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Research Article

Evaluation of yellow mealworm larvae (*Tenebrio molitor*, Insecta, Tenebrionidae) meal as a dietary protein source in Asian Seabass (*Lates calcarifer*) based on growth and some biochemical parameters

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Abstract

This study was conducted to assess the effects of *Tenebrio molitor* larvae meal (TM) in partial substitution of fishmeal (FM), on growth performance, hematological indices, plasma biochemical and antioxidant enzyme activities of Asian Seabass (*Lates calcarifer*). Experiment carried out with 144 pieces of Asian Seabass (mean weight±SE, 54±1.2 g) in a semi-recirculating fish culture system. Fishes were randomly distributed into four groups with three replicates as completely random design. All treatments were fed with iso-nitrogenous and iso-energetic diets at a substitution rate of 0, 20, 40 and 60 % of TM. The experiment was setup for eight weeks and feeding carried out at 3% of body weight/day. Results showed that there is no significant differences (p>0.05) in the growth performance, chemical body composition and protein utilization at the end of the experiment. However, weight gain, lipids and viscerosomatic index showed significant differences among the examined treatments (p<0.05). The red blood cells was not affected by TM diet, but the hemoglobin, hematocrit and mean corpuscular hemoglobin concentration levels decreased with increasing levels of TM (p<0.05). Plasma biochemistry analysis of triglyceride, glucose, cortisol, lysozyme and alkaline phosphatase levels increased significantly with increasing TM in the diet (p<0.05). Increasing the level of TM in fish diets caused an increase in the activity of glutathione peroxidase and catalase, while no significant differences were observed in the activity of superoxid dismutase and malone di aldehyde among the different diets. Overally, this study demonstrates that meal of *T. molitor* can be used as fishmeal replacement up to 40% of diet and is an alternative source of protein in Asian seabass diets.

Keywords: Body composition, Fishmeal replacement, Growth, *Lates calcarifer*, Mealworm, *Tenebrio molitor*

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Introduction

One of the biggest problems in the world is food insecurity due to human population growth and their activities in aquaculture, poultry and ruminants to prepared ingredient to satisfy its high protein requirements (Kohno *et al*., 1986; Matthew, 2009). Animal proteins that represented by fish meal is the most valuable protein source in terms of its containment of essential amino acids and minerals, and despite its use in forming diets, it has some disadvantages, including a high price (NRC, 2011) and lack of sustainability due to the continuous decline of fish stocks (White *et al*., 2004; Panserat, 2009). Therefore, there is an urgent need to find and utilize less expensive and more sustainable protein sources within aquafeeds. Thus, many protein-rich plant sources, such as soybeans have been used in farmed fish diets to replace fish meal (Espe *et al*., 2006; Gatlin *et al*., 2007). However, it was considered a source of concern due to the presence of anti-nutritional factors (Ogunji, 2004; Collins, 2014) that may cause gastrointestinal inflammation (Merrifield *et al*., 2011; Gai *et al*., 2012), high content level of fiber and non-starch polysaccharides, and inadequate fatty acids and amino acids profiles (Gai *et al*., 2012) as well as low palatability of the feed (Papatryphon and Soares, 2001; Gatlin *et al*., 2007).

Recently, the efforts of scientists have turned to use of insect meal as one of the most important available protein alternatives, mostly due to low cost production and high nutritional value. Generally, insects have many advantages; ease to culture on organic matter and other waste materials (Józefiak *et al*., 2016; Józefiak *et al*., 2018), having high feed conversion efficiency (FAO, 2013; Van Huis, 2013), lower levels of greenhouse gases and ammonia emissions (Oonincx *et al*., 2010), and increasing the risk of infection transmission (Van Huis, 2013) and reducing the use of fish meal (Barroso *et al*., 2014). In addition, the diet containing of insect meal are rich in amino acids, fats, vitamins and minerals (Rumpold and Schlüter, 2013; Barroso *et al*., 2014; Nowak *et al*., 2016). Furthermore, the insect larvae can be grown in dense environments (limited spaces) (Makkar *et al*., 2014; Henry *et al*, 2015) as well as fresh and saline water environments (Merritt and Cummins, 1996; FAO, 2013). They are cost-effective in terms of water and energy requirements, there are antifungal agents, and antibacterial peptides in many insects (Nawaz *et al*., 2018).

