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ABSTRACT

Alternative and sustainable fish diets are required by modern aquaculture. We investigated the possibility of using insect (Tenebrio molitor TM, Hermetia illucens HI or Musca domestica MD) larvae meals (as 19.5% of the feed formulation) to replace 30% of the in the fish meal (FM) in a gilthead seabream (Sparus aurata) feed formulated to contain 65% FM. The feeds were isonitrogenous (ca 57% crude protein of dry matter) isolipidic (ca 17% lipid dry matter) and isoenergetic (ca 22 MJ kg− 1 dry matter). To achieve similar energy content among the experimental diets, the fish oil inclusion was adjusted. Fish (average initial weight of 29.5 g) were fed up to apparent satiation three times a day, 7 days per week in a 93-days trial. Each diet was assigned to three 500 L tanks with fish density 2 kg m− 3 . Five fish from the initial population and two fish per tank were taken for whole-body composition analysis. At the end of the experimental period, nine fish per treatment were taken for the analysis of plasma metabolites and liver enzyme activities. Growth performance, feed intake, feed conversion and somatic indices of fish fed the different insect meal diets were similar to the FM fish. However, among the insect meal fish groups, the feeding with the TM diet resulted in higher specific growth rate compared to the HI diet (1.57% and 1.51% per day, respectively). The whole-body proximate composition was similar among experimental groups. Fish fed HI had the lowest fat retention (57.0% compared to 69.3–74.2%). Additionally, the HI group had also lower dry matter and energy retention (30.5% and 36.6%, respectively) compared to the FM group (33.8% and 41.5%, respectively). The whole-body saturated and mono-unsaturated fatty acids content was similar to all the experimental groups. Fish fed diets higher in fish oil (FM and HI) had higher eicosapentaenoic, docosahexaenoic and total ω-3 poly-unsaturated fatty acids content. Whole-body amino acid composition was similar among all experimental groups, while the amino acid retention exhibited significant differences. The plasma metabolites and enzyme activities as well as the hepatic lipogenic enzyme activity were not affected by the different diets. Fish fed the HI diet exhibited higher liver alanine aminotransferase (ALT) activity in comparison to the TM group. Overall, this study shows that FM can be successfully replaced by TM, HI or MD meals in 30% by weight in the diets of gilthead sea bream. Comparing insect meals, HI meal

was inferior in terms of growth performance and dry matter-fat retention compared to TM and MD, respectively.

1. Introduction

Insect meals can be included in aquafeeds as valuable sources of high-quality protein, thereby reducing the reliance on ingredients, such as fish meal, derived from overexploited natural resources. Insect larvae have high nutritional value (Poshadri et al., 2018; Rumpold and Schlüter, 2014), they can be produced in a short time due to their short life cycle (Hua et al., 2019) and they can bioconvert and biotransform organic matter (Fowles and Nansen, 2019; Gasco et al., 2020) with low feed conversion ratio (Oonincx et al., 2015). Therefore, insect larvae production could be performed efficiently next to agri-food manufacturing facilities, by converting side-streams into products of high nutritional value (Smetana et al., 2019) which contribute to limiting environmental degradation (Van Huis, 2013) in the frame of circular economy (Madau et al., 2020). The study of insects and insect meals in aquafeeds has a history that stretches back over 30 years (Bodari and Shepard 1981), but most of the work on farmed fish has been carried out on freshwater species (e.g. Alegbeleye et al., 2012; Barroso et al., 2014; Sogbesan, 2014; St-Hilaire et al., 2007). More recently, research has been conducted on incorporating insects and insect meals into feeds for anadromous species, such as Atlantic salmon (Salmo salar) (Belghit et al., 2018, 2019; Bruni et al., 2020), marine and freshwater crustaceans, such as Pacific white-leg shrimp (Litopenaeus vannamei) and Baltic prawn (Palaemon adspersus) (Rahimnejad et al., 2019; Mastoraki et al., 2020b) and some marine fish species, including the gilthead sea bream (Sparus aurata) (Piccolo et al., 2017; Antonopoulou et al., 2019; Randazzo et al., 2021; Pulido-Rodriguez et al., 2021). In this framework, the aim of the present study is the evaluation of the effect of 30% fish meal substitution with three different insect meals derived from Tenebrio molitor, Hermetia illucens or Musca domestica larvae on growth performance, nutrient utilization and intermediary metabolism of gilthead sea bream Sparus aurata. Although insect meal diets have been extensively studied, comparative studies, investigating the effects of different insect meals in the same trial, are scarce (Jozefiak ´ et al., 2019; Melenchon ´ et al., 2020; Fabrikov et al., 2020). Innovationwise, the study herein is the first one assessing the effects of insect larvae meal from housefly Musca domestica on gilthead sea bream.

2. Materials and methods

The feeding trial was conducted at the Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC) of the Hellenic Centre for Marine Research (Heraklion, Greece) by accredited scientists of the Federation of European Laboratory Animal Science Associations (FELASA). The experiments were authorized by the ethics committee of the region of Crete, Greece (license No 255,340) and were conducted following the guidelines established by the EU Directive 2010/63/EU.

2.1. Composition of diets and experimental conditions

The feeds were formulated to contain fish meal (FM) as the main protein source in the control feed (FM as 65% of the formulation) and insect meals from larval Tenebrio molitor (TM; full-fat), Hermetia illucens (HI; defatted) and Musca domestica MD; full-fat) used to replace 30% of the FM, by including them as 19.5% of the formulation (Tables 1 and 2). To achieve a similar amount of essential amino acids between the different diets, crystalline DL-methionine and L-lysine were incorporated. In addition, to ensure a similar protein and energy content between the diets, the inclusion of the different ingredients, namely fish oil, wheat flour and wheat gluten, were adjusted. A mincing machine with 4 mm die was used for the formation of pellets. Finally, the diets were oven-dried at 40 ◦C for 24 h and were stored in a freezer. Proximate compositions of insects and diets were determined by using standard methods of analysis (AOAC, 1990). Dry matter was determined by drying at 90 ◦C until constant weight and ash by incineration at 700 ◦C for 7 h. Crude fat was determined according to Folch et al. (1957) using extraction with

chloroform-methanol-butylated hydroxytoluene (2:1 v/v + 0.01% w/v BHT). Energy was measured using a bomb calorimeter (6300, Parr Instrument Company, St. Moline, Illinois, USA). Crude fiber was determined by defatting the samples with petroleum ether and sequential boiling with 0.13 mol l − 1 H2SO4 and 0.23 mol l − 1 KOH using Fibretherm (C. Gerhardt GmbH & Co., Konigswinter, ¨ Germany). To quantify lignin, cellulose, chitin and indigestible nitrogen (nitrogen linked to cell-walls or chitin) content of the diets, the analysis of the ash-free acid detergent fiber (ADF) was performed by boiling with 1 N H2SO4 + 2% Cetyl trimethylammonium bromide (CTAB) using Fibretherm, and subtracting the ash content (Bernard et al., 1997; Finke, 2007; Goering and Van Soest, 1979). Crude protein was defined with a nitrogen analyzer (FP-528, Leco corporation, St. Joseph, Michigan, USA) by multiplication of the nitrogen content by 6.25. Crude protein was determined, according to Dumas's method, using a nitrogen analyzer (FP-528, Leco corporation, St. Joseph, Michigan, USA) by multiplication of the nitrogen content by 6.25. In addition, the nitrogen linked to ADF (ADIN) was determined according to Goering and Van Soest (1979) using a nitrogen analyzer. Diets' protein content was adjusted by subtracting the ADIN from the total nitrogen content and then multiplying it by 6.25. The amino acid composition of the diets was analyzed after acid hydrolysis (6 N HCl, 11 ◦C, 24 h), and derivatization by AccQ-Tag™ Ultra according to the amino acid analysis application solution (Waters Corporation, Milford, MA, U.S.A.). DL-Norvaline (Sigma) 2.5 mM was used as an internal standard. UPLC was performed on an Acquity system (Waters Corporation, Milford, MA, U.S.A.) equipped with PDA detector and the detection wavelength was set at 260 nm. The column used was BEH C18 column (100 mm × 2.1 mm i.d., 1.7 µm) from Waters. The flow rate was 0.7 mL/min and the column temperature was kept at 55 ◦C. Peak identification and integration were performed by the software Empower v.2.0 (Waters Corporation, Milford, MA, U.S.A.) using Amino Acid Standard H (Thermo Scientific Pierce) as an external standard. Tryptophan was not quantified due to its susceptibility to acid hydrolysis, whereas cysteine reacts with cysteine to form cystine. Moreover, during acid hydrolysis procedure, asparagine is converted to aspartate and glutamine to glutamate, so the reported values for these amino acids (Asx and Glx) represent the sum of both amino acids. For the analysis of fatty acids, lipid samples were saponified with a NaOH-methanol solution and the resulting fatty acids were methylated by a Boron trifluoride-methanol solution, according to AOCS (1989).

