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The effect of the substitution of fishmeal with mealworm (*Tenebrio molitor*) on the survival, growth and quality of the European seabass (*Dicentrarchus labrax*) reared in Morocco

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ABSTRACT

Insects are considered an alternative and sustainable source of protein in aquaculture diets. Hence, this work aimed to study the nutritional value of the larvae meal of *Tenebrio molitor* (TM) and evaluate the effect of its incorporation by partially substituting fishmeal in diets of seabass. For this purpose, four feeds (TM0, TM17, TM34, and TM50) with 0%, 17%, 34%, and 50% replacement of fishmeal, respectively were formulated, prepared, and tested on 280 fish with an initial average weight of 42.65 ± 0.81g. These fish were randomly divided into four feed groups in duplicate. Each group consisted of two tanks, each of which contained 35 fish individuals. These fish were hand-fed until satiation with one of the experimental diets. The results obtained showed that substitution of 50% of fishmeal by the larvae meal of TM improves the zootechnical performances and has no negative effect either on the feed intake, or on the analytical composition of the fish, or the intestinal microflora. Histological sections of the liver and intestine showed that there were no specific changes neither in the vacuolation of liver lipid cells, nor in the distribution, the number of caliciform cells or the intestinal rods.

INTRODUCTION

The nutritional requirements of farmed fish, especially carnivores, are quite high in terms of quality and quantity of protein in the diet. For this reason, fishmeal, with its excellent protein content and balanced amino acid profile, has traditionally been considered the best protein source useful in the formulation of feeds for farmed fish. However, fishmeal is a product of limited supply **(Oliva-Teles *et al.*, 2015)** and the rapid

development of aquaculture **(FAO, 2020)**. This has led to a heated debate regarding the sustainability of its production **(Hardy, 2010)**.

Therefore, to limit the dependence of aquaculture on fishery products and to preserve fishery resources, it has become necessary to find potentially sustainable alternatives for fish meal in the production of aquaculture feeds. Indeed, in farmed fish, some feeds with a maximized substitution of meals of terrestrial or marine origin (oilseed meals, protein crops, feather meal, algae...) for fishmeal strongly reflect impaired growth performance given the presence of anti-nutritional factors **(Rakotozanany, 2012)**, high levels of fiber and non-starch polysaccharides and inadequate fatty acid and amino acid profiles **(Gai *et al.*, 2017). In addition to those** effects, poor palatability **(Gatlin *et al.*, 2007)** is considered. Thus, the health of farmed fish is affected while causing nutritional deficiencies **(Ballester-Lozano *et al.*, 2015)**.

Over the past decade, an increasing interest has emerged in using insects as a protein source in feed production **(FAO, 2014)** in a wide scale due to the high quality and quantity of protein in many insect species **(Sánchez-Muros, *et al.*, 2014)**. Although insects are not currently produced in sufficient quantities to be used in commercial fish feed production, they hold great promise as sustainable ingredients for the future of the aquaculture feed industry **(Makkar *et al.*, 2014; Tran *et al.*, 2015)**

The larvae of many insect species can be used in the production of insect meal. Among these species is the mealworm *Tenebrio molitor* (Linnaeus, 1758), a globally distributed beetle belonging to the family Tenebrionidae **(Makkar *et al.*, 2014)**. These larvae hold great promise for aquaculture since they are easy to breed and feed and are rich in protein with an adequate amino acid profile **(De Marco *et al.*, 2015)**. Recent studies have reported the use of TM larval meal in fish feeds **(Sánchez-Muros *et al.*, 2014; Mastoraki *et al.*, 2020)** and poultry **(Bovera *et al.*, 2015; 2016; Biasato *et al.*, 2017)**.

Thus, the present study aimed to develop and produce a sustainable aquaculture feed, reducing the dependence on depletable marine resources, while covering the nutritional needs of farmed fish hence ensuring the development of sustainable and reasonable aquaculture. In addition. The current work was conducted to evaluate the effect of using the meal of the larvae of TM as a partial substitution ingredient for fishmeal in the diet of seabass (*Dicentrarchus labrax*) reared in M'diq in northern Morocco, taking into account the zootechnical parameters, the parameters of food use and the analytical composition of fish fed with experimental diets at an increasing rate of substitution.

MATERIALS AND METHODS

The experiment was conducted at the Specialized Center in Zootechnics and Marine Aquaculture Engineering of M'diq of the National Institute of Fisheries Research, located in northern Morocco.

The fish used in this experiment were provided by the fish farm of the company of Aqua M'diq located in the bay of M'diq. This farm is located on the west coast of the Moroccan Mediterranean Sea, between Cape Ceuta (35°54' N, 5°17'10" W) in the North and Cape Negro (35°40' N, 5°16'40" W) in the South. The fish were taken from floating

cages at sea and then transferred and stored in the INRH tanks for one month in order to acclimatize to the new rearing conditions and the new experimental feed.

1. Insect meal and food formulation

The insect meal, incorporated in the formulation of the experimental diets used in the present study, was manufactured from the larvae of TM. The latter were mass reared in the entomology laboratory of the Agronomic and Veterinary Institute Hassan II, a horticultural complex in Agadir, Morocco.

The strain of this insect species was provided by the International Foundation for Wildlife Preservation. Its rearing was maintained in smooth plastic food containers to prevent larvae from climbing on the walls. During the larval and adult stages, the insects were fed the same diet of 60% wheat bran and 40% poultry feed (17.3% protein; 4.3% lipid; 11.5% moisture; 6.7% cellulose; 5.3% minerals; 0.44% calcium and 0.48% phosphorous). Portions of fresh potatoes were distributed every 4 days as a water source. The rearing conditions were maintained at a temperature of 28 ± 1°C under a relative humidity of 70 ± 5% with an optimal fan, and in total darkness except during handling.

A quantity of 30kg of larvae, 70 ± 6 days of old, was collected by sieving the cleared substrate with a 2mm mesh laboratory metal sieve. Then, these larvae were fasted for 24 hours. To use these larvae in good conditions while respecting the welfare recommended by the European Directive (European Directive 2010/63/EU), they were put in a freezer at -18°C for 24 hours, then dried at 75°C for 48 hours. Afterwards, a stainless steel grinder (25,000 rpm) was used to grind them into a homogeneous meal.

The other ingredients used in the formulation of the experimental feeds were purchased. Thus, four experimental diets were formulated: a control diet (TM0) in which fish meal was the main source of protein (TM0: 0% TM larvae meal) and three diets (TM17, TM34 and TM50) in which fish meal was replaced by TM larvae meal at 17%, 34% and 50%, respectively (Table 1).

