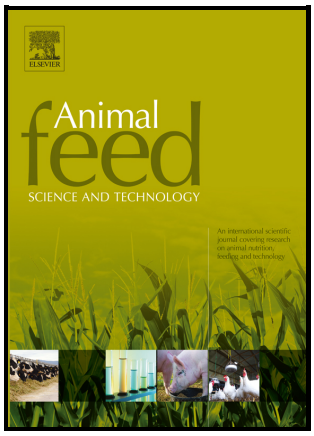
Nutrient digestibility of diets containing five different insect meals in gilthead sea bream (Sparus aurata) and European sea bass (Dicentrarchus labrax)

Journal Pre-proof



Nutrient digestibility of diets containing five different insect meals in gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*)

Maria Mastoraki, Nikolas Panteli, Yannis P. Kotzamanis, Laura Gasco, Efthimia Antonopoulou, Stavros Chatzifotis

PII: S0377-8401(22)00223-1

DOI: https://doi.org/10.1016/j.anifeedsci.2022.115425

Reference: ANIFEE115425

To appear in: *Animal Feed Science and Technology*

Received date: 20 February 2022
Revised date: 7 July 2022
Accepted date: 26 August 2022

Please cite this article as: Maria Mastoraki, Nikolas Panteli, Yannis P. Kotzamanis, Laura Gasco, Efthimia Antonopoulou and Stavros Chatzifotis, Nutrient digestibility of diets containing five different insect meals in gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*), *Animal Feed Science and Technology,* (2022) doi:https://doi.org/10.1016/j.anifeedsci.2022.115425

Nutrient digestibility of diets containing five different insect meals in gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*)

Maria Mastoraki[a,b], Nikolas Panteli[a], Yannis P. Kotzamanis[c], Laura Gasco[d], Efthimia Antonopoulou[a,*], Stavros Chatzifotis[b,*]

[a]Laboratory of Animal Physiology, Department of Zoology, School of Biology, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece. mmastora@bio.auth.gr; nkpanteli@bio.auth.gr; eantono@bio.auth.gr

[b]Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Centre for Marine Research, Gournes Pediados, P.O. Box 2214, 71003 Heraklion, Crete, Greece. stavros@hcmr.gr

[c]Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Centre for Marine Research,46.7 km Athens-Sounion Avenue Anavyssos, Attiki 19013, Greece. jokotz@hcmr.gr

[d]Department of Agricultural, Forest and Food Sciences, University of Turin, Largo P. Braccini 2, 10095 Grugliasco, TO, Italy. laura.gasco@unito.it

[*] Corresponding author:

Efthimia Antonopoulou, Laboratory of Animal Physiology, Department of Zoology, School of Biology, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece, +302310998563, eantono@bio.auth.gr

Stavros Chatzifotis, Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Centre for Marine Research, Gournes Pediados, P.O. Box 2214, 71003 Heraklion, Crete, Greece +302810337873. stavros@hcmr.gr

Abstract

In the recent years, insect meals have been studied as alternative feed ingredients for aquafeeds, due to their adequate nutrient composition and low ecological footprint. These studies involve nutrient digestibility measurements, as they provide valuable information on the ability of fish to utilize insect meal efficiently. In this context, the current study evaluated the nutrient digestibility in European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) fed diets with five different insect meals . For this investigation, diets including 19.5% of each insect meal from yellow mealworm (*Tenebrio molitor* – TM), black soldier fly (*Hermetia illucens* – HI), common housefly (*Musca domestica* – MD), super worm (*Zophobas morio* – ZM) or lesser mealworm (*Alphitobius diaperinus* – AD) and a control fish meal (FM) diet were tested. In European sea bass, TM and MD diets showed similar dry matter, protein, fat, energy, and organic matter ADCs among each other as well as with the FM diet ($P > 0.05$). However, adjusted protein ADC of TM and ZM diets (93.4 – 93.6%) were significantly higher ($P < 0.05$) compared to the FM diet (91.9%). Fat ADC was similar in all dietary groups (88.6 - 92.4%; $P > 0.05$). The HI diet exhibited significantly lower dry matter, protein and organic matter ADCs compared to the FM diet ($P < 0.05$). Moreover, the AD diet presented significantly lower digestibility coefficients of dry matter, organic matter and energy than the FM diet ($P < 0.05$). The dietary contents of crude fiber and ash, and the inclusion of plant feedstuffs correlated negatively with various ADCs ($P < 0.05$). Regarding gilthead sea bream, the ADCs of dry matter, organic matter, protein, adjusted protein and energy were not affected by the different dietary treatments ($P > 0.05$). Fat ADC of the TM diet (73.6%) was significantly lower compared to the other five diets (78.9-84.3%; $P < 0.05$). The individual amino acid ADCs values were found to be very high in all experimental diets (90.9 – 98.0% for European sea bass and 88.9 – 97.2% for gilthead sea bream). In sea bass, the variation in crude protein and adjusted protein ADCs was strongly reflected on the individual amino acid ADCs. Nevertheless, the "true protein digestibility" as expressed by the sum of amino acids was not affected by the insect inclusion compared to the FM diet ($P > 0.05$), while a significant difference was observed between the ZM and HI diets (95.9% and 94.1% respectively; $P < 0.05$). In sea bream, the sum of amino acids and the individual amino acid ADC values did not differ among the experimental diets ($P > 0.05$) except for methionine ($P < 0.05$).

Conclusively, in sea bream all diets exhibited similar overall digestibility. In sea bass, compared to the FM diet, TM and MD diets had similar or even slightly better digestibility, whilst diets HI and AD presented lower overall digestibility.

Keywords: amino acid digestibility, mealworm, black soldier fly, housefly, super worm, lesser mealworm

Abbreviations: Fish meal, FM; *Tenebrio molitor*, TM; *Hermetia illucens*, HI; *Musca domestica*, MD; *Zophobas morio*, ZM; *Alphitobius diaperinus*, AD; Acid Detergent fiber, ADF.

1. Introduction

Nowadays, the aquaculture industry is facing an economic and ecological pressure, which is constantly and progressively increasing due to the sustainability concerns and availability issues of natural marine resources (Hua, 2020). Therefore, research is confronted with a paradox in which aquaculture is presented as a solution to ensure food abundance, while the extended use of fish meal (FM) and fish oil is aggravating the global pressure put on the already over-exploited fish stocks. For this reason, the research around the use of new alternative protein and oil sources for the aquaculture industry is expanding. Initially, studies had been focused on the use of plant or vegetable feedstuffs, especially soybean meals (Daniel, 2018), and animal by-products such as bone, meat and blood meals (Hodar et al., 2020). However, the presence of unknown active compounds, anti-nutritional factors, deficiency in some essential amino acids (Francis et al., 2001)~~Kokou and Fountoulaki, 2018~~, low palatability (Jingting et al., 2020) and the enteritis or inflammation (Merrifield et al., 2011; Gai et al., 2012) associated with plant ingredients have restricted their use as fish feed ingredients.

The utilization of insect meals in aquafeeds has gained great interest over the past years and the recent research shows promising results. The inclusion of insect meals in the aquafeeds has been found to not only maintain similar performance to that of FM based diets but even enhance fish

growth (Belforti et al., 2015; Piccolo et al., 2017; Ido et al., 2019; Rema et al., 2019; Stejskal et al., 2020). When a new ingredient is evaluated for its use in aquafeeds, it is advisable to assess its nutritional value for a given species by measuring its digestibility and therefore the species' ability to utilize efficiently the new ingredient. In gilthead sea bream (*Sparus aurata*), a 25% inclusion of yellow mealworm meal (*Tenebrio molitor* – TM) did not affect the apparent digestibility coefficients of dry matter, crude protein, and ether extract, while when TM was included at 50%, a decrease in digestibility was reported, probably due to the increased dietary chitin (Piccolo et al., 2017). In a comparative study, using both TM and black soldier fly meal (*Hermetia illucens* – HI) in gilthead sea bream, Fabrikov et al. (2020) highlighted that the higher chitin content of HI led to lower protein digestibility compared to TM and the FM diet. The improved digestibility coefficients observed in the TM diets, when compared to the HI diets in European sea bass (*Dicentrarchus labrax*) were also attributed to the lower chitin content of TM (Basto et al., 2020). However, to our knowledge, a digestibility study with the common housefly (*Musca domestica* – MD) has not yet been conducted for sea bass and sea bream. Nevertheless, according to Mastoraki et al. (2020) the inclusion of 19.5% MD meal in European sea bass did not affect the growth performance, the whole body composition and the nutrient retention. Concerning *Zophobas morio* (ZM) inclusion at 7.5% (25% FM substitution) acted beneficially and decreased the feed conversion ratio of Nile tilapia (*Oreochromis niloticus*), while an increase in the inclusion of ZM (30% to completely replace FM) lowered the specific growth rate (Jabir et al., 2012a). On the other hand, Hosseini Shekarabi et al. (2021) using diets with 22% inclusion of defatted ZM, reported inferior specific growth rate and feed conversion ratio in rainbow trout (*Oncorhynchus mykiss*), due to the lower ZM digestibility. In fact, the authors observed a significant decline in dry matter, protein and lipid digestibility when inclusion was only 5.5% (Hosseini Shekarabi et al., 2021).

It becomes clear that several drawbacks have been identified regarding the digestibility of insect meals. Insect larvae contain about 1-10% of acid detergent fiber, depending on the insect species, which consists of chitin and cuticular proteins (Finke, 2002; Finke, 2013; Rumpold and Schlüter, 2014). Chitin solubility is limited and could decrease both the water retention of the intestinal content and gut evacuation time and therefore reduce the efficiency of digestive enzymes on

the substrates (Tharanathan and Kittur, 2003; Sinha et al., 2011). In addition, dietary fiber can bind to protein and minerals, thus reducing the bioavailability and digestibility of these nutrients (Anvo et al., 2017). Finally, chitin consumption has been associated with decreased bile acid levels and therefore inefficient fat absorption (Hansen et al., 2010). However, chitosan, the derivative of chitin, has been found to act as a bacteriostatic, antioxidant, and anti-inflammatory agent as well as an active growth promoter (Abdel-Ghany and Salem, 2020). Despite the limitations arising from the chitin content, the incorporation of insect meal in aquafeeds is beneficial due to their more than sufficient nutritional value and favourable ecological footprint (Rumpold and Schlüter, 2013).