The fatty acid methyl esters (FAMEs) were extracted with isooctane and analyzed through a Shimadzu GC-2010 gas chromatograph (Shimadzu Corporation, Japan), equipped with a flame-ionization detector (GCFID) and a SP-2330 capillary column (30 m × 0.25 mm i.d. × 0.20 µm film thickness (Supelco Inc., Bellfonte, Pennsylvania, USA). Helium was used as carrier gas at 2 mL/min constant flow; the split ratio was 1:50 and the injected volume 1.0 µl. The thermal gradient was 100 ◦C to 160 ◦C at 10 ◦C min− 1 , 160 ◦C to 220 ◦C at 3 ◦C min− 1 and kept for 5 min, and lastly, 220 ◦C to 250 ◦C at 10 ◦C min− 1 and kept for 5 min. The injector and detector temperature were maintained at 260 ◦C and 280 ◦C, respectively. Fatty acids were identified by comparison with a known standard mixture (Supelco 37 Component FAME Mix). FAME contents were expressed as a percentage (%) of total FAMEs basis. Juvenile gilthead sea breams were provided from the IMBBC hatchery. After a light anaesthesia (phenoxyethanol, 150 ppm), 360 fish were individually weighed (29.5 ± 0.7 g) and were randomly divided into 12 open circulation indoor tanks (500 L). Five fish were sacrificed by anaesthesia overdose (phenoxyethanol, 500 ppm) and were stored at − 20 ◦C for a whole-body proximate composition analysis. The feeding trial started the following day. The water temperature throughout the experimental period was 19.9 ± 0.1 ◦C, salinity was 35 ppt, oxygen saturation was constantly over 80%, and the photoperiod was 12 h light/12 h dark. Diets were assigned to triplicate groups and fish were fed by hand until apparent satiation, three times a day, 7 days a week for three consecutive months. Pellets that remained unconsumed were siphoned daily and dried to determine feed intake.

2.2. Growth performance and somatic indices

At the end of the experimental period, the sampling was carried out after a 24 h fast in order to reduce handling stress and ensure an empty gastrointestinal tract. Fish were lightly anaesthetised, individually

weighed and measured accordingly. Three random fish per tank were sacrificed by anaesthesia overdose (phenoxyethanol, 500 ppm). The liver, viscera (including liver and visceral fat) and mesenteric fat were weighed, gut length was measured, while blood and liver were collected for further analysis (see subchapter 2.3). Additionally, two fish per tank at the end of the experiment were sacrificed by anaesthesia overdose and stored at − 20 ◦C for a whole-body proximate composition analysis. Fish for the whole-body analysis were frozen, chopped, lyophilized (Telstar Cryodos, Terrassa, Spain) and homogenized (Retsch ZM200, Haan, Germany). Proximate composition, amino acid composition and fatty acid profile were analyzed, as described in Section 2.1.

The following growth performance and somatic indices were calculated:

- Survival (%) = 100 × final/initial number of fish Weight gain (WG, %) = 100 × (FBW (final body weight, g) –IBW (initial body weight, g))/IBW
- Specific growth rate (SGR,%day− 1) = 100×ln (FBW/IBW) / number of days Daily feed intake (DFI,%body weight day− 1) = number of days' ×total dry feed intake (g)×100/ ((IBW+FBW)×0.5)
- Feed conversion ratio (FCR) = total dry feed intake (g)/weight gain (g)
- Condition factor(CF) = 100×body weight(g)×total length− 3 (cm)
- Hepatosomatic index (HSI) = 100×liver weight(g)/body weight(g)
- Mesenteric fat index (MFI) = 100×perivisceralfat weight(g)/body weight(g)
- Viscerosomatic index (VSI) = 100×viscera weight(g)/body weight(g)
- Relative gut length (RGL) = gut length (cm)/fish total length (cm)
- Nutrient retention efficiency (%) = 100 × (final nutrient quantity in the body (g, wet basis)− initial nutrient quantity in the body (g, wet basis))/nutrient consumed (g, dry basis)

where nutrient can be dry matter, protein, lipid, energy, ash or amino acids.

2.3. Plasma and liver enzyme activities

Blood samples from three fish per tank were taken from the caudal vein with heparin coated syringes and were stored on crushed ice until all samples were collected. Plasma was removed by centrifugation at 3500 ×g for 15 min and stored at − 80 ◦C until analysis. The quantification of plasma glucose, cholesterol, triglycerides, phospholipids and lactate was performed by enzymatic colorimetric methods using commercial kits (BIOSIS Biotechnological Applications L.T.D Greece and Spinreact S.A.U., Spain). Activities of plasma alanine aminotransferase (ALT, EC 2.6.1.2) and aspartate aminotransferase (AST, EC 2.6.1.1) were determined kinetically at 340 nm using commercial kits according to the instructions of the manufacturer (BIOSIS Biotechnological Applications L.T.D, Greece). After sampling, the three livers per tank were stored at − 80 ◦C. A frozen sample of each liver was homogenized using 10 volumes of buffer (4 ◦C) which contained 30 mM HEPES, 0.25 mM saccharose, 0.5 mM EDTA, 5 mM K2HPO4 and 1 mM DTT (pH 7.4). After centrifugation at 1000 ×g for 10 min at 4 ◦C, supernatants were sonicated for 1 min. Following a second centrifugation at 15,000 ×g for 20 min at 4 ◦C, supernatants were collected for the assessment of enzyme activity. Activities of ALT and AST were measured using commercial kits (Spinreact S. A.U., Spain). Glutamate dehydrogenase (GDH, EC 1.4.1.2) activity was assayed as described by Bergmeyer (1974) using 10 mM of L-glutamic acid to a reaction mixture containing 175 mM tris (pH 8.5), 100 mM semicarbazine, 1.1 mM NAD, 1 mM ADP and 5 mM L-Leucine. For the enzymatic activity of glucose-6-phosphate dehydrogenase (G6PD; EC 1.1.1.49), malic enzyme (ME; EC 1.1.1.40) and fatty acid synthetase (FAS; EC 2.3.1.38), liver samples were homogenized using 5 volumes of buffer (4 ◦C) which contained 20 mM Tris-HCl, 0.25 M sucrose, 2 mM EDTA, 0.1 M sodium fluoride, 0.5 mM phenyl methyl sulphonyl fluoride (PMSF) and 10 mM β-mercaptoethanol (pH 7.4). The homogenate was centrifuged at 30,000 ×g for 20 min at 4 ◦C. G6PD activity was measured according to Bautista et al. (1988) by adding 20 mM glucose-6-phosphate to a

reaction mixture containing 1000 mM Tris-HCL buffer (pH 7.8), 200 mM MgCl2, and 10 mM NADP. ME activity was measured according to Ochoa (1955) by adding 15 mM L-malate to a reaction mixture containing 500 mM glycyl-glycine, 50 mM MgCl2, and 10 mM NADP (pH 7.4). FAS activity was assayed by following the method described by Chang et al. (1967) as modified by Chakrabarty and Leveille (1969) by addition of 0.6 mM malonyl-CoA in a reaction mixture of 100 mM potassium phosphate buffer (pH 6.5), 0.1 mM NADPH, and 0.025 mM acetyl-CoA. Enzyme activities were determined at 37 ◦C using a Multiskan GO microplate reader (model 51,119,200; Thermo Scientific, Nanjing, China). Enzyme activities were expressed as nmol of substrate hydrolyzed per min (mU) and per mg of protein.