Table 1. Percentage of ingredient incorporation in the four experimental diets

Ingredient	**TM0**	**TM17**	**TM34**	**TM50**
Fishmeal	30%	25%	20%	15%
TM larvae meal	0%	5%	10%	15%
Soybean meal	28%	28%	28%	28%
Wheat flour	11%	11%	11%	11%
Wheat gluten	6%	6%	6%	6%
Corn gluten	6%	6%	6%	6%
Pea protein concentrate	5%	5%	5%	5%
Fish oil	4%	4%	4%	4%
Sunflower oil	8%	8%	8%	8%
Vitamin-Mineral Premix	2%	2%	2%	2%
Total	100%	100%	100%	100%

Composition of Vitamin-Mineral Premix (Kg-1) Vitamins A 800000 (IU), Vitamins D3 150000 (IU), Vitamins E 16000 (IU), Vitamins B1 1000 (mg), Vitamins B2 1400 (mg), Vitamins B3 or B5 3000 (mg),

Vitamins B6 1000 (mg), Vitamins B9 400 (mg), Vitamins B12 4 (mg), Vitamins K 1000 (mg), Vitamins PP 4000 (mg), Vitamins H2 50 (mg), Vitamins C 15000 (mg), Anti Ox (+), Sorbitol (+), Choline 90 (g), Copper 400 (mg), Iron 2000 (mg), Manganese 4800 (mg), Zinc 5000 (mg), Cobalt 20 (mg), Selenium 25 (mg), Iodine 30 (mg), Magnesium 3000 (mg).

The inclusion rate of fishmeal in the control diet is 30%, which is comparable to the current inclusion of fishmeal in commercial feeds for seabass of 40g. The inclusion rate of TM meal is 5%, 10% and 15% in the three experimental diets. These were prepared in the INRH laboratory. The ingredients were first sieved, weighed and then carefully mixed by hand. Then, water was added to the mixture to obtain the preferred consistency. The resulting mixture was fed into an experimental extruder (Henan, china, type DGP-80, with a dimension of 1750×1500×1350 mm, voltage of 380 volts, and a power of 23 kW) belonging to Aqua M'diq company. The extruded pellets produced had a diameter of 4mm and were dried in a drying oven at 60°C for 10 hours. Then, these extruded feeds were sprayed with a mixture of fish oil and sunflower oil and stirred vigorously in order to guarantee a better absorption of these oils by the granules.

2. Experimental device and fish farming

The rearing system consisted of eight cylindrical tanks with a capacity of 200 liters each; it was maintained under natural thermal and photoperiodic conditions. The supply of filtered sea water (sand filter) is in an open circuit with a renewal rate of 100% per hour. Air pumps feed diffusers were placed at the bottom of each tank to ensure sufficient oxygenation of the water.

The physico-chemical parameters (temperature, dissolved oxygen, salinity and pH) of the seawater in the experimental tanks were daily measured, prior to the distribution of the feed, using a HORIBA U-10 submersible multiparameter probe with accuracies of 0.1°C, 0.01 mg/l, 0.1g/l and 0.01

The total number of seabass used was 280. They were anesthetized with clove oil (0.4 ml/10 liters of seawater) then individually weighed. They were randomly distributed in the eight experimental tanks at a rate of 35 fish per tank, thus forming four duplicate groups each corresponding to an experimental diet (TM0, TM17, TM34 and TM50). Experimental fish were manually fed once a day until apparent satiety and six times a week for six months of the experiment. The amount of feed delivered was determined by weighing the feed boxes of the experimental diets separately before and after each feeding to assess the amount of feed consumed in each tank.

3. Fish sampling

Sampling was conducted monthly non-destructive including all fish tested to evaluate their zootechnical performance. Before each sampling, the seabass were fasted for 24 hours to reduce their stress. They were first carefully fished and then anesthetized with clove oil at a rate of 0.4 ml/10 liter. They were then counted and weighed individually. Their size was measured using an ichthyometer. At the end of the experiment, four fish from each group were randomly sampled to calculate somatic indices; they were sacrificed by overdose of anesthesia and then dissected to collect their livers and viscera.

4. Zootechnical performance and somatic indices

In order to evaluate the growth of seabass during the experiment and observe the efficiency of the use of the experimental feed, the following zootechnical parameters and indices were calculated (Table 2).

Table 2. Formulas for calculating zootechnical performance. Initial number (Ni); Final number (Nf); Average initial weight (Wi), Average final weight (Wf); Rearing time (Δt); Average weight (W); Average length (L); Total fish weight (Wt); Quantity of feed distributed (QFD).

Zootechnical parameter	
Survival rate (SR)	SR (%) = 100 × (Nf of fish / Ni of fish)
Weight gain (WG)	WG (g) = (Wf – Wi)
Relative growth rate (RGR)	RGR (%) = 100 × (Wf– Wi) /Wi
Specific growth rate (SGR)	SGR (%) = 100 × (Ln (Wf) – Ln (Wi)) /Δt
Daily growth rate (DGR)	DGR (g/j) = (Wf – Wi) / Δt
Food use parameter	
Voluntary intake (VI)	VI (g) = QFD/number of fish
Feeding rate (FR)	FR (g/j) = FCI × DGR
Feed conversion index (FCI)	FCI = Quantity of food ingested / GP
Feed efficiency (FE)	FE = 100 × WG / Quantity of food ingested
Protein Efficiency Coefficient (PEC)	PEC = 100 × WG / Wi
Somatic indice	
Condition coefficient (K)	K = 100 × (W/L3)
Hepato-somatic index (HSI)	HSI (%) = 100 × liver weight / Wt
Somatic visceral index (SVI)	SVI (%) = 100 × viscera weight / Wt

5. Analytical composition of ingredients, experimental feed and fish

At the end of the experiment, four fish from the groups fed the control diet (TM0), and those fed the best performing diet (TM50) were sacrificed by overdosing under anesthesia and stored at -20°C for 24 hours for immediate whole body composition analysis. Biochemical analyses of the experimental ingredients and feeds as well as the fish were performed in a private laboratory.

Protein was analyzed according to the Kjeldahl method, lipid according to NM 08.5.044-1996, fatty acid profile according to the adapted method NF EN ISO 12966 and ash according to MO-AA-AG-402 V01-2016.