Recently, most species of insects used in livestock, poultry and aquatic organisms feeds production are *Hermetia illucens* (black soldier fly), *Musca domestica* (common house fly), *Tenebrio molitor* (yellow mealworm), *Bombyx mori* (silkworm) and several locusts (Van Huis, 2013). *T. molitor* meal is considered as a good protein source for fish because of its high protein and essential amino acid content (Rumpold and Schlüter, 2013; Nowak *et al*., 2016). *T. molitor* larvae meal (TM) was used in broiler chickens

(Bovera *et al*., 2015; De Marco *et al*., 2015; Biasato *et al*., 2016) and laying hens (Giannone, 2003; Wang *et al*., 2005). In addition, it has been used in many fish diets as a partial alternative to fish meal; in rainbow trout (*Oncorhynchus mykiss*) (Gasco *et al*., 2014; Belforti *et al*., 2015), African catfish (*Clarias gariepinus*) (Ng *et al*., 2001), common catfish (*Ameiurus melas*) (Roncarati *et al*., 2015), tilapia (*Oreochromis nilotica*) (De Haro *et al*., 2011), Gilthead seabream (*Sparus aurata*) (Piccolo *et al*., 2017) and European sea bass (*Dicentrarchus labrax*) (Gasco *et al*., 2016) and Asian seabass **(***Lates calcarifer* **).** The Asian seabass or barramundi is widely distributed in tropical and subtropical waters of the Pacific Ocean (Katayama and Taki, 1984; Matthew, 2009) and considered as one of the most important cultured fish in the world (FAO, 2006; Glencross, 2006). This species has high nutritional and commercial values and the quality of its firm and flavorful flesh that also grow in brackish and fresh waters (Matthew, 2009). The aim of this study was to assess the replacement of fish meal prepared by yellow mealworm as a dietary protein source for barramondi and determine its effect on growth performance, serum biochemical parameters and liver antioxidant enzymes activity.

Materials and methods

Fish and experimental conditions

This study was carried out at aquaculture facilities in the Isfahan University of Technology, (Isfahan, Iran), equipped with a closed water recirculating system. Asian sea bass (*L. calcarifer*) individuals were purchased from Ramoz Marine Fish Breeding Center (Bushehr, Iran). Before starting the experiment, fish were acclimated from seawater (36 g/L) to saline groundwater (15 g/L) in a circular fiberglass tank (volume of 1 m^3) by gradually decreaseing the salinity over 12 days at a rate of 2 mg/L daily. The chemical composition (mg/L) of used groundwater were borom (2.5), calcium (540.6), potassium (54.7), magnesium (744.8), sodium (3371) and strontium (19). During the two-week acclimatization period, the fish were fed a commercial diet (Dorindaneh Co., Shahrekord, Iran) containing of 12 % moisture, 42 % protein, 18 % lipid, 14.8 % carbohydrate and 10 % ash. A total of 120 fish with initial mean weight of 54±1.2 g (mean±SE) were randomly distributed into 12 recirculating tanks (approximately 300 L; 80 cm in diameter; 70 cm in height), at a density of 10 fish/ tank. The feeding experiment set up for eight weeks and during the experimental period the fish were hand-fed to apparent satiation three times daily (9:00, 13:00 and 17:00). The important water quality parameters were monitored weekly. These parameters including of the water temperature, dissolved oxygen, pH and ammonia content were kept constant at 27°C, 6.5 mg/L, 7.5 and 0.02 mg/L, respectively. The photoperiod of 12:12 (hours light:hours dark) was maintained throughout the experiment. Dead fish were collected and weighed and then

the mortalities were recorded daily.