The present study aims to evaluate and compare the apparent digestibility coefficients of key nutrients (dry matter, protein, fat, amino acids) and energy of diets that included 19.5% of insect meals from *Tenebrio molitor* (TM), *Hermetia illucens* (HI), *Musca domestica* (MD), *Zophobas morio* (ZM) or *Alphitobius diaperinus* (AD) for European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*). As far as we know, this is the first study assessing the effects of *Zophobas morio* and *Alphitobius diaperinus* on nutrient and energy digestibility in diet of European sea bass and gilthead sea bream.

2. Materials and methods

The digestibility trials were conducted at the Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC) of the Hellenic Centre for Marine Research (Heraklion, Greece) by FELASA accredited scientists (functions A-D), following the European Directive 2010/63/EU.

Whole freeze-dried larvae from TM, ZM and AD were obtained from Kreca Ento-feed (Ermelo, the Netherlands), dried larvae from MD were acquired from Reptilia Nostra (Athens, Greece) and partially defatted HI meal was purchased from Hermetia Deutschland GmbH & Co. KG (Baruth/Mark, Germany). The larvae were finely ground in a knife mill (Grindomix GM200, Retsch GmbH, Haan, Germany. Six experimental diets were formulated for each fish species; the control diet (FM) in which fish meal was the main protein source (650 g/kg) and five diets which incorporated 195 g/kg of insect meals to replace 30% of the fish meal (Table 1). Celite was added as an external

inert marker (10 g/kg). Celite is an acid-washed, diatomaceous silica powder, which is widely used as an external digestibility marker due to its indigestible, non-toxic and completely inert nature. Using celite (the acid insoluble ash method - AIA) for the digestibility assessments has many advantages such as the low cost and ease of measurement (Goddard and McLean, 2001). The AIA method was chosen over the chromium oxide method because the latter is suspected to cause disturbance in the digestion and to move through the gut separately from the digesta (Jobling, 2001).

The inclusion of the secondary ingredients (fish oil, wheat meal, wheat gluten) was adjusted to achieve similar protein and energy content among the experimental diets (for each fish species). Moreover, experimental diets were supplemented with DL-methionine and L-lysine to ensure a similar amount of essential amino acids amongst the different diets for each fish species. After a thorough mix of the ingredients ~~by hand~~, water was added to the mixture (approximately 2:1) to obtain the preferred consistency. Subsequently, the mixture was pelletized using a mincing machine (4 mm die), the pellets were oven-dried at 40 °C for 24 h and stored in a freezer at -20 °C until used. Juvenile *D. labrax* and *S. aurata* were obtained from the IMBBC hatchery. Two separate trials were conducted, one for each fish species. Open-circulation borehole water, with renewal of 200% per hour, provided adequate levels of dissolved oxygen and the temperature was maintained constantly at 20.2 ± 0.4 °C for the *D. labrax* and 19.6 ± 0.2 °C for the *S. aurata* trial. Eighteen groups of 15 fish (average individual weight of 35.1 ± 8.2 g for *D. labrax* and 49.9 ± 0.7 g for *S. aurata*) were randomly distributed in 250 l cylindroconical indoor tanks (one group per tank), equipped with a settling column. For acclimatization to the diets, fish were fed by hand until apparent satiation, three times daily, for three weeks. Apparent satiation was achieved by terminating the feeding when the feeding activity became lengthier and the pellets remained uneaten at the bottom of the tank. During the experimental trial, fish were fed three times a day (until apparent satiation). After the last feeding, any uneaten pellet was collected in the settling columns. The columns were removed, cleaned thoroughly and readjusted to the tanks until the next morning. Before the first daily feeding, faeces were collected from the settling columns each morning, centrifuged at 2,000 g for 10 min (Megafuge 1.0, Heraeus Instruments, GmBH, Hanau, Germany). Then the faeces were pooled immediately from each tank and the new fecal material was added to the container which contained the previously

collected material per tank and stored back to a freezer (-20oC). At the end of the trial, faeces were freeze-dried for 48 h (Telstar Cryodos, Terrassa, Spain).

Diets and faeces were analyzed for dry matter and ash according to AOAC (1990; methods 934.01 and 942.05 respectively). Crude lipids were determined according to Folch et al. (1957) by chloroform - methanol extraction. Crude protein was assessed using a nitrogen analyzer (FP-528, Leco corporation, St. Joseph, Michigan, USA) employing the 6.25 conversion factor to calculate the % crude protein. Energy was measured using a bomb calorimeter (6300, Parr Instrument Company, St. Moline, Illinois, USA). For the analysis of the ash-free acid detergent fiber (ADF), the samples were boiled in an acid solution using Fibretherm (C. Gerhardt GmbH & Co., Königswinter, Germany) and the ash content of the product was subtracted (Goering and Van Soest, 1979). In addition the nitrogen linked to ADF was measured by analyzing the nitrogen content of the boiling product of the previous analysis using a nitrogen analyzer (Goering and Van Soest, 1979). The crude fiber of the diets was determined by defatting the samples with petroleum ether, sequential boiling with H_2SO_4 and KOH solutions using Fibretherm and subtracting the products' ash content. Celite content in diets and faeces was determined with the acid insoluble ash (AIA) method by boiling the samples in a HCl solution as described by Vogtmann et al. (1975). Chitin in insect meals was estimated by subtracting the acid detergent insoluble protein (ADIN x 6.25) from the ash-free ADF (Marono et al., 2015). Crude protein was adjusted for the ADIN, by subtracting the ADIN from the crude nitrogen and then multiplying by 6.25, to correct for non-protein nitrogen such as chitin and for the indigestible protein attached to lignin and chitin (Goering and Van Soest, 1970; Bernard et al., 1997; Finke, 2007). Organic matter was determined by subtracting the crude ash content from the total dry matter. The amino acid composition of fish and diets were analyzed after acid hydrolysis (6 N HCl, 110 °C, 22 h) and derivatization by AccQ-Tag™ Ultra (Waters Corporation, Milford, MA, U.S.A.). UPLC was performed on an Acquity system (Waters Corporation, Milford, MA, U.S.A.) with DL-Norvaline as an internal standard, as described in Kotzamanis et al. (2020).

Apparent digestibility coefficients (ADCs) of nutrients and energy of the diets were determined by the following formula:

Apparent Digestibility Coefficient (ADC) = 100 - 100 x (AIA in the diet/ AIA in faeces) x (nutrient or energy in faeces/ nutrient or energy in the diet)

Data were tested for normality and equality of variances with Kolmogorov-Smirnov and Levene's tests, respectively. One-way analysis of variance (ANOVA) was performed to determine if significant differences existed among the dietary treatments (results were considered statistically significant at $P < 0.05$), while individual means were compared using the post-hoc Tukey's test. Correlations were performed using the non-parametric Spearman correlation analysis. All statistical analyses were carried out using SigmaStat 3.5 (Systat Software, Inc., San Jose, California, USA).

3. Results

The nutrient ADCs of the experimental diets in European sea bass and gilthead sea bream are presented in Table 2. Regarding European sea bass, the partial substitution of FM with insect meals from TM and MD did not affect dry matter ADC (76.6 – 77.4%, $P > 0.05$). However, the dietary inclusion of HI, ZM and AD resulted in significantly lower dry matter ADC (69.9 – 73.1%, $P < 0.05$). The ZM diet showed significantly higher protein ADC (93.2 ± 0.3%) compared to FM diet (91.6 ± 0.1%, $P < 0.05$), while the lowest protein ADC (89.6 ± 0.5%) was observed in HI diet ($P < 0.05$). Protein ADC was significantly increased when protein in diets and faeces was adjusted for the nitrogen in ADF (adjusted protein ADC 91.1 – 93.6% compared to protein ADC 89.6 – 91.5%, $P < 0.05$). In specific, protein ADC of TM and ZM diets were significantly higher compared to FM ($P < 0.05$), while HI was similar to FM diet ($P > 0.05$). The ZM was the only insect diet that had similar ADF ADC to FM diet ($P > 0.05$), while a significant reduction was observed in the other four diets ($P < 0.05$). Energy ADC was significantly lower in ZM and AD diets (82.1 – 82.3%) compared to FM, TM, and MD diets (85.1 – 86.6%, $P < 0.05$). Finally, organic matter ADC of the FM diet was significantly higher (83.8 ± 0.3%) compared to HI, ZM and AD diets (78.4 – 79.9%, $P < 0.05$). The content of crude fiber in the diets was negatively correlated with the ADCs of dry matter, fat, energy, and organic matter ($r = -0.843$, -0.756, -0.762 and -0.881 respectively, $P < 0.05$). Protein and adjusted protein ADC showed a negative correlation with dietary ash content ($r = -0.536$ and -0.643 respectively, $P < 0.05$). Plant inclusion in the diets also showed a negative correlation with dry matter,

fat, energy and organic matter ADCs (r = -0.473, -0.498, -0.530 and -0.51 respectively, P < 0.05). The ADC of individual amino acids among the experimental diets differed in 13 out of the 18 amino acids studied here (P < 0.05). Moreover, in European sea bass the ADC of individual amino acids showed a significant positive correlation (P < 0.05) with the adjusted protein ADC in all amino acids except taurine. The ADC of the sum of amino acids was significantly higher in the ZM diet (95.9 ± 0.2%) compared to HI diet (94.1 ± 0.6%, P < 0.05), while the rest of the diets exhibited similar total amino acid ADC among each other (P > 0.05). Lastly, the dietary amino acid content affected positively (P < 0.05) the corresponding amino acid ADC in seven out of the 13 amino acids that exhibited significant differences.

In gilthead sea bream, the ADCs of dry matter, protein, adjusted protein, energy and organic matter were similar among the experimental diets (P > 0.05). Removal of the nitrogen linked to ADF from feed and faeces resulted in significant increase of the protein ADC (adjusted protein ADC 89.6 - 92.4% and protein ADC 89.0 - 91.5%, P < 0.05). The fat ADC of TM diet (73.6 ± 0.7%) was significantly lower compared to all the other diets (78.9 - 84.3%, P < 0.05), while HI, MD, ZM and AD diets had similar fat ADCs among each other as well as with FM diet (P > 0.05). The substitution of FM with insect meals did not affect ADF ADC (P > 0.05). However, among the insect diets, ZM had the highest ADF ADC (P < 0.05). Dietary ADF content was positively correlated with dry matter and energy ADC (r = 0.567 and 0.536 respectively, P < 0.05). The individual amino acids ADCs among the experimental diets was similar (P > 0.05), except for methionine. The ADC of the latter appeared to be positively correlated with the adjusted protein ADC (r = 0.556, P < 0.05), and no correlation was observed with dietary methionine content (r = 0.445, P > 0.05). The ADC of the sum of amino acids was similar among the different diets (P > 0.05).