Extracts' protein concentration was determined using Sigma-Aldrich protein assay kit (B6916), with bovine serum albumin as standard, according to Bradford (1976).

2.4. Statistical analysis

Data were tested for normality with the Kolmogorov-Smirnov test and for equality of variances with the Levene's test. One-way analysis of variance (ANOVA) was performed to determine whether significant differences existed among dietary treatments. Data which did not follow the ANOVA assumptions were analyzed by Kruskal Wallis' tests. The results were considered statistically significant at $p < 0.05$ and individual means were compared using the Tukey's test. Correlational analyses were performed using the Spearman correlation. All statistical analyses were carried out using SigmaStat 3.5 (Systat Software, Inc., San Jose, California, USA).

3. Results

3.1. Growth performance

Survival was similar in all experimental groups, and fish quadrupled their initial weight (29.5 g) during the 93-day feeding trial (Table 3). Fish fed with insect meal diets had similar final body weight compared to the fish meal group ($p > 0.05$, Table 3). However, the inclusion of TM resulted in significantly higher weight gain and SGR (331.3 ± 7.5% and 1.57 ± 0.02% per day, respectively) compared to the HI inclusion (305.9 ± 5.2% and 1.51 ± 0.01% per day, respectively). Insect meals inclusion did not affect the daily feed intake (1.36–1.46% of body weight per day) as well as the feed conversion ratio (1.04–1.12) ($p > 0.05$, Table 3).

Somatic indices (condition factor, hepatosomatic index, viscerosomatic index, mesenteric fat index and the relative gut length) were similar to all fish groups ($p > 0.05$, Table 3).

3.2. Whole-body proximate composition and nutrient retention

The whole-body contents of dry matter, protein, fat, ash and energy were not affected by the partial substitution of fish meal with different insect meals ($p > 0.05$, Table 4). Regarding whole-body amino acid profiles, no statistically significant differences were observed in the essential and non-essential amino acid content of fish fed with the different experimental diets ($p > 0.05$, Table 4). Fatty acid composition of the experimental diets (Table 2) affected significantly the fish whole-body fatty acid content, with differences being observed in the content of 15 of the 19 individual fatty acid methyl esters studied (Table 5). A significant positive correlation between whole-body fatty acid content and feed was observed for 11 out of 19 fatty acids analyzed (Table 5). Additionally, there was also a significant positive correlation between dietary fish oil and whole-body fatty acid content for 11 fatty acids. The HI group had generally similar fatty acid profile compared to fish fed diet FM with the only differences being observed in lauric acid (C12:0) and linolenic acid (C18:2 ω-6), that presented higher contents in HI fed fish. Total saturated fatty acid (SFA) and mono-unsaturated fatty acid (MUFA) content was not affected by the different dietary treatments (22.3–

24.4% and 43.9–47.4%, respectively). Fish fed with the FM and HI diets had higher poly-unsaturated fatty acid (PUFA) content compared to the fish fed with TM and MD diets (32.8–33.5% compared to 27.5–28.1%), which was driven by the significantly higher ω-3 content (17.8–18.6% compared to 12.0–13.5%). The highest oleic acid (C18:1 ω-9) of the TM diet led to a significantly higher oleic acid content in the fish fed with TM (38.0 ± 0.8%). The ω-3 PUFA, EPA (eicosapentaenoic acid C20:5 ω-3) and DHA (docosahexaenoic acid C22:6 ω-3) content of the fish increased over time (higher content than the initial fish) in all experimental groups. However, fish fed diets with higher fish oil inclusion (FM and HI) had a more pronounced increase. Protein retention (Table 6) was similar to all experimental groups (29.3–31.3%; $p > 0.05$). Fish fed with HI had significantly lower dry matter, fat and energy retention compared to the FM group. In addition, the HI group had significantly lower fat retention (57.0%) compared to the fish fed with TM and MD (73.2% and 69.3%, respectively). Dry matter, energy and fat retention were negatively correlated with the crude fiber content of the diets ($r = -0.777$, -0.820 and -0.799, respectively; $p < 0.05$). Moreover, a negative correlation was found between fat retention and diet's ADF content ($r = -0.756$, $p < 0.05$). Ash retention was significantly lower in fish fed with the FM diet (22.4%) and was positively correlated with SGR ($r = 0.699$, $p < 0.05$). The fish meal substitution with insect meals resulted in significant differences in the amino acid body retention/deposition for 10 out of 17 amino acids measured (Table 6). Within the 10 amino acids that exhibited significant differences in body retention/deposition, seven of them (arginine, methionine, phenylalanine, cysteine, glycine, proline and tyrosine) correlated negatively ($p < 0.05$) with the corresponding dietary amino acid content (i.e., the higher the amino acid content in diet, the lower the retention/deposition).

3.3. Plasma metabolites and liver enzyme activities

Plasma glucose, cholesterol, triglycerides, phospholipids, and lactate levels were not affected by the different dietary treatments (Table 7). In addition, plasma ALT and AST activities were similar to all the experimental groups (Table 7). Liver ALT activity was significantly lower in fish fed TM (368.9 ± 5.4 mU mg protein^{-1}) compared to fish fed with HI (457.9 ± 5.5 9 mU mg protein^{-1} ; Table 8). The partial substitution of fish meal with the different insect meals did not affect liver AST and GDH activities. No significant differences were observed in the activities of the multienzyme complex of FAS and the activities of ME and G6PD among the different experimental groups (Table 8).

4. Discussion

4.1. Growth performance

Similar daily feed intake for all the experimental groups with 30% fish meal substitution with insect meals was well accepted by the fish, complying with the known nutritional requirements and diets of gilthead sea bream (Tibaldi and Kaushik, 2005; Wilson, 2003). Insect meal fed fish had similar growth performance compared to the fish fed the fishmeal diet. Piccolo et al. (2017) have reported an improvement of SGR and FCR with 25% TM inclusion in the diet of gilthead sea bream (50% FM in the control diet, 105.2 g initial body weight). In our trial, despite the similar growth performance with the FM group, the inclusion of TM resulted in higher weight gain and SGR compared to HI without affecting FCR and feed intake. The difference observed could be explained by the higher crude fiber and ADF of the HI diet compared to the TM diet (for HI 3.9 and 8.8, respectively; for TM 2.5 and 5.6, respectively) which might decrease nutrient digestibility. In addition, the HI diet had the lowest adjusted crude protein content which could have negatively affected growth performance. Fabrikov et al. (2020) by using TM or HI to replace 15% or 30% of fish meal in the diet of gilthead sea bream (5.1–10.9% inclusion of insect meal, 36.8% FM in the control diet, 6.4 g initial body weight) have reported similar growth performance and FCR. However, these experimental diets had a lower insect meal inclusion compared to the present study and similar crude fiber content (1.3–1.9%).