Fish diets preparation

The dried *T. molitor* larvae and fish waste meal were purchased from a local breeder (Isfahan Province, Iran) and Rizdaneh company, Isfahan). The *T. molitor* larvae was ground into meal an electric blender (A11, IKA, Germany) at nutrition laboratory of Isfahan University of Technology. Then, four isoenergetic and isonitrogenic experimental diets were prepared for Asian seabass based on previous researches (Aquacop-Cuzon *et al*., 1989; Ambasankar *et al*., 2009). These diets were prepared using a commercial meat grinder with a 2 mm screen which were air-dried for 24 h, ground to a suitable size and stored at -2°C in air-tight plastic bags until used. Replacement levels were as follows: a control diet (TM0) without mealworm larvae and three diets in which fish waste meal was partially replaced with TM at 20% (TM20), 40% (TM40) and 60% (TM60). The diets ingredients and chemical composition of TM and FWM diets are presented in Table 1.

Table1: Ingredients (%) and proximate composition (%) of the experimental diets, *Tenebrio molitor* meal (TM) and fish waste meal (FWM).

			Experiment diets			
	FWM	**TM**	**control**	**TM 20**	**TM 40**	**TM60**
Ingredients (g /kg)						
Mealworm larvae			-	4.34	8.48	13.02
Fish waste meal[a]			21.7	17.36	13.02	8.68
Soybean meal			21.7	21.7	21.7	21.7
Wheat gluten			21.7	21.7	21.7	21.7
Wheat flour			21.9	21.9	21.9	21.9
Inactivated baker's yeast			3	3.2	3.5	4
Glumatine [b]			0.5	0.5	0.5	0.5
Lysine			0.5	0.5	0.5	0.5
Methionine			0.5	0.5	0.5	0.5
Salt			1	1	1	1
Molasses			3	3	3	3
Vitamin-mineral premix [c]			1	1	1	1
Vitamin C			0.5	0.5	0.5	0.5
Soybean oil			3	2.8	2.5	2
Proximate composition (g/ kg)						
Dry matter	93.34	97.72	98.32	97.57	98.52	97.5
Crude protein	48.78	48.32	45.87	45.96	45.83	45.72
Lipid	18.73	33.9	8.79	8.86	9.15	9.26
Ash	20.37	3.1	8.42	7.99	6.66	6.72
Carbohydrate[c]	5.56	12.71	34.48	34.94	35.48	36.49
Gross Energy (kcal/kg)	-	-	4128.13	4140.8	4185.6	4231.41

[a] Wastes from the production of fish fillets, and fresh fish supply stores (fish head, visceral by-products)

[b] Glumatine is a manufacturing by-product, which is used as a binder in aquatic food (Karimi *et al*., 2018). [c] Vitamins (mg/kg diet): cholecalciferol, 2,000 (IU/kg diet); retinol, 18,000 (IU/kg diet); menadione sodium bisulphate, 10; α-tocopherol, 35; riboflavin, 25; thiamine, 15; nicotinic acid, 200; Ca pantothenate, 50; pyridoxine, 5; cyanocobalamin, 0.02; folic acid, 10; biotin, 1.5; ascorbyl monophosphate, 50; inositol, 400.
Minerals (mg/kg diet): copper sulphate, 19.6; cobalt sulphate, 1.91; iron sulphate, 200; potassium iodide, 0.78; sodium fluoride, 2.21; manganese oxide, 26; magnesium oxide, 830; sodium selenite, 0.66; zinc oxide, 37.5; dibasic calcium phosphate, 5.93 (g/kg diet); potassium chloride, 1.15 (g/kg diet); sodium chloride, 0.44 (g/kg diet). (According to the label of vitamin-mineral premix)

* The net energy of the diet Calculated based on carbohydrate= 4.01, protein= 4.01, and lipid= 9.03 (Kellner and Patience, 2017; Tacona, 1990).