4. Discussion

The present study highlights the differentiation in digestibility of innovative insect meal diets in a species-specific manner. In European sea bass, TM and MD diets had similar or even slightly better digestibility than FM, due to the higher adjusted protein ADCs while the other coefficients

were similar. The ZM diet had lower dry matter ADC yet higher protein and adjusted protein ADCs in comparison to FM. On the contrary, diets HI and AD presented inferior overall digestibility values compared to FM based diet.

Dry matter, fat, organic matter and energy ADCs exhibited negative correlation with the dietary plant inclusion. It has been found that diet extrusion can improve complex carbohydrates' bioavailability (e.g. starch) and increase diet digestibility (Anvo et al., 2017; Caimi et al., 2020). All the experimental diets in the present study were not extruded and the incorporation of plant feedstuffs, which contained complex carbohydrates, may have reduced their nutrient digestibility. Furthermore, it has been reported that dietary ash can adversely affect protein digestibility (Robaina et al., 1997). Following 20% inclusion of insect meals in the diet of European sea bass, Basto et al. (2020) observed an improved dry matter, protein, energy and fat digestibility in the TM diets compared to the HI, whereas locust inclusion led to a lower digestibility. Those authors attributed their results to the higher ash and chitin content of HI meal and to the higher ADF, crude fiber and ash of locust meal (Basto et al., 2020). The inclusion of grasshopper meal (*Zonocerus variegatus*) up to 16.4% had no negative effects on nutrient digestibility, while further inclusion resulted in reduced dry matter, protein, and fat digestibility of African catfish (*Clarias gariepinus*) due to the increased incorporation of digestion resistant components, such as ash and chitin (Alegbeleye et al., 2012). The reduction of protein and energy digestibility of diets with 15% MD in Nile tilapia , as well as the reduction of fat and dry matter digestibility of diets with 30% MD, were also attributed to the high ash content of MD diets (Ogunji et al., 2008). In the present study, the differences observed in protein and adjusted protein ADCs in European sea bass could be, to some extent, due to the dietary ash content as indicated by their negative correlation coefficients. In addition, dietary crude fiber was negatively correlated with the ADCs of dry matter, fat, energy and organic matter. The lower dry matter, protein, and fat digestibility of red tilapia (*Oreochromis* spp.) fed with 30% ZM was attributed to the low protein content and the poor amino acid composition of ZM meal (Jabir et al., 2012b). In the present study, the incorporation of insect meals did not affect the amino acid composition of the diets and therefore the observed differences are not likely to be a result of the amino acid differences.

According to literature, the reduction of digestibility is greatly related to the presence of chitin. An *in vitro* digestibility experiment showed that insects chitin is a major factor in protein digestibility reduction (Marono et al., 2015). In the diets of hybrid tilapia (*O. niloticus* x *O. aureus*), dry matter digestibility showed a reduction as the inclusion of chitin was increased (Shiau and Yu, 1999). Chitin digestion could assist in alleviating its effects on digestibility. It has been found that lysozyme, an antimicrobial enzyme which is present in all fish species and forms part of their innate immune system, can use chitin as a substrate (Fines and Holt, 2010), although an effective chitin digestion requires the presence of chitinase and chitobiase (Henry et al., 2015). Some studies confirmed the presence of chitinase on stomach and gut mucosa, pyloric caeca and pancreas of some fish species (Fontes et al., 2019), for example in Atlantic cod (*Gadus morhua*; Danulat and Kausch, 1984) and cobia (*Rachycentron canadum*; Fines and Holt, 2010). Nevertheless, the ability to hydrolyse chitin is limited or absent in most fish species. Fish include chitin in their natural diet through consumption of crustaceans as well as insects in the case of freshwater fish. However, chitin fibers in both crustaceans and insects are arranged in the cuticles by forming bonds with protein, minerals, fat or other compounds thus reducing the accessibility of digestive enzymes to these nutrients as described in Henry et al. (2015). According to previous studies, dietary chitin resulted in the reduction of dry matter, protein and energy digestibility of insect meal diets in Nile tilapia (30% MD inclusion; Ogunji et al., 2009) and meagre (*Argyrosomus regius*, 10-30% HI; Guerreiro et al., 2020), lower protein, fat and amino acids digestibility in Atlantic salmon (*Salmo salar*, 60% HI; Belghit et al., 2018), reduced fat digestibility in rainbow trout (20% HI; Dumas et al., 2018) and lower protein digestibility in Siberian sturgeon (*Acipenser baerii*, 18.7-37.5% defatted HI; Caimi et al., 2020) and tench (*Tinca tinca*, 5% TM; Fabrikov et al., 2020). However, it is important to emphasize that chitin content may differ among insect species as well as within species depending on the developmental stages (Erdogan and Kaya, 2016). Higher dry matter, protein, adjusted protein, energy and chitin digestibility were observed in diets with beetles' insect meals (TM and ZM) compared to insect meals from cockroaches and crickets (*Nauphoeta cinerea*, *Gromphadorhina portenosa* and *Gryllus assimilis*) in Nile tilapia, due to the lower chitin content (Fontes et al., 2019).

Despite the differences observed in the present study on the nutrient digestibility of HI diet in European sea bass, it could be suggested that the digestibility decrease was very small (4.3%, 0.9% and 3.9% decrease in dry matter, adjusted protein and organic matter ADCs compared to FM diet) and could not affect growth performance. In addition, the coefficients of variation of the nutrient ADCs between diets HI and FM were very low, ranging from 1% for protein and adjusted protein to 4% for dry matter digestibility. Indeed, in a three-month growth trial with a similar diet design in European sea bass, fish fed the HI diet performed equally well as the FM group (Mastoraki et al., 2020). Therefore, it is likely that the growth performance parameters of European sea bass fed diets with 19.5% inclusion of ZM or AD would be similar to the FM group if they were tested in a growth trial, given that herein the protein and adjusted protein digestibility were similar (AD) or higher (ZM) compared to the FM diet.

Regarding gilthead sea bream, the ADCs of dry matter, organic matter, protein, adjusted protein and energy were not affected by the different dietary treatments. In accordance with our results, the incorporation of insect meals did not alter nutrient digestibility in carp (*Cyprinus carpio*, 30% MD; Ogunji et al., 2009), European sea bass (19.5% HI; Magalhães et al., 2017), Nile tilapia (43% MD for total fishmeal replacement; Wang et al., 2017), rainbow trout (up to 50% TM or up to 40% HI; Belforti et al., 2015; Renna et al., 2017; Rema et al., 2019; Chemello et al., 2020; Fabrikov et al., 2020), Atlantic salmon (30% inclusion of HI in low FM diets; Fisher et al., 2020), and Siberian sturgeon (30% HI; Rawski et al., 2020). In addition, dry matter, protein, and fat digestibility in gilthead sea bream was similar between the FM diet and the diet with 25% TM (Piccolo et al., 2017). However, a higher dietary inclusion level resulted in decreased digestibility, due to the increased chitin content which acted dose-dependently (Piccolo et al., 2017). Furthermore, protein digestibility of diets in which FM was substituted with TM was higher compared to the HI diets in gilthead sea bream, probably due to the higher chitin content of HI (Fabrikov et al., 2020).

In contrast to our results, Piccolo et al. (2017) feeding larger size gilthead sea breams (86.97 g) in higher water temperature (22 ± 1°C), observed higher dry matter and fat digestibility of the FM diet (84.3%, and 87.8% respectively) compared to the present results (71.07% and 80.59% respectively), while the protein digestibility herein was higher (90.26% in the present study, 85.5% in Piccolo et al.,

2017). The observed differences could be due to the different fish size, the different experimental conditions, the protocol of collection and processing of faeces and the quality of the dietary ingredients (Hua, 2020). Despite the low values of fat and energy digestibility (83.2% and 82.7% respectively) observed in sea bream, the results are in agreement with a previous study in which gilthead sea bream was fed with a diet which also contained 650 g/kg FM (83.4% and 82.3% respectively; Davies et al., 2009).

Herein, fat ADC of the TM diet was significantly lower compared to the other five diets. Taurine has been reported to contribute to bile salts formation and facilitate fat digestion (Gómez-Requeni et al., 2004). However, in the present study, a correlation between fat ADC and dietary taurine content was not found probably due to the low taurine concentration of diets (Kotzamanis et al. 2020). The reduced fat digestibility observed in turbot (*Psetta maxima*) fed HI was attributed to the higher content of the HI diets in saturated fatty acids and chitin (Kroeckel et al., 2012). Dietary fatty acid composition, especially the ratio of saturated, mono-unsaturated and poly-unsaturated fatty acids can affect fat digestibility. Individual fatty acids digestibility depends on the level of saturation, the carbon chain length and the melting point (Basto et al., 2020; Hua, 2020). Higher digestibility is observed in unsaturated fatty acids, while saturated fatty acids have reduced digestibility, especially when their carbon chain length is increased (Panini et al., 2017). Moreover, it has been reported that fish lipase has higher affinity to long-chain poly-unsaturated fatty acids, which increases their absorption (Magalhães et al., 2017). On the contrary, chitin has great ability to form ionic bonds with fat and bile, which reduces their hydrolysis by lipase and therefore, reduces fat digestibility (Piccolo et al., 2017). Additionally, chitin consumption reduces bile salts formation and by extension fat digestibility, given that bile acid is essential for the activation of lipase and efficient fat absorption (Piccolo et al., 2017). According to the above, a reduced fat digestibility was expected in HI and MD diets due to their higher chitin (Table 1) and saturated fatty acids content (Mastoraki et al., 2020) compared to TM and FM diets. However, no such observations were noted herein and the reason for the reduced fat digestibility of the TM diet is unclear.