6. Enumeration of the intestinal microflora

At the end of the experiment, three fish from the groups fed the control diet (TM0) and those fed the best performing diet (TM50) were sacrificed by overdosing under anesthesia for microbiological analysis of the gut microflora. The total weight and gut weight of sampled fish were recorded.

For the quantitative study, finely chopped intestines, from each sampled fish, were diluted in 90ml of physiological water; the mixture was homogenized with a stomacher for 1-2 minutes. A dilution series was prepared from the homogenate (10-1) to a dilution of 10-5. From each tube, 1ml of dilution was transferred to Petri dishes, and then the PCA

medium was poured into these dishes, which were incubated at 30°C for 72 hours. Bacterial colonies that developed after incubation were counted and expressed as (CFU)/g according to the following formula:

$$N = \Sigma\left(\frac{\text{Colonies}}{\text{V} \times (\text{n1} + 0,1\ \text{n2}) \times \text{D}}\right)$$

Where, N: Number of CFU/g or per ml of the initial product; Σ Colonies: Sum of the colonies of the interpretable petri dishes; V: Volume of solution deposited in each petri dish (1 ml); n1: Number of petri dish considered in the first dilution retained; n2: Number of petri dish considered in the second dilution retained, and D: Factor of the first dilution retained.

7. Histological sections of liver and intestine of fish

At the end of the experiment, two fish from the groups fed with the control diet (TM0), and those fed with the best performing diet (TM50) were sacrificed by overdosing under anesthesia to perform histological sections. The weight of the fish, their livers and intestines were recorded.

The samples of livers and intestines taken for the histological study were fixed with 10% Davidson's solution, dehydrated in alcohol and soaked in kerosene at the histopathology laboratory of the Specialized Center in Zootechnics and Marine Aquaculture Engineering of INRH in Tangier. On the kerosene blocks, 4µm sections were made with a Leica Jung 2050 rotary microtome. These sections were stained by the hematoxylin and eosin method. The sections were observed and photographed using a photomicroscope (CETI 5MP Digi-Pad Microscope Tablet Camera with 9.7" screen).

8. Statistical analysis

Data were tested for normal distribution (Anderson-Darling test) and equality of variances by the Bartlet method. A one-factor analysis of variance (ANOVA) was performed, followed by Tukey's multiple comparison test, which was performed to determine whether there were significant differences between the experimental diets (results are considered statistically significant at $P < 0.05$). In addition, orthogonal polynomial contrasts were used to further determine the linear and quadratic effects of the experimental diets. Correlation analyses were performed using Pearson correlation. The one-sample t-test was applied for the fish analyses. All statistical analyses were performed using Minitab 20.3.0.0. Thus, the data were presented as mean ± standard error of the groups.

RESULTS

1. Physicochemical parameters

The evolution of water temperature, dissolved oxygen content, salinity as well as pH and their average, maximum, minimum values, and standard error are grouped in Table (3). The physico-chemical parameters of the water, at the level of the experimental tanks, depended on the renewal, the frequency of siphoning and the fish load.

Table 3. Average, maximum, minimum values, and standard error of measured physicochemical parameters

Variable	Average	Minimum	Maximum	Standard error
Temperature (°C)	17.03	15.90	20.00	0.57
Dissolved oxygen (mg/l)	5.73	5.00	6.70	0.25
Salinity (%)	38.37	38.10	38.70	0.08
pH	7.68	7.60	7.80	0.02

2. Experimental diets

All ingredients used in the formulation of the experimental diets were analyzed for protein and lipid content (Table 4). The experimental diets (TM0, TM17, TM34, and TM50) were formulated to adequately meet the nutritional requirements of the seabass. Their biochemical compositions and energy values are shown in Table (5).

The inclusion of the TM larvae meal resulted in a higher crude lipid content, especially for the TM50 diet (20.90%) compared to the TM0 control diet (15.80%) due to the lipid content of TM larvae meal (30.60%) (Table 5) and a higher crude protein content of the TM17, TM34, and TM50 diets (43.96%, 43.40%, and 43.31% respectively) was slightly lower than the TM0 control diet (44.69%), as the protein content of the TM larvae meal (47.10%) was lower than that of the fish meal (60.73%) (Table 4).

Table 4. Analytical composition of ingredients used g/100 g

Ingredient	Protein %	Lipid %
Fishmeal	60.73	11.10
Mealworms larvae meal	47.10	30.60
Soybean meal	53.12	4.10
Wheat flour	14.72	6.80
Wheat gluten	70.04	4.60
Corn gluten	59.64	1.00
Pea protein concentrate	74.16	1.60

In addition, linear and quadratic responses revealed in the obtained values of the analytical composition of the experimental feed according to the rate of replacement of fishmeal with the meal of TM larvae ($p \geq 0.05$), as shown in Table (5). The obtained values of protein, lipid, and ash levels in the experimental feeds showed that there was no significant difference between the substitution diets ($P \geq 0.05$). Crude energy was positively correlated with lipid content and negatively correlated with protein content ($r = 0.999$; - 0.871, respectively; $P \geq 0.05$).

Table 5. Analytical composition of the four manufactured experimental diets. Means not sharing any letters are significantly different by one-sample t-test at the 0.05 significance level. Non-nitrogenous extracts (NNE) ; Protein/lipid (P/L); Protein/crude energy (P/E)

Analytical composition	Experimental diets				p Value	
	TM0	TM17	TM34	TM50	Trend L (R2 Adjust)	Trend Q (R2 Adjust)
Crude protein (%)	44.69[a]	43.96[a]	43.40[a]	43.31[a]	0.037 (0.892)	0.119 (0.997)
Crude lipid (%)	15.80[a]	17.10[a]	18.30[a]	20.90[a]	0.026 (0.922)	0.356 (0.956)
Crude ash (%)	8.56[a]	8.49[a]	8.12[a]	7.31[a]	0.084 (0.759)	0.113 (0.985)
NNE (%)	21.86[a]	21.75[a]	21.15[a]	19.06[a]	0.122 (0.657)	0.190 (0.941)
Crude energy (MJ/kg)	20.96[a]	21.28[a]	21.52[a]	22.16[a]	0.033 (0.904)	0.416 (0.929)
Crude energy (Kcal/100g)	500.16	507.85	513.54	528.98	0.033 (0.901)	0.419 (0.925)
P/L	2.83	2.57	2.37	2.07	0.003 (0.990)	0.926 (0.981)
P/E (mg/Kcal)	89.35	86.56	84.51	81.37	0.006 (0.982)	0.627 (0.975)