[C] Carbohydrate = 100- (protein+ lipid + Ash + Moisture)

Growth performance

At the end of the experiment (eight weeks), fishes were starved for one day and then three fish from each tank were randomly sacrificed through an overdose of anesthetic (tricaine methanesulfonate- MS222: 200 mg/ L**).** The growth performance indices, the protein efficiency ratio (PER), protein productive value (PPV), viscerosomatic index (VSI), hepatosomatic index (HSI) and the body weight gain (BW) were calculated according to the following equations (Li *et al*., 2009):

Feed conversion ratio (FCR) = feed intake (g)/weight gain (g)
Protein efficiency ratio (PER) = weight gain (g)/total protein given (g)
Protein productive value (PPV) = $(W_t \times P_2 – W_0 \times P_1)/ (I_d \times P)$.
Feed intake (FIg/day) =100× total amount of the feed consumes (g)/$[(W_0+W_t)/2]/t$

Where W_0 is the initial body weight (g), W_t is the final body weight (g), P is the crude protein percentage of the diets, P_2 is the crude protein percentage of the carcass at the end of the experiment, P_1 is the crude protein percentage of the carcass at the beginning of the experiment, I_d is the amount of food consumed and t is the experiment period (day). The survival rate was estimated as follows:

Survival rate = (final number of fish/initial number of fish) × 100
BW = final body weight (g) – initial body weight (g)
Weight gain (WG %)=[(final body weight–initial body weight)/ initial body weight]×100
Specific growth rate (SGR %/day) = [(Ln final body weight – Ln initial body weight)/ number of feeding days] × 100

Finally, somatic indices such as hepato-somatic (HSI), viscero-somatic (VSI) and the condition factor (CF) index were calculated as follows:

Condition Factor (CF %) = (weight of fish/ (length of fish)^3) × 100
Hepatosomatic index (HSI %) = (weight of liver/weight of fish) × 100
Viscerasomatic index (VSI %) = (weight of viscera/weight of fish) × 100

Biochemical analyses of diets and fish carcass

At the beginning of the experiment, carcasses of three fish were weighed and kept at freezer at –20°C for initial composition analysis. At the end of the trial, all fishes were starved for one day (to empty the digestive tract). After the biometry of the fishes, the carcasses of three fish were randomly collected in each treatment and then dried, and ground for analyze the approximate chemical composition of the carcass. The proximate analyses of the carcass of the fish, mealworm larvae, fish waste meal and the four experimental diets

were determined according to AOAC (2005) (Table 1). The samples were oven-dried at 105°C for 24 hours to reach a constant weight. Crude protein by using the Kjeldahl method (Kejeltec V40 Auto Analyzer, Bakhshi, Iran), crude lipid by using the Soxhlet extraction method (model 6XI Extraction Unit, Bakhshi, Iran), and ash by incinerating dried samples at 550°C for 4 h, using a Nabertherm muffle furnace (Model:K, Germany) were estimated.

Plasma biochemical parameters

Three fish in each tank were randomly selected and anesthetized using clove powder (200 mg/L) at the end of the experiment. The blood samples were taken from the caudal vein using heparinized plastic syringes, then plasma samples were separated by centrifugation at 3500 rpm at a temperature of 4°C for 10 min, and then it was kept at -20°C for biochemical analyzes. Plasma parameters were measured for concentrations of total protein (TP), triglyceride (TG), cholesterol (CHOL), aspartate aminotransferase activity (AST), alanine aminotransferase activity (ALT), alkaline phosphatase activity (ALK), albumin (ALB), lysozyme, lactate dehydrogenase (LDH), glucose (Glu) and cortisol (Cort) on an automated blood analyzer (Mindray BS 400, Al-Zahra Laboratory Isfahan, Iran) by using of Pars Azmoun Commercial Kits (Karaj, Iran).