Significantly higher ADF ADC was observed in ZM diet, probably due to the higher ADF content of ZM as indicated by the positive correlation. Moreover, a positive correlation was observed

among dietary ADF and dry matter, organic matter and energy ADCs. Therefore, FM substitution in the diets of gilthead sea bream with these insect meals may have been successful even in higher levels of inclusion (higher ADF), without any negative effects or even with a positive effect on the digestibility. Many studies have indicated that insect meal inclusion may improve digestibility, for example the incorporation of 30% and 7.6 - 26.1% silkworm meal (*Bombyx mori*) in carp (Nandeesha et al., 1990) and rohu (*Labeo rohita*; Begum et al., 1994) respectively, the inclusion of 30% cricket meal (*Gryllus bimaculatus*) in African catfish (Taufek et al., 2016), the 15% substitution of plant proteins with HI in European sea bass (Pérez-Pascual et al., 2020) and the inclusion of 25% TM in European sea bass (Gasco et al., 2016).

The individual amino acid ADCs were very high in all experimental diets (90.9 – 98.0% for European sea bass and 88.9 – 97.2% for gilthead sea bream) indicating a sufficient uptake by both fish species. Additionally, in sea bream the average essential amino acid ADC of the insect meal diets (91.9% - 94.5%) was similar to each other and slightly higher compared to premium quality FM (90.2%; Davies et al., 2009). In contrast, the average essential amino acid ADC of the insect meal diets in sea bass (94.2% - 95.8%) was slightly lower than premium quality FM (96.3%; Davies et al., 2009) and also similar to each other. Lysine and methionine are the first limiting amino acids in most formulated diets for aquaculture (Halver and Hardy, 2002). In both fish species, lysine and methionine digestibilities were very high and ranged between 93.1% - 97.2% and 93.2% - 98.0% for sea bream and sea bass, respectively. Methionine is pivotal for several processes of fish physiology including proper protein synthesis (Wilson, 2003). Inclusion of insect meals did not negatively affect methionine digestibility. Contrariwise, both TM and ZM diets in sea bass and AD in sea bream favoured the digestibility of this specific amino acid. In both fish species, the highest digestible amino acids were methionine, lysine and arginine. An antagonistic interaction has been previously observed between arginine and lysine during the transport across the brush border membrane (Berge et al., 1999). Arginine and lysine have similar structure and share a common carrier for absorption by the intestinal epithelium (Berge et al., 1999). Excess dietary lysine can reduce arginine uptake and vice versa, however arginine displays a stronger inhibitory effect on lysine uptake due to its higher affinity for the carrier (Murillo-Gurrea et al., 2001; Hoseini et al., 2020). Herein, no such observations were

detected and both lysine and arginine were highly digestible. The reduced phenylalanine ADCs in both fish species could be related to the involvement of the aromatic amino acids in the chitin-protein links (Muthukrishnan et al., 2012), which could reduce the bioavailability of the aforementioned amino acid. Regarding formulated diets containing non-protein nitrogen, the determination of the total amino acid digestibility may give an insight into the "true protein digestibility" (Booth and Pirozzi, 2021). In sea bream the sum of amino acids ADC did not differ among the experimental diets. On the other hand, in sea bass the "true protein digestibility" as expressed by the sum of amino acids was not affected by the insect inclusion compared to the FM diet, however a significant difference was observed between the ZM and HI diets. Similarly, in a study with Atlantic salmon fed diets containing up to 35% HI meal or paste, the total amino acid digestibility was not affected, while protein digestibility was displayed as reduced, due to interference of chitin in the overall results (Weththasinghe et al., 2021). In European sea bass the variation in crude protein and adjusted protein digestibility was strongly reflected on the amino acid digestibility among the experimental diets, as the significant positive correlations indicated. Despite the significant differences in the amino acid digestibility, the numerical differences amongst the experimental values were marginal.

In order to explore digestibility, it is imperative to highlight that digestive processes are directly mediated by fish gut microbiota. In specific, the prominent role of gut in nutrient digestion and absorption is highly contributed to enzyme-producing bacteria, which facilitate the utilization of feed ingredients that host-organism would not otherwise be able to digest (Ray et al., 2012). Bacteria belonging to several genera, e.g. *Acinetobacter*, *Enterobacter*, *Pseudomonas* and *Aeromonas*, have been reported to produce a variety of digestive enzymes such as lipases and proteases (Ray et al., 2012; Egerton et al., 2018). Different consumed diets may influence the bacterial metabolic contribution, since diet is considered to be a key modifiable factor in shaping and maintaining a functional gut microbiota (Miyake et al., 2015). According to Panteli et al. (2021), dietary inclusion of TM, HI and MD at 19.5% led to the enhancement of *Aeromonas* and *Pseudomonas* in the gut of *D. labrax* and *S. aurata*. Thus, alterations in digestibility, e.g., increased protein digestibility, that were observed in our study may be associated with differential responses of fish microbiota to the insect meals.

5. Conclusions

In conclusion, our results demonstrated that the 30% substitution of fish meal with five different insect meals in the diets of European sea bass and gilthead sea bream had minimal impact on the nutrient, amino acid and energy digestibility. In European sea bass, compared to the FM diet, TM and MD diets had similar or even slightly better digestibility, while ZM diet had higher protein and adjusted protein digestibility. Diets HI and AD presented slightly inferior overall digestibility compared to FM, with lower or similar nutrient digestibility coefficients, probably due to a combination of plant ingredients inclusion, dietary ash and crude fiber content. However, the differences were minute. The variation in crude protein and adjusted protein ADCs was strongly reflected on the individual amino acid ADCs in sea bass, while the sum of amino acids ADC was not affected by the insect meals inclusion compared to the FM diet. In gilthead sea bream all nutrient digestibility coefficients were similar among the experimental diets except for TM which exhibited lower fat digestibility. Overall, amino acid digestibility was not affected by the different experimental diets. The present study demonstrated that diets including 19.5% of the five examined insect meals did not negatively affect nutrient digestibility in sea bass and sea bream.

Acknowledgments: This research was partially funded by the European Union and the Greek national funds through the National Strategic Reference Framework 2014–2020 (GR & EU), Special Actions Aquaculture-Industrial Materials-Open innovation in culture (project code: MIS 5045857), entitled “Insect meal as an alternative source of protein in the aquafeed for fish farming” (acronym: Entomo4fish), and by Greece and the European Union (European Social Fund- ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project “Strengthening Human Resources Research Potential via Doctorate Research” (MIS-5000432), implemented by the State Scholarships Foundation (IKY) to Maria Mastoraki. This project has also partially received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 652831 (AQUAEXCEL2020, AQUAculture infrastructures for EXCELlence in European fish research towards 2020) to Laura Gasco. This output

reflects the views only of the author(s), and the European Union cannot be held responsible for any use which may be made of the information contained therein.

CRediT authorship contribution statement

Maria Mastoraki: Funding Acquisition, Investigation, Formal analysis, Writing - original draft. **Nikolas Panteli** Investigation, Writing - review & editing. **Yannis P. Kotzamanis**: Investigation, Writing - review & editing. **Laura Gasco**: Writing - review & editing. **Stavros Chatzifotis**: Conceptualization, Writing - review & editing, Funding Acquisition. **Efthimia Antonopoulou**: Conceptualization, Funding Acquisition, Writing - review & editing.

References

Abdel-Ghany, H.M., Salem, M.E.S., 2020. Effects of dietary chitosan supplementation on farmed fish; a review. Rev. Aquac. 12, 438-452. https://doi.org/10.1111/raq.12326.

Alegbeleye, W.O., Obasa, S.O., Olude, O.O., Otubu, K., Jimoh, W., 2012. Preliminary evaluation of the nutritive value of the variegated grasshopper (*Zonocerus variegatus* L.) for African catfish *Clarias gariepinus* (Burchell. 1822) fingerlings. Aquacult. Res. 43, 412-420. http://doi.org/10.1111/j.1365-2109.2011.02844.x.

Anvo, M.P.M., Aboua, B.R.D., Compaoré, I., Sissao, R., Zoungrana-Kaboré, C.Y., Kouamelan, E.P., Toguyéni, A., 2017. Fish meal replacement by *Cirina butyrospermi* caterpillar's meal in practical diets for *Clarias gariepinus* fingerlings. Aquacult. Res. 48, 5243-5250. https://doi.org/10.1111/are.13337.

AOAC, 1990. Official Methods of Analysis, 15th ed. Association of Official Analytical Chemists Inc, Washington, DC, USA.

Basto, A., Matos, E., Valente, L.M.P., 2020. Nutritional value of different insect larvae meals as protein sources for European sea bass (*Dicentrarchus labrax*) juveniles. Aquaculture 521, 735085. https://doi.org/10.1016/j.aquaculture.2020.735085.

Begum, N.N., Chakraborty, S.C., Zaher, M., Abdul, M.M., Gupta, M.V., 1994. Replacement of fishmeal by low-cost animal protein as a quality fish feed ingredient for indian major carp, *Labeo rohita*, fingerlings. J. Sci. Food Agric. 64, 191-197. https://doi.org/10.1002/jsfa.2740640207.

Belforti, M., Gai, F., Lussiana, C., Renna, M., Malfatto, V., Rotolo, L., De Marco, M., Dabbou, S., Schiavone, A., Zoccarato, I., 2015. *Tenebrio molitor* meal in rainbow trout (*Oncorhynchus mykiss*) diets: effects on animal performance, nutrient digestibility and chemical composition of fillets. Ital. J. Anim. Sci. 14, 669-676. https://doi.org/10.4081/ijas.2015.4170.

Belghit, I., Liland, N.S., Waagbø, R., Biancarosa, I., Pelusio, N., Li, Y., Krogdahl, Å., Lock, E.-J., 2018. Potential of insect-based diets for Atlantic salmon (*Salmo salar*). Aquaculture 491, 72-81. http://doi.org/10.1016/j.aquaculture.2018.03.016.

Berge, G.E., Bakke-McKellep, A.M., Lied, E., 1999. *In vitro* uptake and interaction between arginine and lysine in the intestine of Atlantic salmon (*Salmo salar*). Aquaculture 179, 181-193. https://doi.org/10.1016/S0044-8486(99)00161-1.

Bernard, J.B., Allen, M.E., Ullrey, D.E., 1997. Feeding captive insectivorous animals: Nutritional aspects of insects as food, Scientific Advisory Group to the American Zoo and Aquarium Association. Nutrition Advisory Group Handbook, Fact Sheet, pp. 1-7.

Booth, M.A., Pirozzi, I., 2021. The digestibility of raw materials by barramundi *Lates calcarifer*: Emphasis on the effect of inclusion rate on the digestibility of soybean meal and soy protein concentrate. Anim. Feed Sci. Technol. 273, 114800. https://doi.org/10.1016/j.anifeedsci.2020.114800.