NNE = 100 - (%protein + %lipid+ %ash + %moisture + %cellulose); Crude energy (MJ/kg) = (23.7 × %protein) + (39.5 × %lipid) + (17.2 × (% NNE +%Cellulose)); Crude energy (Kcal/100 g) = (5.66 × %protein) + (9.44 × %lipid) + (4.11 × (% NNE + %Celluloses))

3. Zootechnical performance and somatic indices

During the 180 days of rearing, all mortality was marked as random; diets then had no significant effect on the survival rate. Zootechnical performance in terms of weight gain, relative growth rate, specific growth rate, and daily growth rate showed linear and quadratic responses to increasing rates of fishmeal substitution with TM larval meal (Table 6; $p \geq 0.05$). The final mean weight of fish fed the TM50 diet was higher (120.5 ± 3.80 g) compared to fish fed the TM0, TM17, and TM34 diets, which have a final mean weight of (118.60 ± 3.60 g), (118.70 ± 1.40 g), and (118.90 ± 1.90 g), respectively. The difference between the final mean weights of the different groups of fish fed the experimental diets was not significant ($p \geq 0.05$). Thus, fish fed with the TM50 diet recorded the best weight gain (77.70 ± 2.60) compared to other fish fed with the TM0 control diet (75.65 ± 3.35).

Growth performance (weight gain, relative growth rate, specific growth rate, and daily growth rate) showed a negative correlation with protein content (r = -0.860; -0.996; -0.888; and -0.815, respectively) and a positive correlation with lipid content (r = 0.993; 0.862; 0.959; and 0.966, respectively; $p \geq 0.05$).

Linear and quadratic responses were also detected in feed utilization efficiency according to the substitution rate of fishmeal by mealworms larvae meal for ($p \geq 0.05$), as shown in Table 6. Feed utilization efficiency is improved with increasing substitution rate of fishmeal. The feeding rate was not affected by the substitution of TM larvae meal for fishmeal (0.51 - 0.56 g. day-1). Substituting 50% of fishmeal in the TM50 diet resulted in the lowest feed conversion rate (1.18 ± 0.06) compared to the fishmeal control diet (1.33 ± 0.11). On the other hand, the feed conversion index had a strong positive correlation with crude protein content and a negative correlation with crude lipid content (r = 0.957; -0.849 respectively; $p \geq 0.05$), while the protein efficiency coefficient was negatively

correlated with crude protein content and positively correlated with crude lipid content ($r = -0.965$; 0.884 respectively; $p \geq 0.05$).

The hepato-somatic index, and somatic visceral index values showed that there was no significant difference between the groups of fish fed with the different experimental diets (Table 6; $p \geq 0.05$). Fish fed the TM50 diet had the lowest K-condition factor value (1.14 ± 0.04; $p \geq 0.05$). Crude protein content of the diets was positively correlated with the condition factor ($r = 0.647$, $p \geq 0.05$) and with the somatic visceral index ($r = 0.984$; $p \geq 0.05$), whereas crude lipid content was positively correlated with the hepato-somatic index ($r = 0.985$; $p \geq 0.05$).

4. Amino acid content

The TM50 performance diet was analyzed to determine its amino acid content and to compare it with the approximate content of the control diet TM0 (Table 7).

Table 7. Amino acid composition (g/100 g) of TM0 control diet and TM50 diet

Amino acids	TM0	TM50
Essential amino acids		
Lysine	4.93	14.00
Methionine	1.64	1.56
Threonine	3.16	7.80
Leucine	6.65	15.20
Isoleucine	3.52	9.10
Histidine	2.20	5.80
Arginine	5.50	10.80
Phenylalanine	3.94	7.10
Valine	4.05	15.50
Tryptophan	0.94	0.90
Non-essential amino acids		
Aspartic acid	7.74	7.80
Glutamic acid	15.58	17.00
Cysteines	1.21	2.24
Glycine	4.37	3.99
Tyrosine	2.81	5.10
Serine	3.77	6.50
Proline	4.95	23.10
Alanine	4.48	21.50

The amino acid composition of the TM0 and TM50 diets was not affected by the substitution of fishmeal with mealworms, but lysine, leucine, arginine, valine, and proline were higher in the TM50 diet. Therefore, the TM50 diet has a higher content of essential amino acids compared to the TM0 diet (Fig. 1). Based on the comparison of TM0 and

TM50 diets, the substitution of 50% of the fishmeal does not cause a dietary deficiency in amino acids.

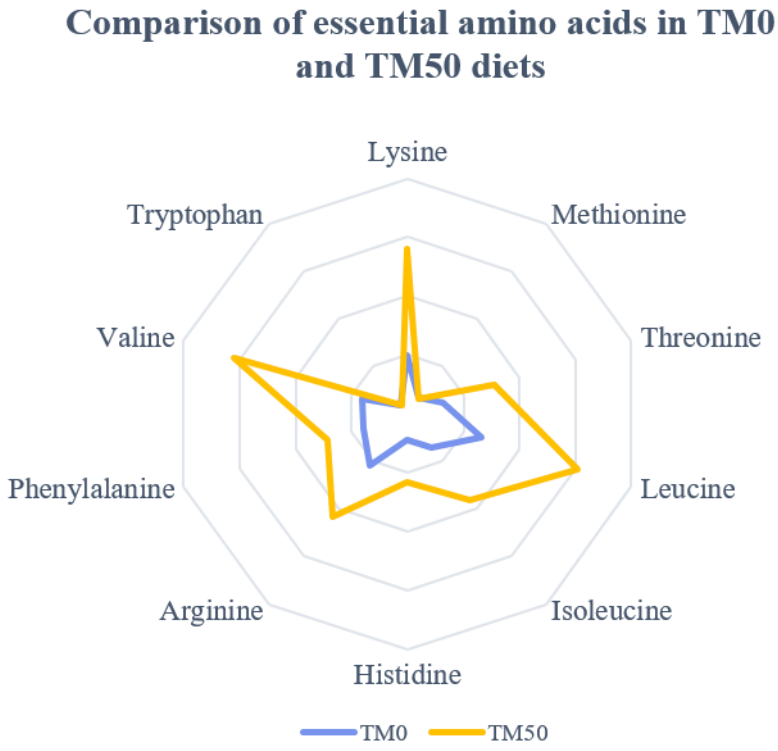

Fig. 1. Comparison graph between the essential amino acid composition of TM0 and TM50 diets

Table 6. Zootechnical performance and somatic indices calculated in the groups of fish fed with the experimental diets. Values are the mean of duplicate groups, and are presented as the mean ± standard error of the groups. Means not sharing any letters are significantly different by ANOVA at $p < 0.05$. Survival rate (SR); Average initial weight (Wi); Average final weight (Wf); Weight gain (WG); Relative growth rate (RGR); Specific growth rate (SGR); Daily growth rate (DGR); Voluntary intake (VI); Feeding rate (FR); Feed conversion index (FCI); Feed efficiency (EA); Protein efficiency coefficient (PEC); Condition factor (K); Hepato-somatic index (HSI); (SVI) Somatic visceral index.