Liver antioxidant capacity

Liver antioxidant activity was determined as superoxide dismutase (SOD) (Kono, 1978), catalase (CAT) (Koroluk *et al.*, 1988), the malondialdehyde (MDA) (Placer *et al*., 1966). The glutathione peroxidase (GPx) was measured using the RADOX (UK) Kit.

Statistical analysis

The experiment was carried out by a completely randomized design. The normality and homogeneity of data were assessed prior to any statistical analysis. SPSS 25.0 Software was used for all statistical analysis of the data. Normality of data was evaluated using Kolmogorov-Smirnov test. The One-Way ANOVA was used for significant differences of treatments. Difference between different means was determined by Duncan multiple range test at 95% confidence limit.

Results

Growth performance and biometry

The effects of treated diets during the growth trial are shown in Table 2. There were no significant differences ($p>0.05$) among the groups treated with different levels of TM, neither specific growth rate (SGR), feed conversion ratio (FCR), feed intake (FI), protein efficiency ratio (PER), productive protein value (PPV) nor for the survival rate as compared to the control diet. However, the final body weight (FBW), body weight increase (BW) and weight gain (WG %) showed significant differences among the treatments

(p<0.05), and the weight of the fish grew almost three times at the end of the experiment. In relation to biometric indices, no significant differences (p>0.05) were found in both condition factor (CF) and hepatosomatic index (HSI) when the TM included in the diets (Table 2). On the contrary, viscerosomatic index (VSI) showed significant differences among the treatments (p<0.05).

Table 2: Growth performances, survival rate and somatic indices of Asian seabass (*Lates calcarifer*) fed the experimental diets. Data are means ±SE, n=3.

Growth performance	TM0	TM20	TM40	TM60
Initial body weight (g)	54.65±0.9	55.03±0.79	52.91±0.19	56.12±0.17
Final body weight (FBW) (g)	109.25±6.46^{c}	124.61±3.46^{b}	129.49±5.25^{a}	131.41±2.15^{a}
BW (g)	54.6±5.5^{c}	69.57±4.25 ^{b}	76.58±5.05 ^{a}	75.28±2.6 ^{a}
WG (%)	99.74±8.5^{c}	127.09±5.49^{ab}	144.6±7.29^{a}	119.56±3.86^{b}
SGR (%.day^{-1})	1.25±0.07	1.45±0.3	1.61±0.2	1.23±0.17
FCR	1.06±0.02	1.12±0.13	0.92±0.06	1.01±0.17
FI (g/day)	1.28±0.01	1.45±0.02	1.36±0.01	1.42±0.09
Protein utilization				
PER	2.04±0.04	2.02±0.24	2.13±0.12	1.87±0.36
PPV (%)	17.16±1.05	15.45±3.42	15.74±1.96	15.89±2.87
Survival (%)	83.85±3.55	88.89±0.0	88.89±0.0	81.66±1.66
Biometric indexes				
CF (g.cm^{-3})	1.08±0.01	1.08±0.003	1.1±0.04	1.13±0.01
HSI (%)	1.75±0.25	1.36±0.06	1.9±0.22	1.95±0.01
VSI (%)	7.22±0.18^{b}	7.39±0.11^{b}	8.59±0.36^{a}	8.91±0.41^{a}

Different letters in each raw show significant difference at 95% confidence limit among the experimental treatments ($p<0.05$). Four dietary treatments: TM0: fish waste meal group; TM20, TM40 and TM60: *Tenebrio molitor* larvae meal at 20, 40 and 60% substitution rate of FM groups, respectively.

Chemical composition of carcasses

The results of the carcass proximate composition of the fish fed with TM presented in Table 3. The chemical analysis of carcass showed no significant differences in moisture, dry matter, protein and ash among the treatments (p>0.05). In comparison, there were significant differences in the lipid content (p<0.05) which increased gradually with the increased TM concentration in the feeding groups.

Table 3: Effect of TM meal on proximate composition in carcasses of Asian seabass (*Lates calcarifer*). Data are means ±SE, n = 3.