Tenebrio molitor has been extensively used as a fish meal replacement in literature. In European sea bass (Dicentrarchus labrax), Gasco et al. (2016) have not observed any negative effects on growth performance and feed conversion with 36% fish meal substitution with full fat TM (25% inclusion), while Mastoraki et al. (2020a) have reported an increased FCR with 30% fish meal substitution (19.5% inclusion). It is known that growth performance and feed efficiency are not affected when up to 28% of TM was included in the diets of rainbow trout (Jeong et al., 2020) and yellow catfish (Pelteobagrus fulvidraco; Su et al., 2017). When defatted TM is used, a complete replacement of FM is possible in larger rainbow trout (78.3 g, 20% inclusion; Chemello et al., 2020) without negative effects on growth performance. However, in smaller rainbow trouts (5.01 g; Rema et al., 2019) and juvenile red seabreams (Pagrus major; Ido et al., 2019), the inclusion of 25% and 65% of TM replacing fish meal has completely improved their growth performance.

Regarding the substitution of fish meal with partially defatted Hermetia illucens, our results agree with those of Abdel-Tawwab et al. (2020) and Mastoraki et al. (2020a) reporting 50% and 30% replacement of fish meal (14.8% and 19.5% inclusion, respectively) in European sea bass without any effect on growth performance. Furthermore, HI diets have performed in the same fashion as fish meal diets in rainbow trout (21% inclusion; Cardinaletti et al., 2019) in Nile tilapia (30% inclusion to complete fish meal replacement; Muin et al., 2017), in grass carp (Ctenopharyngodon idellus, 13.4% inclusion; Lu et al., 2020), in Eurasian perch (up to 60% inclusion; Stejskal et al., 2020), and in Siberian sturgeon (up to 18.5% inclusion; Caimi et al., 2020).

To date, this is the first study that reports the effects of Musca domestica inclusion in the diet of gilthead sea bream. In the present study, no differences were observed in growth performance and feed conversion when 30% of fish meal was substituted with MD. Successful fish meal substitution with MD has also been achieved in barramundi (Lates calcalifer, 10% inclusion; Lin and Mui, 2017), Heteroclarias (Clarias × Heterobranchus, 15–50% inclusion; Ekelemu, 2015; Omoruwou and Edema, 2011; Sogbesan, 2014), and in Nile tilapia (33–68% inclusion; Ezewudo et al., 2015; Ogunji et al., 2007; Wang et al., 2017).

Regarding the somatic indices, the present study observed no differences in the hepatosomatic index (values <2%) Liver is the primary metabolic tissue, and hepatosomatic indices exceeding 2% can indicate an impairment of glucose and/or fat metabolism or vitamin deficiency (Chemello et al., 2020). Fish from the different dietary groups of this study had similar viscerosomatic and mesenteric fat indices. Dietary fat has been reported to affect fat storage in the viscera and liver of fish (Huang et al., 2016). Furthermore, when fish meal is substituted with insect meals, the reduction of dietary ω-3 fatty acids and the increase of dietary linoleic and linolenic acids can lead to an imbalance in the ω-3/ ω-6 fatty acid ratio which can result in increased liver fat deposition (Mikołajczak et al., 2020; Xu et al., 2020). In the present study, the dietary fat was similar among the experimental diets, not affecting visceral and liver fat deposition. The lack of differences observed in the fat deposition of the liver and perivisceral cavity was further supported by similar activities of the liver lipogenic enzymes between the experimental groups. Contrary to the study of Piccolo et al. (2017) who have reported significantly higher relative gut length in gilthead sea bream fed diets with 50% inclusion of TM, no differences were observed herein when fish meal was substituted at lower level (19.5%) with different insect meals. Similarly to our study's inclusion level, the same pattern has also been observed in the relative gut length of European sea bass (Mastoraki et al., 2020a).

4.2. Whole-body proximate composition and nutrient retention

Whole-body dry matter, protein, fat, ash, and energy contents of the experimental fish were similar across dietary treatments. Our results are in line with other studies with similar or higher dietary insect meal inclusion; for example, fish meal substitution with HI or MD in barramundi (Katya et al., 2017; Lin and Mui, 2017), with HI in Atlantic salmon (Salmo salar; Belghit et al., 2018) or with TM in European sea bass (Gasco et al., 2016).A general trend of increasing body fat is observed in different studies when fish meal is substituted with insect meals, sometimes accompanied with a decrease in protein content. The higher fat

content observed in insect meal-fed fish is usually explained by the higher dietary content of saturated fatty acids and by the change in the ω-3/ω-6 ratio which can enhance lipogenesis (Alves et al., 2020). On the other hand, insect fat which is rich in medium-chain fatty acids is not stored but readily utilized for energy production (Tocher, 2015), leading to lower body fat contents in salmon and Jian carp (Belghit et al., 2019; Cyprinus carpio var. Jian; Li et al., 2016). In this study, the lack of significant differences in the whole-body fat content was corroborated by the similar lipogenic enzymes activities. The whole-body amino acid profile was not affected by the inclusion of the different insect meals. Similarly, no effect was observed in the muscle of grass and Jian carp both fed HI (Zhou et al., 2018; Lu et al., 2020). Studies have also shown that the inclusion of insect meals does not affect the essential amino acid content of European sea bass fed TM, HI, or MD (Mastoraki et al., 2020a), Jian carp fed silkworm meal (Ji et al., 2015), and rainbow trout fed TM (Jeong et al., 2020). Additionally, Jeong et al. (2020) report no adverse effects on muscle proximate and essential amino acid composition. Major differences were observed in the whole-body fatty acid content driven by the different fatty acid profiles and fish oil levels of the experimental diets. Thus, due to the defatted nature of HI meal used in the present study, higher percentage of fish oil was included in the HI diet to ensure similar lipid and energy contents among the dietary treatments. The inclusion of insect meals did not seem to affect fish total whole-body SFA content, in line with previous reports employing other fish species using TM or MD (Gasco et al., 2016; Iaconisi et al., 2018; Mastoraki et al., 2020a). In contrast, an increasing SFA content was observed in salmon using full fat HI, along with the increase in the HI meal inclusion due to the higher content of lauric (C12:0) and myristic (C14:0) acids of the meal (Bruni et al., 2020). In this study, the wholebody total MUFA content was similar across experimental groups, in agreement withother studies in European sea bass fed TM, HI, or MD (Mastoraki et al., 2020a). On the contrary, higher MUFA content has been reported in the fillets of rainbow trout fed TM (Iaconisi et al., 2018), and lower ones in rainbow trout (Secci et al., 2018) and Eurasian perch (Stejskal et al., 2020) fed HI; this is due to the differences in the respective fatty acid profiles of the insect meals used in these investigations. In our study, higher inclusion of fish oil in the FM and HI diets resulted in higher total ω-3 PUFA, EPA and DHA whole-body content in the respective groups. This fact is also in agreement with Mastoraki et al. (2020a) employing a highly defatted HI meal in the diet of European sea bass. However, the contents of ω-3 PUFA are reported to decline when the inclusion of fish oil is lower due to the use of partially defatted (Stejskal et al., 2020) or full fat insect meals (present study and S´ anchez-Muros et al., 2016). Protein retention was across the experimental groups of this study; however, dry matter and energy retentions were lower in fish fed HI compared to the FM group. Moreover, the HI fed sea breams had the lowest fat retention. Dry matter and energy retentions were found to be negatively correlated ($p < 0.05$) to dietary crude fiber, while fat retention was found to be negatively correlated to dietary crude fiber and ADF. Therefore, the lower fat retention of the HI group could be attributed to the presence of chitin in the ADF fraction which was higher compared to the other diets and it is reported to inhibit fat absorption (Kroeckel et al., 2012) and fatty acid synthesis (Coz-Rakovac et al., 2005). However, in the present study, the liver lipogenic enzymes including FAS were not affected by the dietary treatments. Recently, Panteli et al. (2021) using a 30% substitution of fish meal with HI meal for the diet of gilthead sea bream, have observed a decrease in the abundance of the beneficial bacteria of the phylum Firmucutes in the intestine of the fish fed HI compared to the control group. This decrease could probably be responsible for the reduction in the fatty acid absorption effectiveness of the gut (Panteli et al., 2021) and the lower fat retention; this in turn may explain the decreased fat retention of the HIfed sea breams without any differences in the lipogenic enzymes of the present study. The nutrient utilization of different fish species fed with different insect meals were diversified in this research. The complete replacement of FM with HI (35% inclusion) in African catfish may increase fat retention without affecting protein deposition (Huda et al., 2020), while 26.4% inclusion of HI in the diet of rainbow trout may increase fat retention and decrease protein deposition (Dumas et al., 2018). Enhanced protein retention is observed in rainbow trout fed TM (Rema et al., 2019), whereas the 20% inclusion of TM in mandarin fish (Siniperca scherzeri) is reported to improve protein and fat deposition (Sankian et al., 2018). Several factors can be involved in these