Performances zootechniques	**Experimental diets**				**p Value**	
	TM0	**TM17**	**TM34**	**TM50**	**Trend L (R2 Adjust)**	**Trend Q (R2 Adjust)**
Zootechnical parameters						
SR (%)	97 ± 0.00[a]	97 ± 0.03[a]	97 ± 0.03[a]	97 ± 0.00[a]	1.000 (0.000)	1.000 (0.000)
Wi	42.90 ± 0.20[a]	42.60 ± 0.50[a]	42.30 ± 1.35[a]	42.80 ± 1.20[a]	0.693 (0.000)	0.281 (0.505)
Wf	118.60 ± 3.60[a]	118.70 ± 1.40[a]	118.90 ± 1.90[a]	120.50 ± 3.80[a]	0.154 (0.574)	0.249 (0.876)
GP (g)	75.65 ± 3.35[a]	76.10 ± 1.90[a]	76.65 ± 0.55[a]	77.70 ± 2.60[a]	0.029 (0.913)	0.010 (1.000)
RGR (%)	1.77 ± 0.08[a]	1.79 ± 0.07[a]	1.81 ± 0.05[a]	0.82 ± 0.01[a]	0.051 (0.849)	0.281 (0.945)
SGR (%)	0.56 ± 0.02[a]	0.57 ± 0.01[a]	0.57 ± 0.01[a]	0.58 ± 0.01[a]	0.053 (0.844)	0.988 (0.689)
DGR (g/j)	0.42 ± 0.02[a]	0.42 ± 0.01[a]	0.43 ± 0.01[a]	0.44 ± 0.02[a]	0.059 (0.827)	0.235 (0.955)
Food use parameters						
VI (g)	98.50 ± 3.50[a]	90.50 ± 1.50[a]	91.00 ± 2.00[a]	89.50 ± 1.50[a]	0.168 (0.539)	0.397 (0.685)
FR (g/j)	0.56 ± 0.02[a]	0.52 ± 0.02[a]	0.51 ± 0.03[a]	0.50 ± 0.01[a]	0.123 (0.653)	0.409 (0.750)
FCI	1.33 ± 0.11[a]	1.22 ± 0.07[a]	1.21 ± 0.04[a]	1.18 ± 0.06[a]	0.091 (0.739)	0.380 (0.835)
FE	0.76 ± 0.06[a]	0.82 ± 0.05[a]	0.83 ± 0.03[a]	0.86 ± 0.05[a]	0.064 (0.814)	0.384 (0.880)
PEC	2.53 ± 0.20[a]	2.81 ± 0.03[a]	2.85 ± 0.04[a]	2.95 ± 0.14[a]	0.065 (0.812)	0.416 (0.861)
Somatic indices						
K	1.16 ± 0.00[a]	1.17 ± 0.04[a]	1.15 ± 0.01[a]	1.14 ± 0.04[a]	0.204 (0.451)	0.440 (0.554)
HSI (%)	1.67 ± 0.12[a]	1.48 ± 0.14[a]	1.33 ± 0.10[a]	1.30 ± 0.10[a]	0.022 (0.934)	0.222 (0.985)
SVI (%)	8.23 ± 0.20[a]	7.13 ± 0.77[a]	6.72 ± 0.13[a]	6.69 ± 0.17[a]	0.095 (0.727)	0.076 (0.992)

5. Analytical composition of fish flesh

Fish fed the control diet (TM0) based on fishmeal and those fed the best performing diet (TM50) were analyzed for protein, lipid and fatty acid profiles (Table 8).

Table 8. Analytical composition of the flesh of fish fed the TM0 and TM50 experimental diets. Means not sharing any letters are significantly different by one-sample t-test at the 0.05 significance level

Analytical composition	Diets	
	TM0	**TM50**
Crude protein (%)	16.68[a]	15.88[a]
Crude lipid (%)	12.10[a]	14.10[a]
Saturated fatty acids (%)	2.12[a]	3.13[a]
Unsaturated fatty acids (%)	9.98[a]	10.98[a]
Monounsaturated fatty acids (%)	5.63[a]	6.66[a]
Polyunsaturated fatty acids (%)	4.35[a]	4.32[a]
Omega 3 (%)	0.31[a]	0.26[a]
Omega 6 (%)	3.60[a]	3.86[a]
Omega 9 (%)	3.90[a]	4.90[a]
Omega 3/ Omega 6	0.09[a]	0.07[a]

Overall, the substitution of fishmeal with TM meal slightly affected the protein and fatty acid content of the fish flesh. The obtained values of protein, lipid and fatty acid profiles of fish fed with the TM0 control diet and those fed with the TM50 diet show that there was no significant difference ($p \geq 0.05$).

6. Enumeration of total intestinal microflora

Table 9 shows the intestinal microflora counts of fish fed the control diet TM0 and those fed the top performing diet TM50. Total intestinal flora counts were 1.57×10^3 CFU/g in fish fed the TM0 diet, and 1.55×10^3 CFU/g in fish fed the TM50 diet. The difference between the results obtained in the two groups of fish fed the TM0 and TM50 diets was not significant according to one-factor ANOVA at the significance level of $\alpha = 0.05$ ($P \geq 0.05$; with $P = 0.822$).

Table 9. Results of intestinal microflora enumeration of seabass fed TM0 and TM50 diets. Means not sharing any letters are significantly different by ANOVA at the 0.05 significance level. Total weight of fish (Wt); Weight of intestine (Wi); Number of the colony (N).