	TM0	TM20	TM40	TM60
Moisture (%)	71.04±0.81	70.82±0.53	69.68±0.43	70.79±0.1
Protein (g/100 g DM)	70.17±1.18	68.42±1.23	62.68±1.6	64.18±4.23
Lipids (g/100 g DM)	16.25±0.36^{a}	18.13±0.19^{ab}	19.81±0.75^{bc}	20.64±0.19^{c}
Ash (g/100 g DM)	14.05±0.61	11.61±1.96	13.4±0.62	12.06±0.46

Different letters in each raw show significant difference at 5% level among experimental treatments ($p<0.05$). Four dietary treatments: TM0: fish waste meal group; TM20, TM40 and TM60: *Tenebrio molitor* larvae meal at 20, 40 and 60% substitution rate of FM groups, respectively.

Blood indices

Hematological indices of Asian seabass cultured on TM meal diets were mentioned in Table 4. Results showed that there were no significant differences in red blood cells, mean erythrocyte volume (MCV), and mean hemoglobin (MCH) ($p>0.05$). However, there were significant differences in the white blood cells, mean concentration of hemoglobin in red blood cells (MCHC) and hemoglobin content (Hb), and hematocrit (Hct) ($p<0.05$).

Table 4: Effect of TM meal on blood indicators of Asian seabass (*Lates calcarifer*). Data are means ±SE, n=3.

Index	C	TM20	TM40	TM60
RBC (10^6/mm^3)	6.6×10^6	4.1×10^6	6×10^6	5.3×10^6
WBC (10^4/mm^3)	3.4×10^{4b}	4.6×10^{4b}	5.8×10^{4ab}	7.5×10^{4a}
MCV (fl)	63.94±19.93	58.71±5.83	60.87±27.03	58.2±2.90
MCH (pg/cell)	9.66±2.12	18.56±3.25	10.8±3.71	11.53±1.76
MCHC (g/dL)	15.93±1.81^b	31.44±3.84^a	19.63±2.33^b	19.73±2.47^b
Hb (g/dL)	5.76±0.68^{ab}	7.51±0.8^a	4.95±0.56^b	6.07±0.61^{ab}
Hct (%)	32.16±2.58^a	24±0.51^b	25.5±1.33^b	31.16±0.9^a

Different letters in each raw show significant difference at 5% level among experimental treatments ($p<0.05$).

Plasma biochemistry

Triglyceride (TG), glucose, cortisol and alkaline phosphatase (ALK) levels as plasma biochemical parameters were measured in Asian seabass (Table 5). These factors were increased with an increase in TM of the diet. The fishes offered the diet containing the highest TM level (TM60) showed significantly higher TG, glucose and ALK values compared to than those fed the TM-free control diet (TM0). Dietary treatment had no significant influence on any of the other biochemical parameters measured in the present study ($p>0.05$).

Table 5: Effects of TM meal on plasma biochemical indices of Asian seabass (*Lates calcarifer*). Data are means ±SE, n=3.

Index	TM0	TM20	TM40	TM60
Glu (mg/dL)	45.66±11.05^b	42±4.51^b	54.33±8.33^{ab}	77.33±3.92^a
TG (mg/dL)	77.33±7.83^b	117.33±9.49^{ab}	104.66±18.09^{ab}	151±21.65^a
CHOL (mg/dL)	223±7.51	220.33±7.05	222.33±17.24	212.33±8.19
AST (IU/L)	29±3.51	34±7.51	24.66±6.96	30±6.08
ALT (IU/L)	16.66±3.17	18±1.52	18.66±2.18	15.33±3.84
ALK (IU/L)	212.66±13.44^{ab}	253.33±22.15^a	176±14.00^b	264±24.44^a
Alb(g/dL)	1.51±0.04^a	1.48±0.04 ^{a}	1.24±0.07^b	1.28±0.04 ^{b}
TP(g/dL)	4.43±0.26	4.4±0.21	4.16±0.2	4.3±0.25
LDH (IU/L)	472.33±35.48	782±99.41	786±128.57	833.33±143.65
Cort (mg/dL)	1.05±0.31^b	1.47±0.42^b	1.17±0.53^b	2.85±0.17^a

Different letters in lines show significant difference at 5% level among experimental treatments ($p<0.05$).