inconsistent effects, such as the species identity and age of the fish involved, the different insect species used, the design and formulation of the diet or even the quality of the ingredients. Moreover, since the production of insect meals is not a standardized process, the different substrates and processing methods of the insects can also affect the insect meal quality (Becker and Yu, 2013; Reyes et al., 2020). In the present study, the inclusion of different insect meals affected the retention/deposition of 10 amino acids out of 17 studied in total. The observed differences in the amino acid depositions could be attributed to the different dietary amino acid content based on the detected negative correlations.

4.3. Plasma and liver enzyme activities

Regarding plasma metabolites, fish meal substitution had no negative effects on plasma glucose and lactate, and the levels observed were in line with the up to date reported values, for unstressed gilthead sea bream (Peres et al., 2013; Rotllant et al., 2001). Cholesterol and triglycerides were higher in the fish fed FM and HI diets (differences not statistically significant though)and the same is true for European sea bass cholesterol levels (Mastoraki et al., 2020a). This trend could be attributed to the higher content of these energetic metabolites in the fish oil (Mastoraki et al., 2020a). Additionally, it has been reported that insect linoleic acid promotes the breakdown of cholesterol and triglycerides (Song et al., 2018). Therefore, the higher linoleic content of the TM and MD diets could have led to lower plasma cholesterol and triglycerides in fish fed with those two insect meals. Fish meal substitution with insect meals in the diets of Japanese sea bass (Lateolabrax japonicus, HI), Korean rockfish (Sebastes schlegeli, TM), mirror carp (Cyprinus carpio var. specularis, hydrolyzed silkworm), African catfish (TM), pearl gentian grouper (Epinephelus lanceolatus × E. fuscoguttatus, TM), European sea bass (HI) and mandarin fish (TM) are reported to result in lower plasma cholesterol and/or triglycerides, an effect attributed to the presence of chitin (Fawole et al., 2020; Khosravi et al., 2018; Li et al., 2017; Magalhaes ˜ et al., 2017; Sankian et al., 2018; Song et al., 2018; Wang et al., 2019; Xu et al., 2018). Increased plasma aminotransferase activities are related to liver-cell function impairment in cases of severe steatosis, and this is positively correlated with the degree of tissue necrosis (Lemaire et al., 1991). Plasma ALT and AST activities did not differ among the studied groups herein. The levels of both aminotransferases were within the reported range for healthy gilthead sea bream (Peres et al., 2013). In the liver, the ALT activity of fish fed HI was significantly higher compared to the TM group, whereas the activities of other amino acid catabolizing enzymes (GDH and AST) were not affected by the dietary treatment. The amino acid catabolizing enzyme activities are positively correlated with growth performance and therefore an increase in the activity can indicate better protein utilization (Kumar et al., 2017; Lin and Luo, 2011). In addition, high dietary protein content promotes amino acid catabolism (Ballantyne, 2001; Fynn-Aikins et al., 1995). On the other hand, a diet rich in fat may decrease the activity of amino acid catabolizing enzymes due to the sparing effect of fat on amino acids used for energy production (Ballantyne, 2001). In the present study, the growth performance exhibited converse results, with the HI group presenting lower SGR compared to TM. Moreover, the HI diet had the lowest protein content and the highest fat content which would have lowered liver ALT activity. A possible explanation for this could be the higher dietary fiber content, which may have lowered even further the digestible energy of a diet with an already lower energy content. A lower energy availability might have led to a higher demand for amino acids so as to be catabolized for energy production (Kumar et al., 2010), and consequently, this may have increased the activity of ALT in the liver of HI-fed fish. Given that the biological significance of the hepatic ALT activity is still unclear, it has been suggested that the substitution of fish meal with defatted TM in rainbow trout (Chemello et al., 2020), with HI in meagre (Guerreiro et al., 2020), as well as hydrolysed TM in sea trout (Hoffmann et al., 2020) do not actually affect the activity of liver amino acid catabolic enzymes. On the contrary, in rainbow trout, the inclusion of TM may result in higher AST activity compared to the inclusion of HI, without affecting ALT and GDH (Melenchon ´ et al., 2020). Lipogenic enzymes' activity can be affected by the level and quality of dietary protein (Alvarez et al., 1998; Dias et al., 2005; Wacyk et al., 2012) and fat (Alvarez et al., 1998; Jordal et al., 2007). Menoyo et al. (2004) have reported a decrease in liver lipogenesis with vegetable oils' replacement (80%) in the diets of gilthead

sea bream. Peng et al. (2017) have attributed the increase in FAS activity of turbot (Scophthalmus maximus) fed plant oils to the increase of the saturated and monounsaturated fatty acid content of the diets. Furthermore, Menoyo et al. (2003) have reported a lowering effect of dietary ω-3 fatty acids on G6PD and ME activity of Atlantic salmon. In a feeding trial with rainbow trout, the dietary inclusion of up to 20% partially defatted TM have led to comparable dietary ω-3 fatty acid contents, resulting in no differences of the lipogenic enzymes' activities (Chemello et al., 2020). Despite the 44% replacement of fish oil by insect meals in the present study, no differences were observed in the liver lipogenic enzyme activities; this may imply an adequate fish oil supply or a non-detrimental effect of insect meal fat at this rate of inclusion.

5. Conclusion

Under the experimental conditions examined herein, all three insect meal diets performed equally well compared to the fish meal diet, in terms of growth performance, feed consumption and feed conversion. However, among the insect meal diets, HI was slightly inferior compared to TM and MD. The whole-body composition was not affected by the different diets. Protein retention was similar to all experimental groups, however fat retention was lower in the fish fed HI probably due to the higher fiber content. Differences were observed in the amino acid depositions among the experimental groups which could be attributed to the differences in the dietary amino acid composition. In addition, the TM and MD majorly affected the whole-body fatty acid composition, due to the lower fish oil inclusion compared to the FM and HI diets. Plasma metabolites and liver lipogenic enzymes were not affected by the different diets. Overall, fish meal can be successfully substituted by Tenebrio molitor, Hermetia illucens and Musca domestica meals in the diets of gilthead sea bream at a rate of 30%.

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TABLES

Table 1
Ingredients and proximate composition of insect meals and experimental diets.