Parameters	Diets	
	TM0	**TM50**
Wt	118.20 ± 5.00[a]	124.00 ± 7.85[a]
Wi	5.52 ± 0.30[a]	5.66 ± 0.35[a]
N (CFU/g)	1.57×10^{3a}	1.55×10^{3a}

7. Histological sections of livers and intestines

The microphotographs provided in figures 2 and 3, show that the level of fat accumulation in the liver of fish fed the TM50 diet is similar to that of fish fed the TM0 diet, without reaching steatosis. No fat accumulation was observed in the intestine of any group of fish.

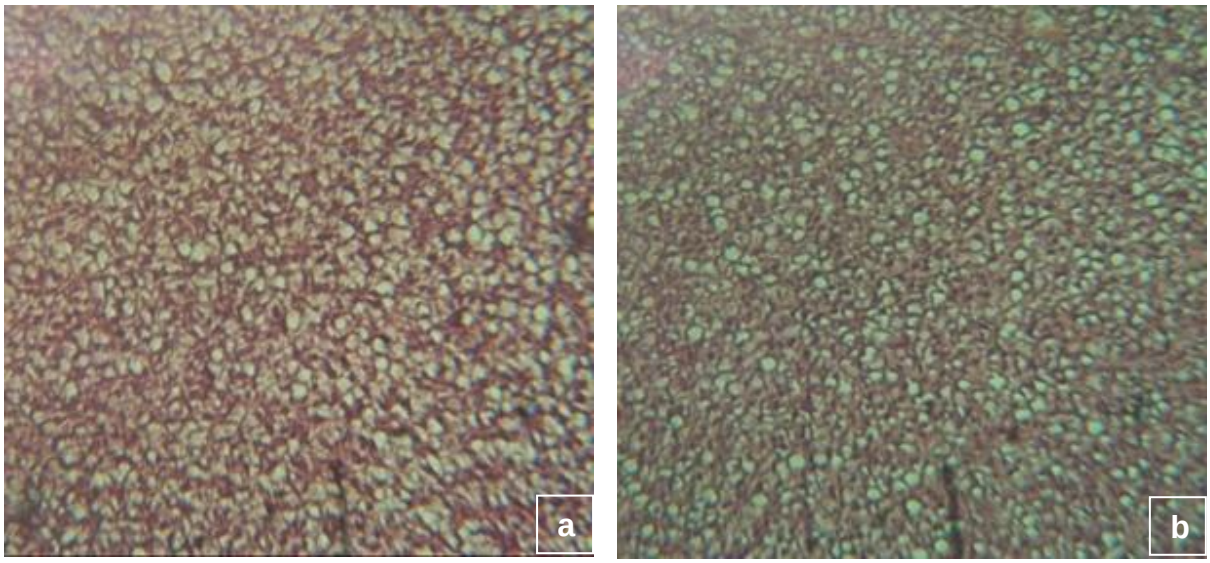

Fig. 2. Histological section of seabass liver (a: fed with TM0 diet; b: fed with TM50 diet)

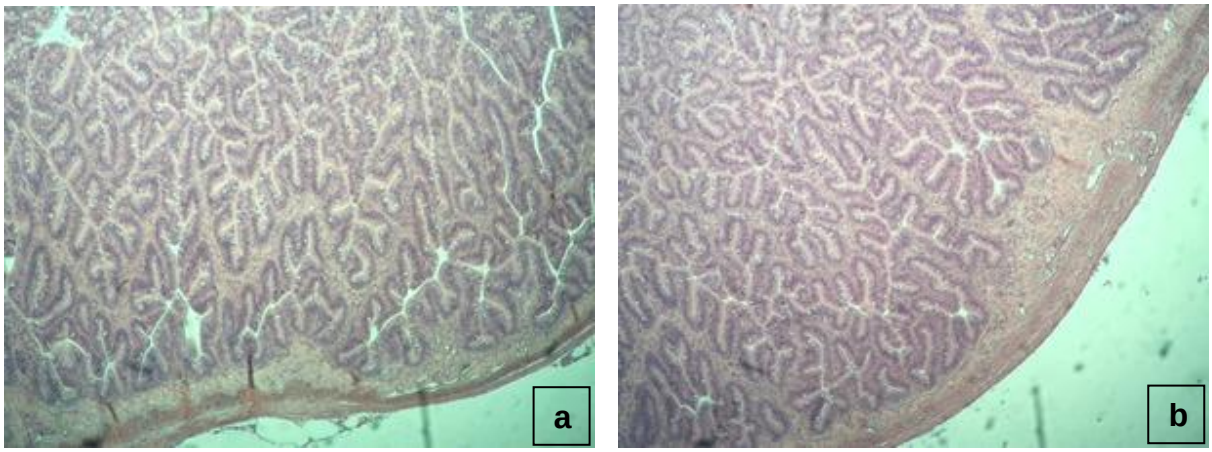

Fig. 3. Histological section of seabass intestine (a: fed with TM0 diet; b: fed with TM50 diet)

The number of caliciform cells was not affected by the diets (Table 10). The staining of the epithelial layer of the gut was homogeneous in fish fed TM50 as well as those fed TM0. The number of granulocytes in the intestinal submucosa of both diets is not exceptional. Intraepithelial lymphocytes are abundant in both diets.

Table 10. Summary of histological characteristics observed in the livers and intestines of seabass fed TM0 and TM50 diets. Histochemical reactivity in tissues was assessed by scoring staining on the following scale: negative (-), mild (+), moderate (++) and marked (+++).

Tissues	Diets	
	TM0	**TM50**
Histological section of liver		
Lipid deposits	+/-	+/-
Steatosis	-	-
Histological section of intestines		
Lipidic deposits	-	-
Caliciform cells	++	++
Enterocytes	+++	+++
Lymphocytes	++	++
Chorion	+	+

DISCUSSION

In carnivorous fishes and in particular the seabass (*Dicentrarchus labrax*), it is mainly proteins and lipids that allow them to cover their energy needs. Therefore, their food ration must be rich in these nutrients. Protein and lipids are expected to comprise (40-55%) and (16-22%) of the seabass diet, respectively **(NRC, 2011).** Most studies focused on replacing fishmeal with insect meal have recommended partial replacement **(Henry *et al.*, 2015; Tran *et al.*, 2015).** Currently, the average feed formulation meeting the nutritional requirements of seabass contains 15-30% fishmeal **(Gouvello, 2017).** Experimental feeds were then designed to meet the nutritional requirements of seabass put into experiment with a protein level that varies between 43.31% and 44.69%, and a lipid level between 18.5% and 20.9%. The essential amino acid content of all experimental diets was consistent with common knowledge of the amino acid requirements of sea bass **(Alliot, 1973; Wilson, 2003).**

