence of various pigments can result in enhanced pigmentation of skin in fish (Belay et al., 1996; Walker and Berlinsky, 2011; Tulli et al., 2012). The different diets tested in this trial do not seem to have had a significant effect on skin colour values, contrary to previous findings related to the use of alternative sources of

protein as partial replacers of the traditional marine sources. Only a trend in lightness increasing with the increase of TM inclusion in the diet was observed, both at the dorsal and ventral regions. The numerical differences highlighted for TM50 group regarding the chromaticity indexes (a* and b*), and amplified in the cumulative index of saturation, did not result significant as a consequence of the large intra-group variability.

However, the colour differences were appreciated by the ΔE ratio. When compared TM0 and TM50, the values registered allow to perceive the colour of the skin of the these two groups as of two different colour by an observer, according to Mokrzycki and Tatol (2011). The differences between TM25 and TM50 colours were less relevant but always noticeable whilst by the comparison of the TM0 and TM25 groups, the ΔE ratio highlighted differences in colour that also inexperienced observer can notice in the dorsal region.

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 is 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 and variations in skin colour.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Table 1. Ingredients, chemical composition and estimated aminoacid profile of experimental diets and *Tenebrio molitor* larvae meal (TM).

	TM	FM	TM25	TM50
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 100 g^{-1})	93.90	95.1	95.2	95.2
Ash (g 100 g^{-1}, as fed)	4.7	8.9	7.1	5.0
CP (g 100 g^{-1}, as fed)	51.9	43.8	43.5	43.0
EE (g 100 g^{-1}, as fed)	23.6	19.3	19.0	19.4
ADF (g 100 g^{-1}, as fed)	7.2	0.8	2.5	4.4
Arg, % CP[e]	3.61	5.7	5.4	5.0
Phe, %CP	4.0	5.1	4.8	4.7
Ile, % CP	2.63	4.4	4.3	4.3
His, % CP	2.11	2.2	2.6	2.9
Leu, % CP	4.52	9.3	9.1	9.2
Lys, % CP	1.68	6.5	6.0	5.7
Met, % CP	1.62	4.1	3.7	3.5
Thr, % CP	2.71	3.8	3.8	3.8
Trp, % CP	1.75	0.9	0.8	0.7
Val, % CP	3.72	4.4	4.9	5.1
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).

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

	TM0	TM25	TM50	*P*-value	RMSE
Number of fish	*51*	*51*	*51*		
Live weight, g					
Initial BW	105.1	105.1	105.4	0.976	2.16
Final BW	239.6[b]	294.6[a]	238.9[b]	0.017	18.9
WG, %	127.9[b]	180.9[a]	126.5[b]	0.033	21.24
DIR	6.30	5.86	6.06	0.473	0.41
SGR	0.50[b]	0.63[a]	0.54[b]	0.021	0.01
FCR	1.34[a]	1.02[b]	1.28[a]	0.038	0.09
PER	1.74[b]	2.26[a]	1.79[b]	0.035	0.11

Abbreviations: TM0: fish meal group; TM25 and TM50: *Tenebrio molitor* larvae meal at 25 and 50% inclusion level groups, respectively; BW: body weight; WG: weight gain DIR: daily intake rate; SGR: specific growth rate; FCR: feed conversion ratio; PER: protein efficiency ratio; RMSE: root mean square error.
a, b: $P < 0.05$.

Table 3. Apparent digestibility coefficients of dry matter (ADC_{DM}), crude protein (ADC_{CP}), and ether extract (ADC_{EE}) of gilthead sea bream fed the experimental diets.

	TM0	TM25	TM50	*P*-value	RMSE
Number of fish	*24*	*24*	*24*		
ADC_{DM}	87.02^{A}	8.44^{A}	78.46^{B}	<0.0001	1.24
ADC_{CP}	89.97^{A}	87.26^{A}	79.19^{B}	<0.0001	1.47
ADC_{EE}	91.12^{A}	89.93^{A}	82.39^{B}	<0.0001	1.56

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.
A, B: $P < 0.01$.

Table 4. Morphometric and marketable traits of gilthead sea bream fed the experimental diets.

	TM0	TM25	TM50	*P*-value	RMSE
Number of fish	*51*	*51*	*51*		
Intestinal length, cm	12.30^{B}	16.79^{A}	16.13^{A}	<0.0001	3.13
IL/FTL	0.49^{B}	0.63^{A}	0.65^{A}	<0.0001	0.013
CF	1.51^{b}	1.60^{a}	1.58^{a}	0.020	0.14
Dressed yield, %	93.88^{A}	93.31^{A}	92.12^{B}	<0.0001	1.06
HSI, %	1.22^{C}	1.64^{B}	2.16^{A}	<0.0001	0.43
VSI, %	5.09^{B}	5.52^{B}	6.77^{A}	<0.0001	0.96
Number of fish	*10*	*10*	*11*		
Total body, g	247.00^{b}	292.50^{a}	226.91^{b}	0.008	44.92
Right fillet, g	54.00^{ab}	59.13^{a}	45.93^{b}	0.023	10.39
Left fillet, g	51.35^{b}	64.82^{a}	48.76^{b}	0.012	12.15
Right skin, g	11.31^{ab}	14.06^{a}	8.77^{b}	0.028	4.25
Frame, g	35.32^{a}	39.02^{a}	28.53^{b}	0.009	7.28
Fins, g	6.56	8.74	6.46	0.140	2.83
Head, g	69.98^{ab}	82.66^{a}	63.16^{b}	0.021	15.06
Fillet with skin yield, %	45.09	45.00	44.91	0.986	2.51
Fillet without skin yield, %	34.73	30.96	32.86	0.282	5.17
Frame, %	14.21	13.42	12.51	0.076	1.64
Fins, %	2.65	3.02	2.90	0.737	1.08
Head, %	28.41	28.13	27.82	0.868	2.53
Total wastes, %	45.27^{a}	44.57^{ab}	43.24^{b}	0.028	1.66

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.
IL/FTL: Intestinal Length/Fish Total Length; CF: condition factor; HSI: hepatosomatic index; VSI: viscerosomatic index; A, B: $P < 0.01$; a, b: $P < 0.05$.

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

	TM0	TM25	TM50	*P*-value	RMSE
Number of fish	*10*	*10*	*11*		
Dorsal region					
L*	35.93	39.58	46.35	0.375	17.058
a*	3.67	4.18	-0.03	0.388	7.580
b*	-8.65	-8.38	-3.60	0.448	10.203
Chroma*	10.90	10.69	4.39	0.367	11.837
Hue*	219.58	206.03	219.74	0.566	27.822
ΔE^a	3.20	8.93	11.93		
Ventral region					
L*	66.23	67.63	71.28	0.446	9.309
a*	1.57	1.19	-0.64	0.416	4.048
b*	-4.64	-4.00	-1.92	0.590	6.290
Chroma*	7.08	6.05	3.17	0.334	6.180
Hue*	176.72	182.36	191.36	0.709	35.393
ΔE^b	1.33	4.27	5.56		

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;

[a] A standard observer sees the difference in color as follows: $0 < \Delta E < 1$ - observer does not notice the difference, $1 < \Delta E < 2$ - only experienced observer can notice the difference, $2 < \Delta E < 3{:}5$ - unexperienced observer also notices the difference, $3{:}5 < \Delta E < 5$ - clear difference in color is noticed, $5 < \Delta E$ - observer notices two different colors.