Caimi, C., Renna, M., Lussiana, C., Bonaldo, A., Gariglio, M., Meneguz, M., Dabbou, S., Schiavone, A., Gai, F., Elia, A.C., 2020. First insights on black soldier fly (*Hermetia illucens* L.) larvae meal dietary administration in Siberian sturgeon (*Acipenser baerii* Brandt) juveniles. Aquaculture 515, 734539. https://doi.org/10.1016/j.aquaculture.2019.734539.

Chemello, G., Renna, M., Caimi, C., Guerreiro, I., Oliva-Teles, A., Enes, P., Biasato, I., Schiavone, A., Gai, F., Gasco, L., 2020. Partially defatted *Tenebrio molitor* larva meal in diets for grow-

out rainbow trout, *Oncorhynchus mykiss* (Walbaum): Effects on growth performance, diet digestibility and metabolic responses. Animals 10, 229. https://doi.org/10.3390/ani10020229.

Daniel, N., 2018. A review on replacing fish meal in aqua feeds using plant protein sources. Int. J. Fish. Aquat. Stud. 6, 164-179.

Danulat, E., Kausch, H., 1984. Chitinase activity in the digestive tract of the cod, *Gadus morhua* (L.). J. Fish Biol. 24, 125-133. https://doi.org/10.1111/j.1095-8649.1984.tb04784.x.

Davies, S.J., Gouveia, A., Laporte, J., Woodgate, S.L., Nates, S., 2009. Nutrient digestibility profile of premium (category III grade) animal protein by-products for temperate marine fish species (European sea bass, gilthead sea bream and turbot). Aquacult. Res. 40, 1759-1769. https://doi.org/10.1111/j.1365-2109.2009.02281.x.

Dumas, A., Raggi, T., Barkhouse, J., Lewis, E., Weltzien, E., 2018. The oil fraction and partially defatted meal of black soldier fly larvae (*Hermetia illucens*) affect differently growth performance, feed efficiency, nutrient deposition, blood glucose and lipid digestibility of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 492, 24-34. https://doi.org/10.1016/j.aquaculture.2018.03.038.

Egerton, S., Culloty, S., Whooley, J., Stanton, C., Ross, R.P., 2018. The gut microbiota of marine fish. Front. Microbiol. 9, 873. https://doi.org/10.3389/fmicb.2018.00873.

Erdogan, S., Kaya, M., 2016. High similarity in physicochemical properties of chitin and chitosan from nymphs and adults of a grasshopper. Int. J. Biol. Macromol. 89, 118-126. https://doi.org/10.1016/j.ijbiomac.2016.04.059.

Fabrikov, D., Sánchez-Muros, M.J., Barroso, F.G., Tomás-Almenar, C., Melenchón, F., Hidalgo, M.C., Morales, A.E., Rodriguez-Rodriguez, M., Montes-Lopez, J., 2020. Comparative study of growth performance and amino acid catabolism in *Oncorhynchus mykiss*, *Tinca tinca* and *Sparus aurata* and the catabolic changes in response to insect meal inclusion in the diet. Aquaculture 529, 735731. https://doi.org/10.1016/j.aquaculture.2020.735731.

Fines, B.C., Holt, G.J., 2010. Chitinase and apparent digestibility of chitin in the digestive tract of juvenile cobia, *Rachycentron canadum*. Aquaculture 303, 34-39. https://doi.org/10.1016/j.aquaculture.2010.03.010.

Finke, M.D., 2002. Complete nutrient composition of commercially raised invertebrates used as food for insectivores. Zoo Biol. 21, 269-285. https://doi.org/10.1002/zoo.10031.

Finke, M.D., 2007. Estimate of chitin in raw whole insects. Zoo Biol. 26, 105-115. http://doi.org/10.1002/zoo.20123.

Finke, M.D., 2013. Complete nutrient content of four species of feeder insects. Zoo Biol. 32, 27-36. https://doi.org/10.1002/zoo.21012.

Fisher, H.J., Collins, S.A., Hanson, C., Mason, B., Colombo, S.M., Anderson, D.M., 2020. Black soldier fly larvae meal as a protein source in low fish meal diets for Atlantic salmon (*Salmo salar*). Aquaculture 521, 734978. https://doi.org/10.1016/j.aquaculture.2020.734978.

Folch, J., Lees, M., Sloane Stanley, G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497-509.

Fontes, T.V., de Oliveira, K.R.B., Gomes Almeida, I.L., Maria Orlando, T., Rodrigues, P.B., da Costa, D.V., 2019. Digestibility of insect meals for nile tilapia fingerlings. Animals 9, 181. https://doi.org/10.3390/ani9040181.

Francis, G., Makkar, H.P.S., Becker, K., 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture 199, 197-227. https://doi.org/10.1016/S0044-8486(01)00526-9.

Gai, F., Gasco, L., Daprà, F., Palmegiano, G.B., Sicuro, B., 2012. Enzymatic and histological evaluations of gut and liver in rainbow trout, *Oncorhynchus mykiss*, fed with rice protein concentrate-based diets. J. World Aquacult. Soc. 43, 218-229. https://doi.org/10.1111/j.1749-7345.2012.00557.x.

Gasco, L., Henry, M., Piccolo, G., Marono, S., Gai, F., Renna, M., Lussiana, C., Antonopoulou, E., Mola, P., Chatzifotis, S., 2016. *Tenebrio molitor* meal in diets for European sea bass (*Dicentrarchus labrax* L.) juveniles: Growth performance, whole body composition and in vivo apparent digestibility. Anim. Feed Sci. Technol. 220, 34-45. http://doi.org/10.1016/j.anifeedsci.2016.07.003.

Goddard, J.S., McLean, E., 2001. Acid-insoluble ash as an inert reference material for digestibility studies in tilapia, *Oreochromis aureus*. Aquaculture 194, 93-98. https://doi.org/10.1016/S0044-8486(00)00499-3.

Goering, H.K., Van Soest, P.J., 1970. Forage fiber analysis (apparatus, reagents, procedures, and some applications), Agriculture Handbook United States Department of Agriculture. Agricultural Research Service USDA, Washington, DC, USA, pp. 20.

Gómez-Requeni, P., Mingarro, M., Calduch-Giner, J.A., Médale, F., Martin, S.A.M., Houlihan, D.F., Kaushik, S., Pérez-Sánchez, J., 2004. Protein growth performance, amino acid utilisation and somatotropic axis responsiveness to fish meal replacement by plant protein sources in gilthead sea bream (*Sparus aurata*). Aquaculture 232, 493-510. https://doi.org/10.1016/S0044-8486(03)00532-5.

Guerreiro, I., Serra, C.R., Coutinho, F., Couto, A., Castro, C., Rangel, F., Peres, H., Pousão-Ferreira, P., Matos, E., Gasco, L., 2020. Digestive enzyme activity and nutrient digestibility in meagre (*Argyrosomus regius*) fed increasing levels of black soldier fly meal (*Hermetia illucens*). Aquacult. Nutr. 27, 142-152. https://doi.org/10.1111/anu.13172.

Halver, J.E., Hardy, R.W., 2002. Fish Nutrition. Elsevier, San Diego, CA, USA.

Hansen, J.Ø., Penn, M., Øverland, M., Shearer, K.D., Krogdahl, Å., Mydland, L.T., Storebakken, T., 2010. High inclusion of partially deshelled and whole krill meals in diets for Atlantic salmon (*Salmo salar*). Aquaculture 310, 164-172. https://doi.org/10.1016/j.aquaculture.2010.10.003.

Henry, M., Gasco, L., Piccolo, G., Fountoulaki, E., 2015. Review on the use of insects in the diet of farmed fish: past and future. Anim. Feed Sci. Technol. 203, 1-22. https://doi.org/10.1016/j.anifeedsci.2015.03.001.

Hodar, A.R., Vasava, R.J., Mahavadiya, D.R., Joshi, N.H., 2020. Fish meal and fish oil replacement for aqua feed formulation by using alternative sources: A review. J. Exp. Zool. India 23, 13-21.

Hoseini, S.M., Ahmad Khan, M., Yousefi, M., Costas, B., 2020. Roles of arginine in fish nutrition and health: insights for future researches. Rev. Aquac. 12, 2091-2108. https://doi.org/10.1111/raq.12424.

Hosseini Shekarabi, S.P., Shamsaie Mehrgan, M., Banavreh, A., 2021. Feasibility of superworm, *Zophobas morio*, meal as a partial fishmeal replacer in fingerling rainbow trout, *Oncorhynchus mykiss*, diet: growth performance, amino acid profile, proteolytic enzymes activity and pigmentation. Aquacult. Nutr. https://doi.org/10.1111/anu.13249.

Hua, K., 2020. A meta-analysis of the effects of replacing fish meals with insect meals on growth performance of fish. Aquaculture 530, 735732. https://doi.org/10.1016/j.aquaculture.2020.735732.

Ido, A., Hashizume, A., Ohta, T., Takahashi, T., Miura, C., Miura, T., 2019. Replacement of fish meal by defatted yellow mealworm (*Tenebrio molitor*) larvae in diet improves growth performance and disease resistance in red seabream (*Pagrus major*). Animals 9, 100. https://doi.org/10.3390/ani9030100.

Jabir, M.A.R., Jabir, S.A.R., Vikineswary, S., 2012a. Nutritive potential and utilization of super worm (*Zophobas morio*) meal in the diet of Nile tilapia (*Oreochromis niloticus*) juvenile. Afr. J. Biotechnol. 11, 6592-6598. https://doi.org/10.5897/AJB11.1084.

Jabir, M.D.A.R., Razak, S.A., Vikineswary, S., 2012b. Chemical composition and nutrient digestibility of super worm meal in red tilapia juvenile. Pak. Vet. J. 32, 489-493.

Jingting, Y., Danting, G., Chun, K., Min, J., Xueming, H., 2020. Effect of soybean antigenic protein on feed palatability of fishmeal replaced diets for obscure puffer (*Takifugu fasciatus*) and the alternation of diet preference by domestication. Aquac. Rep. 17, 100332. https://doi.org/10.1016/j.aqrep.2020.100332.

Jobling, M., 2001. Feed composition and analysis. in: Houlihan, D., Boujard, T., Jobling, M. (Eds.), Food intake in fish. Blackwell Science Ltd, Oxford, UK, pp. 1-24.