	FM	TM	HI	MD
Ingredients (%)				
Fish meal (Peru, prime)	65	45.5	45.5	45.5
Insect larvae meal	0	19.5	19.5	19.5
Fish oil	9	5	9.7	6.3
Wheat	17.2	16.6	14.8	16.8
Wheat gluten meal	6	9	6	8.5
Vitamin & mineral mix [a]	2.5	2.5	2.5	2.5
DL-methionine	0.3	0.7	0.7	0
L-lysine	0	1.2	1.3	0.9
Proximate composition of the different insect meals (dry basis) [b]				
Crude Protein (%)		61.0	67.0	58.5
Crude Lipid (%)		28.6	5.7	23.1
Ash %		4.1	7.8	7.4
Gross energy (MJ kg^{-1})		26.9	21.4	24.8
Proximate composition of the experimental diets (dry basis) [b]				
Crude Protein (%)	58.0	57.6	55.8	56.6
Adjusted Crude Protein (%) [c]	56.7	55.5	51.9	54.5
Crude Lipid (%)	17.7	16.1	18.1	16
Ash (%)	12.6	9.2	10.9	10.7
Crude fiber (%)	1.7	2.5	3.9	2.9
Acid detergent fiber (%)	6.2	5.6	8.8	7.1
NFE (%) [d]	10.1	14.5	11.3	13.9
Gross energy (MJ kg^{-1})	22.1	22.4	22.0	22.1
EAA (%)				
Arginine	3.04	2.60	2.69	2.80
Histidine	1.07	1.12	1.03	1.12
Isoleucine	2.28	2.19	2.14	2.18
Leucine	4.15	3.97	3.86	3.99
Lysine	3.81	4.19	4.10	4.38
Methionine	1.54	2.09	1.59	1.60
Phenylalanine	2.16	1.92	1.95	2.38
Threonine	2.36	2.21	2.21	2.34
Valine	2.63	2.73	2.59	2.59
NEAA (%)				
Alanine	3.20	3.42	3.12	3.17
Asx	4.13	4.06	3.97	4.70
Cysteine	0.28	0.24	0.23	0.26
Glx	8.32	8.11	7.83	8.97
Glycine	3.01	2.65	2.67	2.61
Proline	2.88	3.07	2.90	2.83
Serine	2.45	2.29	2.33	2.41
Tyrosine	1.52	1.81	1.61	1.91

Abbreviations: FM, Fish meal; TM, *Tenebrio molitor*; HI, *Hermetia illucens*; MD, *Musca domestica*; EAA, essential amino acids; NEAA, non-essential amino acids; Asx, sum of asparagine and aspartate; Glx, sum of glutamine and glutamate.

[a] Premix (kg ^{1}): Choline 90,000 (mg) Vitamin A 0.3 (MIU), Vitamin D3 0.1 (MIU), Vitamin E 20,000 (IU), Vitamin K 1030 (mg), Vitamin B1 390 (mg), Vitamin B 960 (mg), Nicotinic acid 2600 (mg), Pantothenic acid 4400 (mg), Vitamin B6 890 (mg), Vitamin B12 15 (mg), Folic acid 290 (mg), Biotin 14 (mg), Vitamin C (Stay C 35% MONO) 20,300 (mg), Inositol 15,600 (mg),Total Mn 1200 (mg), Total Ca 72,000 (mg), Total Zn 7000 (mg), Total Cu 450 (mg), Total Se 14 (mg), Total I 100 (mg), Betaine (mg) 71,250 (mg), BHA (E320) 3000 (mg).

[b] Mean of triplicate analyses.

[c] Protein adjusted for the nitrogen linked to acid detergent fiber.

[d] Nitrogen-free extract, NFE = 100 - % crude protein - % crude lipid - % ash -

Table 2
Fatty acid composition (% of total fatty acids) of the experimental diets in which 30% of the fish meal (FM) was substituted with *Tenebrio molitor* (TM), *Hermetia illucens* (HI) or *Musca domestica* (MD) larvae meal.

	FM	TM	HI	MD
12:0	0.12	0.14	2.71	ND
14:0	3.61	3.84	4.48	3.21
16:0	13.38	13.20	14.21	16.84
16:1 ω-7	4.81	4.68	4.94	9.08
17:0	0.78	0.87	1.68	1.50
17:1	0.65	0.76	0.63	0.69
18:0	2.08	2.27	2.52	2.93
18:1 ω-9	23.37	26.57	23.64	25.09
18:2 ω-6	8.03	12.10	9.52	13.10
20:1 ω-9	2.03	1.61	1.94	1.86
18:3 ω-3	6.46	4.20	6.15	4.20
21:0	2.04	1.75	1.79	1.14
20:2 ω-6	0.57	0.72	0.59	0.33
22:1 ω-9	0.64	0.42	0.52	0.54
20:3 ω-3	8.29	5.79	7.74	5.01
20:4 ω-6	0.70	0.51	0.58	0.39
22:2 ω-6	5.70	5.46	4.55	3.56
20:5 ω-3	1.43	1.07	1.13	0.85
22:6 ω-3	9.21	7.50	7.26	5.77
SFA	22.85	22.95	28.44	26.51
MUFA	32.76	35.14	32.95	38.87
PUFA	40.72	37.65	37.87	33.50
ω-3	25.39	18.56	22.28	15.82
ω-6	15.33	19.09	15.59	17.68

Abbreviations: SFA, saturated fatty acids; MUFA, mono-unsaturated fatty acids; PUFA, poly-unsaturated fatty acids; ND, not detected.
Means of duplicate analyses.

Table 3
Effect of 30% substitution of fish meal with different insect meals (TM: *Tenebrio molitor*, HI: *Hermetia illucens* or MD: *Musca domestica*) on growth performance and somatic indices of gilthead sea bream.

	FM	TM	HI	MD
Survival (%)	100	99.1 ± 1.0	100	100
IBW (g)	29.7 ± 0.2	29.1 ± 0.6	29.4 ± 0.5	29.8 ± 0.2
FBW (g)	121.4 ± 2.1	125.3 ± 0.8	119.2 ± 2.3	126.6 ± 0.4
WG (%)	308.3 ± 4.3^{ab}	331.3 ± 7.5^{a}	305.8 ± 5.2^{b}	324.8 ± 4.4^{ab}
SGR (% day^{-1})	1.51 ± 0.01^{ab}	1.57 ± 0.02^{a}	1.51 ± 0.01^{b}	1.56 ± 0.01^{ab}
DFI (% BW day^{-1})	1.36 ± 0.03	1.41 ± 0.02	1.46 ± 0.01	1.38 ± 0.02
FCR	1.04 ± 0.03	1.06 ± 0.03	1.12 ± 0.02	1.04 ± 0.01
Somatic indices				
CF	1.55 ± 0.01	1.66 ± 0.02	1.59 ± 0.02	1.61 ± 0.05
HSI (%)	1.24 ± 0.04	1.45 ± 0.06	1.41 ± 0.12	1.40 ± 0.04
VSI (%)	5.40 ± 0.01	5.70 ± 0.10	5.68 ± 0.20	5.66 ± 0.12
MFI (%)	0.90 ± 0.06	0.97 ± 0.04	1.12 ± 0.08	1.01 ± 0.07
RGL	1.69 ± 0.07	1.85 ± 0.03	1.83 ± 0.05	1.77 ± 0.02

Abbreviations: IBW, initial body weight; FBW, final body weight; WG, weight gain; SGR, specific growth rate; DFI, daily feed intake; FCR, feed conversion ratio; CF, condition factor; VSI, viscerosomatic index; HSI, hepatosomatic index; MFI, mesenteric fat index; RGL, relative gut length.
Mean ± standard error, n = 3 tanks per diet. Different letters in the same row denote statistically significant difference ($p < 0.05$).

Table 4
Effect of 30% substitution of fish meal with different insect meals (TM: *Tenebrio molitor*, HI: *Hermetia illucens* or MD: *Musca domestica*) on whole-body proximate and amino acid composition.