The results of a gradual substitution of fishmeal with TM larval meal (17, 34, and 50%) in the diet of *Dicentrarchus labrax* were evaluated. Daily feed intake was similar in all fish groups, indicating that all diets were palatable and well accepted. Substitution of fishmeal by insect meal had no negative effect on growth performance. The same conclusion was reported by **Gasco *et al.* (2016)** and **Mastoraki *et al.* (2020)** when using 30% and 36% substitution of TM meal for fishmeal in sebass. In contrast, using 50% TM meal in the diet of Nile tilapia (*Oreochromis niloticus*) significantly reduced the feed efficiency and growth performance of this freshwater species **(Sánchez-Muros *et al.*, 2016).**

Our results showed that substitution rates of 17, 34 and 50% of fishmeal with TM larvae meal in *Dicentrarchus labrax* diets had no negative effect on the growth performance of this fish. However, the TM50 diet gave the best results in terms of final body weight, weight gain, FCI and PEC. The same results were reported for 33% and

50% substitution rates of TM meal for fishmeal in the diets of gilthead seabream **(Piccolo *et al.*, 2017)** and in those of rainbow trout **(Belforti *et al.*, 2015),** generating an improvement in SGR, FCI, and PEC. In the same sense, **Chakraborty *et al.* (2021)** were able to substitute up to 75% of fishmeal with earthworm meal, without this new feed affecting the growth and immunity of catfish *Ompok pabda*

In the present study, there was no significant difference in feed conversion index (FCI) between the experimental groups. The crude protein content of the diets showed a strong positive correlation with the FCI.

In addition, the results obtained indicated no differences in the hepato-somatic and Somatic visceral indices. According to **Dernekbaşı (2012),** the hepato-somatic index (HSI) is used to study the effect of diet on the functionality of the liver, which is an important organ for metabolism. The standard values of the HSI vary between 1 to 2%; beyond these values, it indicates that the diet causes disorders in fish, especially in carbohydrate and fat metabolism, the existence of oxidized food in the diet, excess carbohydrates and vitamin deficiency. In the present study, the HSI observed in all food groups was between 1.67 and 1.30% < 2%, which means that there is no metabolic disorder in fish. Similar results were obtained for the HSI in rainbow trout **(Belforti *et al.*, 2015),** with a decrease in this value at increasing levels of TM in the diets.

The results obtained showed that the TM0 and TM50 experimental diets resulted in similar body compositions, except the lipid content of fish fed TM50, which was slightly higher than that of TM0 fish (14.10% and 12.10% respectively) but statistically insignificant. **Gasco *et al.* (2016)** reported no difference between the proximate compositions of European seabass fed fishmeal and TM diets (25% and 50% substitution). Total fatty acid content was not affected by the inclusion of TM larval meal, as previously reported in studies using whole TM meal in European sea bass **(Gasco *et al.*, 2016)** and rainbow trout **(Iaconisi *et al.*, 2018)** diets.

Total intestinal flora counts show that there is no significant difference between fish fed the TM0 and TM50 diets. Bacteria entering the fish diet upon ingestion can adapt in the gastrointestinal tract and form a symbiotic association within the fish digestive tract, in which a large number of microbes are present. This indicates that the fish digestive tract provides favorable ecological niches for these organisms **(Uwem & Nathaniel 2019).**

Liver tissue sections from seabass fed the TM0 and TM50 experimental diets revealed normal histological characteristics. The liver of seabass revealed a normal histological structure and no sign of specific pathology apart from minor fat deposition at the end of the test period. The same result was reported by **Davies *et al.* (2020)** for *Dicentrarchus labrax* feeding with fish silage.

CONCLUSION

The research on the partial substitution of fishmeal bay TM larvae meal in the diet of seabass (*Dicentrarchus labrax*), is a contribution to the development of a feed that meets the nutritional needs of this species and the requirements of the development of a sustainable and reasonable aquaculture, allowing the preservation of fishery resources and their natural food chain. Indeed, the present study shows that it is possible to replace

50% of the fishmeal by the TM larvae meal in the diet of the seabass (*Dicentrarchus labrax*) without compromising its zootechnical performance in farming, or its nutritional quality. The results obtained provide tangible and measurable evidence for the use of TM larvae meal as an important ingredient by the aquaculture feed industry. The use of insect meals should be strongly encouraged not only to reduce the dependence on limited feed ingredients, but also to improve the production performance and health status of farmed fish.

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REFERENCES

Alliot, E.; Febvre, A.; Metailler, R. and Pastoureaud, A. (1973). Besoins nutritifs du bar (*Dicentrarchus labrax l.*) Etude du taux de protéine et du taux de lipide dans le régime. Archimer Actes Colloq.

Ballester-Lozano, G.F.; Benedito-Palos, L.; Estensoro, I.; Sitjà-Bobadilla, A.; Kaushik, S. and Pérez-Sánchez, J. (2015). Comprehensive biometric, biochemical and histopathological assessment of nutrient deficiencies in gilthead sea bream fed semi-purified diets. Br J Nutr 114:713–726.

Belforti, M.; Gai, F.; Lussiana, C.; Renna, M.; Malfatto, V.; Malfatto, L.; De Marco, M.; Dabbou, S.; Schiavone, A.; Zoccarato, I. and Gasco, L. (2015). *Tenebrio Molitor* Meal in Rainbow Trout (*Oncorhynchus Mykiss*) Diets: Effects on Animal Performance, Nutrient Digestibility and Chemical Composition of Fillets. Italian Journal of Animal Science 14:4170.

Biasato, I.; Gasco, L.; De Marco, M.; Renna, M.; Rotolo, L.; Dabbou, S.; Capucchio, M.T.; Biasibetti, E.; Tarantola, M.; Bianchi, C.; Cavallarin, L.; Gai, F.; Pozzo, L.; Dezzutto, D.; Bergagna, S. and Schiavone, A. (2017). Effects of yellow mealworm larvae (*Tenebrio molitor*) inclusion in diets for female broiler chickens: implications for animal health and gut histology. Animal Feed Science and Technology 234:253–263.

Bovera, F.; Piccolo, G.; Gasco, L.; Marono, S.; Loponte, R.; Vassalotti, G.; Mastellone, V.; Lombardi, P.; Attia, Y.A. and Nizza, A. (2015). Yellow mealworm larvae (*Tenebrio molitor*, L.) as a possible alternative to soybean meal in broiler diets. British Poultry Science 1–7.