Kotzamanis Y., Tsironi T., Brezas A., Grigorakis K., Ilia V., Vatsos I., Romano N., Eys V.J., Kumar V., 2020. High taurine supplementation in plant protein-based diets improves growth and organoleptic characteristics of European sea bass (*Dicentrarchus labrax*). Scientific Reports, Nature Research. 10, 12294 (2020). https://doi.org/10.1038/s41598-020-69014-x.

Kroeckel, S., Harjes, A.-G.E., Roth, I., Katz, H., Wuertz, S., Susenbeth, A., Schulz, C., 2012. When a turbot catches a fly: Evaluation of a pre-pupae meal of the Black Soldier Fly (*Hermetia*

illucens) as fish meal substitute - Growth performance and chitin degradation in juvenile turbot (*Psetta maxima*). Aquaculture 364, 345-352. http://doi.org/10.1016/j.aquaculture.2012.08.041.

Magalhães, R., Sánchez-López, A., Leal, R.S., Martínez-Llorens, S., Oliva-Teles, A., Peres, H., 2017. Black soldier fly (*Hermetia illucens*) pre-pupae meal as a fish meal replacement in diets for European seabass (*Dicentrarchus labrax*). Aquaculture 476, 79-85. http://doi.org/10.1016/j.aquaculture.2017.04.021.

Marono, S., Piccolo, G., Loponte, R., Di Meo, C., Attia, Y.A., Nizza, A., Bovera, F., 2015. In vitro crude protein digestibility of *Tenebrio molitor* and *Hermetia illucens* insect meals and its correlation with chemical composition traits. Ital. J. Anim. Sci. 14, 3889. https://doi.org/10.4081/ijas.2015.3889.

Mastoraki, M., Ferrándiz, P.M., Vardali, S.C., Kontodimas, D.C., Kotzamanis, Y.P., Gasco, L., Chatzifotis, S., Antonopoulou, E., 2020. A comparative study on the effect of fish meal substitution with three different insect meals on growth, body composition and metabolism of European sea bass (*Dicentrarchus labrax* L.). Aquaculture, 735511. https://doi.org/10.1016/j.aquaculture.2020.735511.

Merrifield, D.L., Olsen, R.E., Myklebust, R., Ringø, E., El-Shemy, H., 2011. Dietary effect of soybean (*Glycine max*) products on gut histology and microbiota of fish. in: El-Shemy, H. (Ed.), Soybean and nutrition. InTech, Rijeka, Croatia, pp. 231-250.

Miyake, S., Ngugi, D.K., Stingl, U., 2015. Diet strongly influences the gut microbiota of surgeonfishes. Mol. Ecol. 24, 656-672. https://doi.org/10.1111/mec.13050.

Murillo-Gurrea, D.P., Coloso, R.M., Borlongan, I.G., Serrano, A.E., 2001. Lysine and arginine requirements of juvenile Asian sea bass *Lates calcarifer*. J. Appl. Ichthyol. 17, 49-53. https://doi.org/10.1046/j.1439-0426.2001.00242.x

Muthukrishnan, S., Merzendorfer, H., Arakane, Y., Kramer, K.J., 2012. Chitin metabolism in insects. in: Gilbert, L.I. (Ed.), Insect molecular biology and biochemistry. Elsevier, pp. 193-235.

Nandeesha, M.C., Srikanth, G.K., Keshavanath, P., Varghese, T.J., Basavaraja, N., Das, S.K., 1990. Effects of non-defatted silkworm-pupae in diets on the growth of common carp, *Cyprinus carpio*. Biol. Wastes 33, 17-23. https://doi.org/10.1016/0269-7483(90)90118-C.

Ogunji, J., Toor, R.-U.-A., Schulz, C., Kloas, W., 2008. Growth performance, nutrient utilization of Nile tilapia *Oreochromis niloticus* fed housefly maggot meal (magmeal) diets. Turkish J. Fish. Aquat. Sci. 8, 141 - 147.

Ogunji, J., Pagel, T., Schulz, C., Kloas, W., 2009. Apparent digestibility coefficient of housefly maggot meal (magmeal) for Nile tilapia (*Oreochromis niloticus* L.) and carp (*Cyprinus carpio)*. Asian Fish. Sci. 22, 1095-1105. https://doi.org/10.33997/j.afs.2009.22.4.004.

Panini, R.L., Freitas, L.E.L., Guimarães, A.M., Rios, C., da Silva, M.F.O., Vieira, F.N., Fracalossi, D.M., Samuels, R.I., Prudêncio, E.S., Silva, C.P., 2017. Potential use of mealworms as an alternative protein source for Pacific white shrimp: digestibility and performance. Aquaculture 473, 115-120. https://doi.org/10.1016/j.aquaculture.2017.02.008.

Panteli, N., Mastoraki, M., Lazarina, M., Chatzifotis, S., Mente, E., Kormas, K.A., Antonopoulou, E., 2021. Configuration of gut microbiota structure and potential functionality in two teleosts under the influence of dietary insect meals. Microorganisms 9, 699. https://doi.org/10.3390/microorganisms9040699.

Pérez-Pascual, D., Estellé, J., Dutto, G., Rodde, C., Bernardet, J.-F., Marchand, Y., Duchaud, E., Przybyla, C., Ghigo, J.-M., 2020. Growth performance and adaptability of European sea bass (*Dicentrarchus labrax*) gut microbiota to alternative diets free of fish products. Microorganisms 8, 1346. https://doi.org/10.3390/microorganisms8091346.

Piccolo, G., Iaconisi, V., Marono, S., Gasco, L., Loponte, R., Nizza, S., Bovera, F., Parisi, G., 2017. Effect of *Tenebrio molitor* larvae meal on growth performance, in vivo nutrients digestibility, somatic and marketable indexes of gilthead sea bream (*Sparus aurata*). Anim. Feed Sci. Technol. 226, 12 - 20. http://doi.org/10.1016/j.anifeedsci.2017.02.007.

Rawski, M., Mazurkiewicz, J., Kierończyk, B., Józefiak, D., 2020. Black soldier fly full-fat larvae meal as an alternative to fish meal and fish oil in Siberian sturgeon nutrition: The effects on

physical properties of the feed, animal growth performance, and feed acceptance and utilization. Animals 10, 2119. https://doi.org/10.3390/ani10112119.

Ray, A.K., Ghosh, K., Ringø, E., 2012. Enzyme-producing bacteria isolated from fish gut: a review. Aquacult. Nutr. 18, 465-492. https://doi.org/10.1111/j.1365-2095.2012.00943.x.

Rema, P., Saravanan, S., Armenjon, B., Motte, C., Dias, J., 2019. Graded incorporation of defatted yellow mealworm (*Tenebrio molitor*) in rainbow trout (*Oncorhynchus mykiss*) diet improves growth performance and nutrient retention. Animals 9, 187. https://doi.org/10.3390/ani9040187.

Renna, M., Schiavone, A., Gai, F., Dabbou, S., Lussiana, C., Malfatto, V., Prearo, M., Capucchio, M.T., Biasato, I., Biasibetti, E., 2017. Evaluation of the suitability of a partially defatted black soldier fly (*Hermetia illucens* L.) larvae meal as ingredient for rainbow trout (*Oncorhynchus mykiss* Walbaum) diets. J. Anim. Sci. Biotechnol. 8, 57. https://doi.org/10.1186/s40104-017-0191-3.

Robaina, L., Moyano, F.J., Izquierdo, M.S., Socorro, J., Vergara, J.M., Montero, D., 1997. Corn gluten and meat and bone meals as protein sources in diets for gilthead seabream (*Sparus aurata*): nutritional and histological implications. Aquaculture 157, 347-359. https://doi.org/10.1016/S0044-8486(97)00174-9.

Rumpold, B., Schlüter, O., 2014. Nutrient composition of insects and their potential application in food and feed in Europe. Food Chain 4, 129-139. http://doi.org/10.3362/2046-1887.2014.013.

Rumpold, B.A., Schlüter, O.K., 2013. Potential and challenges of insects as an innovative source for food and feed production. Innov. Food Sci. Emerg. Technol. 17, 1-11. https://doi.org/10.1016/j.ifset.2012.11.005.

Shiau, S.-Y., Yu, Y.-P., 1999. Dietary supplementation of chitin and chitosan depresses growth in tilapia, *Oreochromis niloticus* × *O. aureus*. Aquaculture 179, 439-446. https://doi.org/10.1016/S0044-8486(99)00177-5.

Sinha, A.K., Kumar, V., Makkar, H.P.S., De Boeck, G., Becker, K., 2011. Non-starch polysaccharides and their role in fish nutrition – A review. Food Chem. 127, 1409-1426. https://doi.org/10.1016/j.foodchem.2011.02.042.

Stejskal, V., Tran, H.Q., Prokesova, M., Gebauer, T., Giang, P.T., Gai, F., Gasco, L., 2020. Partially defatted *Hermetia illucens* larva meal in diet of Eurasian perch (*Perca fluviatilis*) Juveniles. Animals 10, 1-17. https://doi.org/10.3390/ani10101876.

Taufek, N.M., Muin, H., Raji, A.A., Razak, S.A., Yusof, H.M., Alias, Z., 2016. Apparent digestibility coefficients and amino acid availability of cricket meal, *Gryllus bimaculatus*, and fishmeal in African catfish, *Clarias gariepinus*, diet. J. World Aquacult. Soc. 47, 798-805. https://doi.org/10.1111/jwas.12302.

Tharanathan, R.N., Kittur, F.S., 2003. Chitin—the undisputed biomolecule of great potential. Crit. Rev. Food Sci. Nutr. 43, 61-87. https://doi.org/10.1080/10408690390826455.

Vogtmann, H., Pfirter, H.P., Prabucki, A.L., 1975. A new method of determining metabolisability of energy and digestibility of fatty acids in broiler diets. Br. Poult. Sci. 16, 531-534. https://doi.org/10.1080/00071667508416222.

Wang, L., Li, J., Jin, J.N., Zhu, F., Roffeis, M., Zhang, X.Z., 2017. A comprehensive evaluation of replacing fishmeal with housefly (*Musca domestica*) maggot meal in the diet of Nile tilapia (*Oreochromis niloticus*): Growth performance, flesh quality, innate immunity and water environment. Aquacult. Nutr. 23, 983-993. https://doi.org/10.1111/anu.12466.