	Initial	FM	TM	HI	MD
Dry matter	27.4	33.3 ± 0.3	32.7 ± 0.6	32.5 ± 0.4	32.4 ± 0.4
Crude protein	16.3	17.6 ± 0.1	17.7 ± 0.2	17.8 ± 0.1	17.8 ± 0.0
Crude fat	6.5	11.9 ± 0.1	11.1 ± 0.6	10.4 ± 0.4	10.3 ± 0.3
Ash	4.8	3.4 ± 0.1	3.6 ± 0.1	3.6 ± 0.1	3.6 ± 0.1
Gross energy (MJ kg^{-1})	6.1	8.7 ± 0.1	8.5 ± 0.3	8.3 ± 0.2	8.3 ± 0.1
EAA					
Arginine	3.21	3.03 ± 0.07	3.02 ± 0.11	3.25 ± 0.12	3.12 ± 0.03
Histidine	1.21	1.35 ± 0.05	1.32 ± 0.07	1.49 ± 0.06	1.40 ± 0.04
Isoleucine	2.27	2.15 ± 0.01	2.15 ± 0.09	2.24 ± 0.05	2.19 ± 0.04
Leucine	4.27	3.93 ± 0.04	3.93 ± 0.17	4.06 ± 0.09	4.01 ± 0.06
Lysine	4.80	4.54 ± 0.05	4.58 ± 0.18	4.40 ± 0.01	4.61 ± 0.15
Methionine	1.56	1.53 ± 0.05	1.50 ± 0.06	1.66 ± 0.07	1.55 ± 0.01
Phenylalanine	2.02	2.01 ± 0.08	1.92 ± 0.10	2.18 ± 0.09	2.04 ± 0.06
Threonine	2.63	2.39 ± 0.03	2.41 ± 0.10	2.47 ± 0.06	2.44 ± 0.02
Valine	2.65	2.48 ± 0.01	2.49 ± 0.10	2.57 ± 0.06	2.54 ± 0.04
NEAA					
Alanine	3.62	3.31 ± 0.04	3.29 ± 0.09	3.37 ± 0.03	3.45 ± 0.03
Asx	5.38	5.16 ± 0.08	4.94 ± 0.30	5.12 ± 0.02	5.36 ± 0.10
Cysteine	0.29	0.28 ± 0.01	0.27 ± 0.01	0.30 ± 0.01	0.27 ± 0.01
Glx	7.81	7.15 ± 0.06	7.02 ± 0.35	7.08 ± 0.06	7.38 ± 0.13
Glycine	3.48	3.41 ± 0.24	3.32 ± 0.13	3.85 ± 0.12	3.65 ± 0.17
Proline	2.43	2.24 ± 0.08	2.25 ± 0.05	2.39 ± 0.06	2.35 ± 0.05
Serine	2.51	2.19 ± 0.03	2.21 ± 0.08	2.30 ± 0.06	2.27 ± 0.02
Tyrosine	1.41	1.59 ± 0.08	1.52 ± 0.09	1.78 ± 0.09	1.64 ± 0.05

Abbreviations: EAA, essential amino acids; NEAA, non-essential amino acids; Asx, sum of asparagine and aspartate; Glx, sum of glutamine and glutamate.
Percentage (%) on wet basis unless otherwise stated. Mean ± standard error, n = 3 tanks per diet. No statistically significant differences were observed.

Table 5
Fatty acid composition of whole-body of gilthead sea bream fed diets in which 30% of the fish meal was substituted with different insect meals (TM: *Tenebrio molitor*, HI: *Hermetia illucens* or MD: *Musca domestica*) and correlational analysis with dietary fatty acids and dietary fish oil inclusion.

	Initial	FM	TM	HI	MD	r feed	r fish oil
12:0	0.13	0.06 ± 0.01^{b}	0.06 ± 0.01^{b}	1.00 ± 0.01^{a}	0.08 ± 0.01^{b}	0.389	0.518
14:0	3.66	3.11 ± 0.07^{ab}	2.82 ± 0.13^{ab}	3.49 ± 0.15^{a}	2.66 ± 0.24^{b}	0.605*	0.756**
16:0	17.75	12.90 ± 0.15	15.72 ± 0.45	12.36 ± 0.32	14.98 ± 1.01	−0.086	−0.842***
16:1 ω-7	5.87	6.70 ± 0.10^{c}	5.76 ± 0.11^{d}	6.82 ± 0.07^{bc}	9.30 ± 0.30^{a}	0.907***	0.324
17:0	0.82	1.07 ± 0.03	0.72 ± 0.10	1.00 ± 0.02	1.24 ± 0.34	−0.043	0.367
17:1	1.56	0.79 ± 0.06^{a}	0.45 ± 0.04^{b}	0.62 ± 0.00^{ab}	0.68 ± 0.07^{ab}	−0.626*	0.626*
18:0	4.21	2.24 ± 0.08^{ab}	2.77 ± 0.10^{a}	1.97 ± 0.06^{b}	2.71 ± 0.23^{a}	0.065	−0.885*
18:1 ω-9	43.08	32.36 ± 0.30^{b}	38.04 ± 0.76^{a}	32.87 ± 0.20^{b}	33.89 ± 1.18^{b}	0.734**	−0.626*
18:2 ω-6	2.69	9.04 ± 0.17^{c}	12.63 ± 0.25^{a}	10.52 ± 0.22^{b}	11.66 ± 0.30^{a}	0.799***	−0.756**
20:1 ω-9	0.14	1.92 ± 0.07^{ab}	1.68 ± 0.02^{bc}	2.01 ± 0.06^{a}	1.56 ± 0.06^{c}	0.648*	0.756**
18:3 ω-3	3.37	5.04 ± 0.12^{a}	3.28 ± 0.02^{c}	4.97 ± 0.05^{a}	3.82 ± 0.17^{b}	0.907***	0.842***
21:0	0.87	1.80 ± 0.08^{a}	1.40 ± 0.09^{ab}	1.51 ± 0.08^{ab}	0.71 ± 0.37^{b}	0.907***	0.475
20:2 ω-6	6.76	0.50 ± 0.03	0.23 ± 0.12	0.45 ± 0.03	0.34 ± 0.01	−0.022	0.713**
22:1 ω-9	ND	0.53 ± 0.01^{a}	0.36 ± 0.01^{c}	0.53 ± 0.01^{a}	0.42 ± 0.01^{b}	0.561	0.885***
20:3 ω-3	1.76	4.83 ± 0.11^{a}	2.93 ± 0.03^{b}	4.78 ± 0.02^{ab}	3.54 ± 0.02^{ab}	0.713**	0.842***
20:4 ω-6	ND	1.67 ± 0.98	0.39 ± 0.04	0.68 ± 0.03	0.30 ± 0.12	0.820***	0.734**
22:2 ω-6	1.33	3.36 ± 0.03^{a}	2.06 ± 0.02^{b}	3.19 ± 0.10^{a}	2.16 ± 0.13^{b}	0.475	0.734**
20:5 ω-3	0.77	1.19 ± 0.01^{a}	0.87 ± 0.01^{c}	1.10 ± 0.02^{ab}	1.01 ± 0.05^{b}	0.691*	0.691*
22:6 ω-3	0.72	7.55 ± 0.07^{a}	4.88 ± 0.06^{b}	6.90 ± 0.12^{ab}	5.12 ± 0.32^{ab}	0.600*	0.713**
SFA	28.79	22.28 ± 0.26	24.41 ± 0.87	22.33 ± 0.44	23.41 ± 2.07	0.000	−0.518
MUFA	51.67	43.92 ± 0.52	47.30 ± 0.72	44.44 ± 0.21	47.40 ± 1.52	0.691*	−0.691**
PUFA	17.92	33.46 ± 0.57^{a}	27.50 ± 0.27^{b}	32.77 ± 0.37^{a}	28.14 ± 0.96^{b}	0.756**	0.799***
ω-3	6.62	18.60 ± 0.30^{a}	11.97 ± 0.08^{c}	17.75 ± 0.09^{a}	13.50 ± 0.43^{b}	0.777**	0.777**
ω-6	11.31	14.86 ± 0.87	15.53 ± 0.20	15.02 ± 0.31	13.27 ± 1.13	0.410	−0.626*

Abbreviations: SFA, saturated fatty acids; MUFA, mono-unsaturated fatty acids; PUFA, poly-unsaturated fatty acids; ND, not detected.
Percentage (%) of total fatty acids. Mean ± standard error, n = 3 tanks per diet. Different letters in the same row denote statistically significant difference ($p < 0.05$). In the correlational analysis an asterisk (*) indicates significance at the 0.05 level, ** at the 0.01 level and *** at the 0.001 level.