Bovera, F.; Loponte, R.; Marono, S.; Piccolo, G.; Parisi, G.; Iaconisi, V.; Gasco, L. and Nizza, A. (2016). Use of *Tenebrio molitor* larvae meal as protein source in broiler diet: Effect on growth performance, nutrient digestibility, and carcass and meat traits. Journal of Animal Science 94:639–647.

Chakraborty, P.; Islam, M.R.; Hossain, M.A.; Fatema, U.K.; Shaha, D.C.; Alam Sarker, M.S. and Akter, T. (2021). Earthworm meal (*Perionyx excavatus*) as an alternative protein source to fish meal in feed for juvenile butter catfish (*Ompok pabda*). Aquacult Int 29, 2119–2129.

Davies, S.J.; Guroy, D.; Hassaan, M.S.; El-Ajnaf, S.M. and El-Haroun, E. (2020). Evaluation of co-fermented apple-pomace, molasses and formic acid generated Sardine based fish silages as fishmeal substitutes in diets for juvenile European sea bass (*Dicentrachus labrax*) production. Aquaculture 521:735087.

De Marco, M.; Martínez, S.; Hernandez, F.; Madrid, J.; Gai, F.; Rotolo, L.; Belforti, M.; Bergero, D.; Katz, H.; Dabbou, S.; Kovitvadhi, A.; Zoccarato, I.; Gasco, I. and Schiavone, A. (2015). Nutritional value of two insect larval meals (*Tenebrio molitor* and *Hermetia illucens*) for broiler chickens: Apparent nutrient digestibility, apparent ileal amino acid digestibility and apparent metabolizable energy. Animal Feed Science and Technology 209:211–218.

Dernekbaşı, S. (2012). Digestibility and liver fatty acid composition of rainbow trout (*Oncorhynchus mykiss*) fed by graded levels of canola oil. Turkish Journal of Fisheries and Aquatic Sciences 12:105-113.

European Directive 2010/63/EU of the European Parliament and the Council on the protection of animals used for scientific purposes. Official Journal of the European Communities, L 276, 2010.

FAO (2014). Insectes comestibles : perspectives pour la sécurité alimentaire et l'alimentation animale. FAO, Rome

FAO (2020). La situation mondiale des pêches et de l'aquaculture 2020. FAO

Gai, F.; Peiretti, P.; Brugiapaglia, A. and Casco, L. (2017). Effects of dietary protein source and feeding regime on growth performance, nutrient digestibility, fatty acids, and quality characteristics of rainbow trout, *Oncorhynchus mykiss*, Fillets. Journal of the word aquaculture society. 47:449-507.

Gasco, L.; Henry, M.; Piccolo, G.; Marono, S.; Gai, F.; Renna, M.; Lussiana, C.; Antonopoulou, E.; Mola, P. and 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. Animal Feed Science and Technology 220:34–45.

Gatlin, D.M.; Barrows, F.T.; Brown, P.; Dabrowski, K.; Gaylord, T.G.; Hardy, R.W.; Herman, E.; Hu, G.; Krogdahl, A.; Nelson, R.; Overturf, K.; Rust, M.; Sealey, W.; Skonberg, D.; Souza, J.E.; Stone, D.; Wilson, R. and Wurtele, E. (2007). Expanding the utilization of sustainable plant products in aquafeeds: a review. Aquaculture Res 38 :551–579.

Gouvello, R. and Simard F. (2017). Durabilité des aliments pour le poisson en aquaculture : Réflexions et recommandations sur les aspects technologiques, économiques, sociaux et environnementaux. Gland, Suisse : UICN, et Paris, France : Comité français de l'UICN. 296 pp.

Hardy, R.W. (2010). Utilization of plant proteins in fish diets: effects of global demand and supplies of fishmeal. Aquaculture Research 41:770–776.

Henry, M., Gasco, L., Piccolo, G. and 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.

Iaconisi, V.; Bonelli, A.; Pupino, R.; Gai, F. and Parisi, G. (2018). Mealworm as dietary protein source for rainbow trout: Body and fillet quality traits. Aquaculture 484:197–204.

Makkar, H.P.S.; Tran, G.; Heuzé, V. and Ankers, P. (2014). State-of-the-art on use of insects as animal feed. Animal Feed Science and Technology 197:1–33.

Mastoraki, M.; Mollá Ferrándiz, P.; Vardali, S.C.; Kontodimas, D.C.; Kotzamanis, Y.P.; Gasco, L.; Chatzifotis, S. and 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 528 :735511.

NRC M. (2011) National Research Council (NRC): Nutrient requirements of fish and shrimp. Aquacult Int 20:601–602.

Oliva-Teles, A.; Enes, P. and Peres, H. (2015). Replacing fishmeal and fish oil in industrial aquafeeds for carnivorous fish. In: Feed and Feeding Practices in Aquaculture. Elsevier, pp 203–233

Piccolo, G.; Iaconisi, V.; Marono, S.; Gasco, L.; Loponte, R.; Nizza, S.; Bovera, F. and 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*). Animal Feed Science and Technology 226:12–20.

Rakotozanany, V.S. (2012). Effets du remplacement de la farine de poisson par du gluten de blé sur la croissance des alevins d'esturgeon sibérien (Acipenser baerii Brandt, 1869).

Sánchez-Muros, M-J; Barroso, F.G. and Manzano-Agugliaro, F. (2014). Insect meal as renewable source of food for animal feeding: a review. Journal of Cleaner Production 65:16–27.

Sánchez-Muros, M. ; de Haro, C. ; Sanz, A. ; Trenzado, C.E. ; Villareces, S. and Barroso, F.G. (2016). Nutritional evaluation of Tenebrio molitor meal as fishmeal substitute for tilapia (*Oreochromis niloticus*) diet. Aquacult Nutr 22:943–955.

Tran, G.; Heuzé, V. and Makkar H.P.S. (2015). Insects in fish diets. Animal Frontiers 5 :37–44.

Uwem, M. and Nathaniel, I. (2019). Comparative study of bacterial load and species diversity in the African Catfish (*Clarias gariepinus*) cultured in contrasting aquaculture tanks in Uyo, Nigeria. Animal Research International. 16: 3443 – 3449

Wilson, R.P. (2003). 3 - Amino Acids and Proteins. In: Halver JE, Hardy RW (eds) Fish Nutrition (Third Edition). Academic Press, San Diego, pp 143–179