Weththasinghe, P., Hansen, J.Ø., Nøkland, D., Lagos, L., Rawski, M., Øverland, M., 2021. Full-fat black soldier fly larvae (*Hermetia illucens*) meal and paste in extruded diets for Atlantic salmon (*Salmo salar*): Effect on physical pellet quality, nutrient digestibility, nutrient utilization and growth performances. Aquaculture 530, 735785. https://doi.org/10.1016/j.aquaculture.2020.735785.

Wilson, R.P., 2003. Amino acids and proteins. in: Halver, J.E., Hardy, R.W. (Eds.), Fish Nutrition. Academic Press, San Diego, CA, USA, pp. 143-179.

Table 1: Ingredients and proximate composition of the experimental diets

	Dicentrarchus labrax						*Sparus aurata*					
	FM	TM	HI	MD	ZM	AD	FM	TM	HI	MD	ZM	AD
Ingredients (g/kg)												
Fish	650	455	455	455	455	455	650	455	455	455	455	455

meal												
Insect larvae meal	0	195	195	195	195	195	0	195	195	195	195	195
Fish oil	100	60	97	62	13	53	90	50	95	63	11	53
Wheat	164	172	152	175	193	190	159	154	138	156	179	174
Wheat gluten meal	69	84	68	91	113	78	63	92	62	87	106	80
Vitamin & mineral mix[a]	2.5	2.5	2.5	2.5	2.5	2.5	25	25	25	25	25	25
DL-methionine	5	10	9	3	9	9	3	7	7	0	7	6
L-lysine	0	12	12	7	10	8	0	12	13	9	12	2
Celite	10	10	10	10	10	10	10	10	10	10	10	10
Proximate composition (as fed)												
Crude protein	551±1	537±13	541±1	537±11	547±10	557±10	573±14	571±17	565±29	553±36	525±10	543±15
Adjusted protein[b]	537	522	507	516	518	524	562	529	530	533	498	526
Crude Fat	175±8	172±10	155±12	160±4	141±9	117±17	131±4	130±2	132±0	137±0	159±1	146±19
Ash	99±2	89±1	98±5	93±2	83±7	89±0	111±1	101±5	112±1	113±4	99±1	96±1
Crude Fiber	10±1	28±0	31±3	14±1	34±4	41±1	15±1	22±0	36±2	26±2	34±0	45±6
Acid detergent fiber	79±12	41±12	85±4	78±2	85±4	77±2	63±3	49±5	81±2	63±8	79±12	41±12
Energy	20.3±0.00	20.7±0.111	19.6±0.113	19.8±0.114	19.9±0.38	19.5±0.28	22.5±0.10	22.4±0.211	22.5±0.113	22.4±0.114	19.9±0.08	20.5±0.18
Chitin[c]												

Abbreviations: FM, Fish meal; TM, *Tenebrio molitor*; HI, *Hermetia illucens*; MD, *Musca domestica*; ZM, *Zophobas morio*; AD, *Alphitobius diaperinus*

[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 2,600 (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 7,000 (mg), Total Cu 450 (mg), Total Se 14 (mg), Total I 100 (mg), Betaine 71,250 (mg), BHA (E320) 3,000 (mg)

Nutrients expressed as g/kg and energy as MJ kg^{-1}

[b] Protein adjusted for the acid insoluble nitrogen

[c] Chitin estimated based on the insect inclusion and the chitin content of the individual insect meals (TM, 6.34%; HI, 7.06%; MD, 7.73%; ZM, 4.78%; AD, 4.73%).

Table 2: Amino acid composition (g/kg, as fed basis) of the experimental diets

	Dicentrarchus labrax						*Sparus aurata*					
IAAs	FM	TM	HI	MD	ZM	AD	FM	TM	HI	MD	ZM	AD
Arg	24.3±0.2	20.7±0.8	23.3±0.1	24.0±0.2	23.7±0.6	24.4±0.3	30.0±0.6	24.8±0.1	27.3±0.2	27.4±0.7	21.9±0.2	23.8±0.5
His	10.5±0.1	11.2±0.3	11.2±0.1	11.5±0.3	10.6±0.1	11.2±0.0	10.6±0.4	10.6±0.0	10.4±0.1	11.0±0.3	10.0±0.0	11.4±0.1
Ile	18.7±0.1	19.9±0.3	19.0±0.4	18.5±0.1	19.5±0.4	19.9±0.4	22.5±0.3	20.8±0.1	21.7±0.1	21.3±0.1	18.4±0.8	19.3±0.3
Leu	34.6±0.1	35.0±0.4	33.5±0.4	33.6±0.4	35.0±0.7	35.5±0.8	41.0±0.5	37.7±0.4	39.1±0.0	38.9±0.4	32.5±0.4	34.5±0.4
Lys	32.3±0.0	28.5±0.4	28.8±0.4	30.2±1.6	28.4±0.5	31.0±0.7	37.7±0.1	39.9±0.3	41.6±1.1	42.7±1.6	26.6±0.4	30.0±0.2
Met	16.9±0.1	20.2±1.1	18.0±0.1	14.9±0.1	20.2±0.0	20.5±0.7	15.3±0.4	19.9±0.1	16.1±0.4	15.6±0.1	15.8±0.4	18.9±1.4
Phe	19.7±0.0	19.3±0.3	19.3±0.2	23.5±0.7	19.2±0.1	19.9±0.3	21.3±0.6	18.2±0.1	19.8±0.1	23.3±0.6	17.7±0.3	19.2±0.6
Thr	18.9±0.1	17.1±0.3	18.3±0.2	18.7±0.1	18.7±0.4	19.3±0.4	23.3±0.4	21.0±0.1	22.4±0.3	22.9±0.6	17.4±0.1	18.8±0.1
Val	22.5±0.1	24.7±0.3	23.8±0.4	22.6±.1	23.8±0.3	23.3±0.3	26.0±0.3	25.9±0.2	26.3±0.1	25.3±0.3	23.3±1.0	24.1±0.3
DAAs												
Ala	26.0±0.0	28.3±0.1	27.4±0.2	24.0±0.7	29.6±0.5	30.5±0.6	31.6±0.2	32.5±0.4	31.6±0.4	30.9±0.8	28.7±1.1	30.1±0.0
Asx	37.3±0.1	33.9±0.4	36.5±0.3	36.6±2.0	38.5±0.7	41.4±0.4	40.8±0.4	38.6±0.0	40.3±0.8	45.9±3.9	36.7±0.6	40.4±0.1
Cys	2.2±0.1	2.1±0.0	2.0±0.1	2.3±0.0	2.0±0.0	1.9±0.1	2.8±0.0	2.3±0.1	2.3±0.1	2.5±0.0	1.5±0.1	1.6±0.1
Glx	75.8±0.3	76.1±0.3	73.5±0.1	78.8±1.5	86.3±1.0	84.7±0.4	82.2±0.1	77.1±0.1	79.3±2.1	87.6±5.9	81.2±0.5	80.4±0.7
Gly	24.8±0.1	24.4±0.4	24.3±0.3	23.1±0.4	24.4±0.4	25.0±0.4	29.8±0.5	25.2±0.4	27.0±0.5	25.5±0.6	23.3±0.4	24.7±0.4
Pro	23.5±0.1	27.8±0.0	26.0±0.1	25.1±0.1	28.0±0.4	27.3±0.2	28.4±0.2	29.2±0.3	29.4±0.5	27.6±0.2	26.5±0.4	26.4±0.3
Ser	19.0±0.1	17.9±0.2	19.0±0.0	19.4±0.1	20.3±0.4	20.3±0.4	24.2±0.6	21.8±0.2	23.6±0.0	23.5±0.3	18.9±0.1	19.9±0.3
Tyr	12.6±0.1	12.4±0.3	15.1±0.4	16.7±0.5	15.9±0.4	16.6±0.0	15.1±0.4	17.2±0.0	16.3±0.4	18.6±0.9	14.9±0.2	16.8±0.4
Tau	5.5±0.1	4.2±0.0	4.0±0.0	4.1±0.1	3.3±0.0	4.0±0.0	3.8±0.1	3.2±0.1	2.3±0.1	2.4±0.0	3.3±0.3	3.8±0.1

Abbreviations: IAA, indispensable amino acids; DAAs, dispensable amino acids; Asx, sum of asparagine and aspartate; Glx, sum of glutamine and glutamate

Table 3. Apparent digestibility coefficients (ADCs %) of nutrients and energy of diets in which 30% of fish meal (FM) was substituted with different insect meals (TM: *Tenebrio molitor*, HI: *Hermetia*

illucens, MD: *Musca domestica*, ZM: *Zophobas morio* or AD: *Alphitobius diaperinus*)

ADC (%)	FM	TM	HI	MD	ZM	AD
			Dicentrarchus labrax			
Dry matter	77.4±0.3[a]	77.4±0.4[a]	73.1±0.7[bc]	76.6±0.7[ab]	71.7±1.3[c]	69.9±0.6[c]
Protein	91.6±0.1[b]	92.3±0.2[ab]	89.6±0.5[c]	91.8±0.1[b]	93.2±0.3[a]	91.2±0.1[b]
Adjusted Protein [1]	91.9±0.2[bc]	93.4±0.2[a]	91.1±0.6[c]	92.7±0.1[ab]	93.6±0.2[a]	92.0±0.1[b]
Fat	92.2±0.2	92.4±0.1	91.2±0.3	92.2±0.9	88.6±1.8	87.5±1.5
ADF	89.3±0.7[a]	59.2±1.2[d]	67.5±0.8[c]	78.1±0.5[b]	85.1±1.7[a]	75.63±0.8[b]
Energy	86.3±0.2[a]	86.6±0.1[a]	84.5±0.5[ab]	85.1±0.5[a]	82.1±1.0[b]	82.3±0.4[b]
Organic matter	83.8±0.3[a]	82.4±0.2[ab]	79.9±0.6[bc]	82.2±0.7[ab]	78.4±1.1[c]	78.6±0.4[c]
			Sparus aurata			
Dry matter	70.3±0.1	66.5±0.2	73.0±0.6	70.5±1.3	74.0±1.0	72.0±3.4
Protein	89.0±0.5	89.6±1.6	89.5±0.1	90.9±0.1	91.5±0.2	91.1±0.9
Adjusted Protein [1]	89.6±0.5	91.1±1.8	91.1±0.1	92.4±0.2	92.1±0.2	92.1±0.8
Fat	83.2±0.6[a]	73.6±0.7[b]	82.8±1.5[a]	78.9±0.9[a]	84.3±1.6[a]	80.7±2.6[a]
ADF	61.3±5.1[ab]	48.4±4.4[b]	59.8±1.2[b]	53.5±1.2[b]	78.3±0.5[a]	46.7±5.8[b]
Energy	82.7±0.6	79.5±1.2	84.5±0.7	83.1±0.8	82.9±1.0	82.4±2.3
Organic matter	78.2±0.4	74.2±0.9	80.1±0.9	78.1±1.3	80.1±0.9	79.0±2.8