Table 6
Nutrient retention, essential amino acid retention efficiency, non-essential amino acid deposition and correlational analysis with dietary amino acids of gilthead sea bream fed diets in which 30% of the fish meal was substituted with different insect meals (TM: *Tenebrio molitor*, HI: *Hermetia illucens* or MD: *Musca domestica*).

	FM	TM	HI	MD	r
Dry matter	33.8 ± 0.6^{a}	32.5 ± 0.1^{ab}	30.5 ± 0.4^{b}	32.8 ± 0.6^{a}	nd
Crude protein	29.9 ± 0.6	29.8 ± 1.0	29.3 ± 0.2	31.3 ± 0.4	nd
Crude fat	74.2 ± 1.2^{a}	73.2 ± 3.0^{a}	57.0 ± 2.3^{b}	69.3 ± 2.6^{a}	nd
Ash	22.4 ± 1.3^{c}	33.3 ± 1.4^{a}	26.1 ± 1.1^{b}	29.1 ± 0.8^{a}	nd
Gross Energy	41.5 ± 0.9^{a}	39.3 ± 0.8^{ab}	36.6 ± 0.8^{b}	39.3 ± 0.2^{ab}	nd
EAA					
Arginine	27.8 ± 0.7^{b}	32.0 ± 0.9^{a}	29.2 ± 0.4^{ab}	30.4 ± 0.4^{ab}	−0.756**
Histidine	29.7 ± 0.8	28.1 ± 0.8	28.9 ± 0.4	28.6 ± 0.4	−0.324
Isoleucine	26.3 ± 0.7	26.9 ± 0.8	26.0 ± 0.4	27.6 ± 0.4	−0.043
Leucine	27.1 ± 0.7	28.0 ± 0.8	27.1 ± 0.4	28.4 ± 0.4	0.108
Lysine	33.2 ± 0.8^{a}	29.8 ± 0.8^{b}	28.7 ± 0.4^{b}	29.1 ± 0.4^{b}	−0.453
Methionine	26.6 ± 0.7^{a}	19.3 ± 0.6^{c}	24.0 ± 0.3^{b}	25.8 ± 0.3^{ab}	−0.691**
Phenylalanine	24.7 ± 0.6^{bc}	27.4 ± 0.8^{a}	25.4 ± 0.4^{ab}	22.5 ± 0.3^{c}	−0.907***
Threonine	29.3 ± 0.7	31.0 ± 0.9	29.2 ± 0.4	29.7 ± 0.4	0.000
Valine	26.6 ± 0.7	25.3 ± 0.7	25.0 ± 0.4	27.1 ± 0.4	0.043
NEAA					
Alanine	29.9 ± 0.6^{ab}	27.5 ± 0.8^{b}	28.4 ± 0.4^{ab}	30.3 ± 0.4^{a}	−0.173
Asx	34.3 ± 0.9^{a}	34.4 ± 1.0^{a}	33.1 ± 0.5^{ab}	30.3 ± 0.4^{b}	−0.453
Cysteine	27.0 ± 0.7^{b}	31.1 ± 0.9^{a}	30.9 ± 0.4^{a}	29.6 ± 0.4^{ab}	−0.820***
Glx	24.7 ± 0.6	25.0 ± 0.7	24.4 ± 0.3	23.1 ± 0.3	−0.518
Glycine	30.5 ± 0.8^{c}	34.2 ± 1.0^{ab}	31.9 ± 0.5^{bc}	35.3 ± 0.5^{a}	−0.885***
Proline	22.3 ± 0.6^{ab}	20.6 ± 0.6^{b}	20.5 ± 0.3^{b}	22.8 ± 0.3^{a}	−0.756**
Serine	27.0 ± 0.7	28.4 ± 0.8	26.4 ± 0.4	27.6 ± 0.4	−0.108
Tyrosine	24.3 ± 0.6^{a}	20.2 ± 0.6^{b}	21.4 ± 0.3^{b}	19.6 ± 0.3^{b}	−0.864**

Abbreviations: EAA, essential amino acids; NEAA, non-essential amino acids; Asx, sum of asparagine and aspartate; Glx, sum of glutamine and glutamate; nd, not determined.
Mean ± standard error, n = 3 tanks per diet. Different letters in the same row denote statistically significant difference ($p < 0.05$). In the correlational analysis an asterisk (*) indicates significance at the 0.05 level, ** at the 0.01 level and *** at the 0.001 level.

Table 7
Effect of 30% substitution of fish meal with different insect meals (TM: *Tenebrio molitor*, HI: *Hermetia illucens* or MD: *Musca domestica*) on plasma metabolites of gilthead sea bream.

	FM	TM	HI	MD
Glucose (mg dl^{-1})	93.8 ± 15.8	103.4 ± 11.7	98.2 ± 9.1	112.0 ± 8.4
Cholesterol (mg dl^{-1})	332.6 ± 5.6	279.4 ± 5.2	320.1 ± 22.1	262.9 ± 3.5
Triglycerides (mg dl^{-1})	338.8 ± 26.9	337.6 ± 84.8	466.0 ± 48.2	312.8 ± 6.9
Phospholipids (mg dl^{-1})	887.2 ± 31.1	894.8 ± 12.2	879.8 ± 42.9	877.1 ± 37.8
Lactate (mg dl^{-1})	12.2 ± 0.9	17.4 ± 4.4	15.0 ± 1.0	13.8 ± 2.1
ALT (u l^{-1})	29.8 ± 3.1	19.8 ± 2.7	65.4 ± 26.6	28.6 ± 11.1
AST (u l^{-1})	31.5 ± 5.3	59.5 ± 12.4	56.3 ± 13.9	32.4 ± 2.0

Abbreviations: ALT, Alanine aminotrasferase; AST, Aspartate aminotransferase. Mean ± standard error, n = 3 tanks per diet. Different letters in the same row denote statistically significant difference ($p < 0.05$).

Table 8
Effect of 30% substitution of fish meal with different insect meals (TM: *Tenebrio molitor*, HI: *Hermetia illucens* or MD: *Musca domestica*) on liver amino acid catabolism and lipogenic enzymes of gilthead sea bream.

	FM	TM	HI	MD
Alanine aminotrasferase (ALT)	432.4 ± 19.6^{ab}	368.9 ± 5.4^{b}	457.9 ± 25.5^{a}	432.6 ± 6.9^{ab}
Aspartate aminotransferase (AST)	1539.9 ± 8.3	1570.3 ± 52.9	1656.5 ± 85.8	1688.6 ± 54.4
Glutamate dehydrogenase (GDH)	65.7 ± 2.5	64.0 ± 2.8	61.7 ± 1.6	66.0 ± 2.1
Glucose-6-phosphate dehydrogenase (G6PD)	136.1 ± 4.4	163.5 ± 14.9	143.3 ± 10.4	167.9 ± 7.9
Fatty acid synthase (FAS)	17.1 ± 2.9	22.5 ± 1.7	21.8 ± 1.7	22.1 ± 1.0
Malic enzyme (ME)	9.8 ± 1.3	11.8 ± 1.3	11.6 ± 0.4	12.9 ± 0.5

Expressed as mU mg $protein^{-1}$ (nmoles min^{-1} mg $protein^{-1)}$. Mean ± standard error, n = 3 tanks per diet. Different letters in the same row denote statistically significant difference ($p < 0.05$).