Within rows, different letters denote statistically significant difference ($P < 0.05$). Mean ± standard error, n=3 tanks per diet

[1]Adjusted for the acid insoluble nitrogen

Table 4: Amino acid digestibility (ADC %) of diets in which 30% of fish meal (FM) was substituted with different insect meals (TM: *Tenebrio molitor*, HI: *Hermetia illucens*, MD: *Musca domestica*, ZM: *Zophobas morio* or AD: *Alphitobius diaperinus*) in European sea bass *Dicentrarchus labrax*

	FM	TM	HI	MD	ZM	AD	r feed	r adjusted protein digestibility
IAAs								
Arg	95.4±0.6	96.2±0.2	95.5±0.7	96.4±0.1	96.6±0.2	96.1±0.5	-0.317	0.585*
His	94.0±0.7	94.8±0.4	93.9±0.5	95.4±0.2	95.3±0.2	93.7±0.2	0.323	0.736***
Ile	93.1±0.4[b]	94.7±0.2[ab]	93.0±0.7[b]	93.9±0.1[b]	95.3±0.2[a]	94.0±0.2[ab]	0.630**	0.891***
Leu	94.0±0.3[bc]	95.2±0.2[a]	93.7±0.5[c]	94.6±0.1[abc]	95.8±0.3[a]	94.7±0.2[abc]	0.643**	0.911***
Lys	95.7±0.3	96.3±0.1	95.4±0.5	95.9±0.0	96.1±0.2	94.5±0.9	-0.191	0.754***
Met	95.7±0.6[c]	98.0±0.1[a]	96.8±0.5[abc]	96.2±0.0[bc]	97.8±0.1[ab]	97.4±0.4[abc]	0.837***	0.529*
Phe	92.8±0.5[b]	94.9±0.2[a]	93.2±0.8[ab]	94.4±0.1[ab]	94.8±0.2[a]	93.5±0.4[ab]	0.066	0.920***
Thr	93.7±0.4[ab]	94.2±0.2[ab]	93.2±0.6[b]	94.2±0.1[ab]	95.1±0.2[a]	94.0±0.2[ab]	-0.047	0.806***
Val	93.8±0.4[bc]	94.8±0.2[ab]	93.2±0.5[c]	94.4±0.1[abc]	95.4±0.3[a]	93.7±0.2[bc]	0.241	0.922***
DAAs								
Ala	93.9±0.4[ab]	94.8±0.3[ab]	93.2±0.5[b]	94.3±0.0[ab]	95.6±0.3[a]	94.4±0.2[ab]	0.524*	0.889***
Asx	91.8±0.5	93.2±0.3	91.9±0.8	92.8±0.1	93.8±0.2	92.7±0.4	0.179	0.798***
Cys	91.6±0.6[ab]	93.9±0.1[ab]	90.9±0.8[b]	93.8±0.1[ab]	95.1±0.3[a]	94.3±1.4[ab]	-0.348	0.754***
Glx	96.1±0.	97.0±0.	95.8±0.5	96.8±0.0	97.1±0.1	96.2±0.2	0.536*	0.967***

	4	1						
Gly	93.8±0.6[a]	93.9±0.3[a]	92.0±0.5[b]	94.4±0.1[a]	94.7±0.3[a]	93.5±0.3[ab]	-0.298	0.771***
Pro	95.8±0.3[ab]	96.4±0.1[a]	95.0±0.4[b]	96.5±0.0[a]	96.6±0.2[a]	95.5±0.2[ab]	0.335	0.915***
Ser	93.4±0.5[ab]	93.5±0.5[ab]	92.9±0.6[b]	94.5±0.1[ab]	95.3±0.2[a]	93.9±0.4[ab]	0.593**	0.721***
Tyr	93.6±0.6[b]	94.5±0.4[b]	93.6±0.7[b]	95.1±0.1[ab]	96.8±0.2[a]	95.6±0.4[ab]	0.593**	0.620**
Tau	91.9±1.7[ab]	95.3±0.3[a]	93.3±0.0[ab]	94.0±0.1[ab]	91.7±0.5[b]	92.8±0.1[ab]	0.561*	0.201
Sum of AA	94.4±0.5[ab]	95.4±0.2[ab]	94.1±0.6[b]	95.1±0.1[ab]	95.9±0.2[a]	94.7±0.2[ab]	0.498*	0.953***

Abbreviations: IAAs, indispensable amino acids; DAAs, dispensable amino acids; Asx, sum of asparagine and aspartate; Glx, sum of glutamine and glutamate

Mean ± standard error, n = 3 tanks per diet. Different letters indicate statistically significant difference ($P < 0.05$). In the correlation analysis an asterisk (*) indicates significance at the 0.05 level, ** at the 0.01 level and *** at the 0.001 level

Table 5: Amino acid digestibility (ADC %) of diets in which 30% of fish meal (FM) was substituted with different insect meals (TM: *Tenebrio molitor*, HI: *Hermetia illucens*, MD: *Musca domestica*, ZM: *Zophobas morio* or AD: *Alphitobius diaperinus*) in gilthead sea bream *Sparus aurata*

	FM	TM	HI	MD	ZM	AD	r feed	r adjusted protein digestibility
IAAs								
Arg	93.2±0.7	92.7±1.4	94.7±0.5	94.2±0.1	94.2±1.2	94.6±1.0	-0.129	0.437
His	89.1±0.8	89.9±1.7	91.1±0.8	91.3±0.4	92.9±0.5	93.5±1.0	0.085	0.620**
Ile	91.4±1.2	91.0±1.8	92.9±0.6	92.9±0.4	93.8±0.4	94.0±0.8	-0.524*	0.467*
Leu	92.6±1.1	91.6±1.6	93.5±0.6	93.6±0.4	94.7±0.3	94.9±0.7	-0.599**	0.420
Lys	93.9±1.0	94.4±1.3	95.8±0.4	95.4±0.3	95.3±0.4	95.6±0.6	0.135	0.573*
Met	93.1±0.8[b]	95.7±0.9[ab]	95.6±0.5[ab]	94.6±0.1[ab]	96.1±1.0[ab]	97.2±0.6[a]	0.455	0.556*
Phe	89.7±1.1	89.6±1.9	91.7±0.8	90.7±0.5	93.1±0.9	93.3±1.00	-0.442	0.453
Thr	92.5±1.1	91.2±1.5	93.4±0.5	93.4±0.4	93.5±0.2	93.9±0.8	-0.179	0.408
Val	91.7±1.2	90.9±1.7	92.7±0.6	93.0±0.4	93.8±0.3	93.8±0.8	-0.630**	0.534*
DAAs								
Ala	92.5±1.0	91.8±1.5	93.0±0.4	93.8±0.4	94.3±0.3	94.2±0.8	-0.505*	0.657**
Asx	88.8±1.2	89.0±1.7	90.9±0.5	91.6±0.3	92.5±0.6	92.7±1.1	-0.053	0.631**
Cys	93.1±0.6	92.6±0.7	94.6±0.6	95.5±0.3	93.2±1.3	94.6±0.8	-0.041	0.364

Glx	93.5±1.0	92.8±1.6	94.5±0.4	95.0±0.2	95.9±0.4	95.8±0.7	0.141	0.569*
Gly	91.4±0.8	89.7±1.2	90.7±0.4	91.8±0.4	92.6±0.6	92.5±1.2	-0.517*	0.513*
Pro	94.2±0.7	93.3±1.2	94.3±0.3	94.9±0.2	95.0±0.4	94.4±0.8	-0.241	0.585*
Ser	92.3±0.9	90.8±1.4	93.0±0.4	93.4±0.3	93.4±0.7	93.4±1.0	-0.129	0.484*
Tyr	91.8±0.7	92.4±1.4	93.2±0.5	93.1±0.4	95.0±0.9	94.9±0.8	-0.085	0.614**
Tau	93.3±1.2	93.4±0.6	92.2±0.5	94.4±0.6	95.2±0.8	95.6±0.7	0.335	0.321
Sum of AA	92.3±0.9	91.9±1.5	93.5±0.5	93.6±0.3	94.4±0.5	94.5±0.8	-0.386	0.523*

Abbreviations: IAAs, indispensable amino acids; DAAs, dispensable amino acids; Asx, sum of asparagine and aspartate; Glx, sum of glutamine and glutamate

Mean ± standard error, n = 3 tanks per diet. Different letters indicate 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

Authorship contributions, as described in the manuscript:

- Maria Mastoraki: Funding Acquisition, Investigation, Formal analysis, Writing - original draft
- Nikolas Panteli: Investigation, Writing - review & editing
- Yannis P. Kotzamanis: Investigation, Writing - review & editing
- Laura Gasco: Writing - review & editing
- Efthimia Antonopoulou: Conceptualization, Funding Acquisition, Writing - original draft, Writing - review & editing
- Stavros Chatzifotis: Conceptualization, Writing - review & editing, Funding

All authors (M. Mastoraki, N. Panteli, Y. P. Kotzamanis, L. Gasco, E. Antonopoulou, S. Chatzifotis) **approve this** version of the manuscript to be published.

Acknowledgements:
The authors would like to thank all the people facilitating the implementation of this project including students, technical and administrative staff.

Conflicts of Interest:
The authors declare no conflict of interest.

Conflict of Interest and Authorship Conformation Form

The manuscript entitled "Nutrient digestibility of diets containing five different insect meals in gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*)" written by M. Mastoraki, N. Panteli, Y.P. Kotzamanis, L. Gasco, E. Antonopoulou and S. Chatzifotis contains new unpublished information, and it has not been submitted to, nor is under review at, another journal or other publishing venue.

All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version of the present manuscript.

The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript and declare no conflict of interest.

Highlights

- In sea bream overall digestibility was not affected by the different insect meals.
- Diets with *Tenebrio molitor* or *Musca domestica* performed equally well in sea bass.
- *Hermetia illucens* or *Alphitobius diaperinus* diets had lower digestibility.
- Insect meal inclusion did not affect true protein digestibility.