Antioxidant enzyme activities and immune parameters

The antioxidant capacity in liver of Asian seabass showed that fish fed with TM showed difference in the antioxidant parameters of GPX, CAT and Lysozyme, whereas high values in GPX activity was observed in TM60

treatment (Table 6). On the other hand, no significant differences were observed in the activity of any MDA and SOD ($p>0.05$). In addition, the use of different levels of TM meal increased the lysozyme activities, with highest value 40% TM treatment.

Table 6: Effects of TM meal on oxidative enzyme and immune indices of Asian seabass (*Lates calcarifer*). Data are means ±SE, n=3.

Index	C	TM20	TM40	TM60
MDA (μmol/L)	7.36±0.29	7.56±0.44	7.6±0.51	6.85±0.03
GPX (IU/L)	124.84±8.67^{d}	134.76±8.86^{c}	179.14±65.03^{b}	189.52±3.14^{a}
CAT (IU/L)	1.07±0.22^{ab}	1.67±0.22^{a}	0.85±0.25^{b}	0.51±0.02^{b}
SOD (IU/L)	0.86±0.01	0.92±0.03	0.9±0.02	0.9±0.01
Lysozyme (U/ml/min)	17.87±2.38^{d}	53.67±5.62^{b}	87.4±6.64^{a}	37.15±4.18^{c}

Different letters in lines show significant difference at 5% level among experimental treatments ($p<0.05$).

Discussion

In this experiment, the effects of the partial replacement of FM with TM as a new protein source in Asian seabass feed was tested. The results showed that TM could be a valuable source of protein in Asian seabass at TM20, TM40, and TM60 as a substitute for FM, and also showed that replacement of dietary fishmeal with different levels of TM meal not only had no negative or significant effects on carcasse composition and growth performance parameters such as SGR, FCR, FI, PER, PPV, CF and HSI but also improved WG and SGR. One of the reasons for the growth of fish fed the diets containing TM could be attributed to improvement in the efficiency of nutrient utilization by fish, which might possibly be due to the presence of chitinous materials in the diets (Finke, 2007; Marono *et al*., 2015). The chitinous materials can modulate the gastrointestinal microbiota and potentially impart an improvement in growth efficiency when administered in adequate quantities (Alegbeleye *et al*., 2012). In addition, it has been found that animals' somatic growth is regulated by growth hormone and IGF-I.

Similar to our study, the inclusion of insect meal in rainbow trout diets (Melenchón, 2020), juvenile largemouth bass (Gu *et al*., 2022), blackspot sea bream (Iaconisi *et al*., 2017), did not have any negative effects on growth, protein utilization, WGR, SGR or FCR and the physiological state compared to the control group. On the other hand, Li *et al*. (2022), reported that the inclusion of TM meal in the diets of mirror carp (*Cyprinus carpio*) were significantly improved the FBW, SGR, and PE. In addition, Ido *et al*. (2019), reported the highest FBW, SGR and WG in red seabream (*Pargus major*) fed with 100% FM substituted with defatted TM larvae with no significant differences in the FCR and FI among all fish groups. Besides, the inclusion of TM in rainbow trout diets leads to a decrease in the FCR

alongside a rise in the PER with increasing dietary inclusion levels of TM larvae.

The contradictions in the growth performance and whole-body composition of fish fed with the graded levels of TM can be attributed to one or more factors, including TM factors, fish attributes, dietary factors (TM-compensated FM level and approximate composition of diets) and experimental conditions such as water temperature, salinity, and experimental settings, etc. Thus, optimal FM substitution levels could vary among fishes by insect species, rearing conditions, processing methods of insect meal, and duration of the experimental trials (Osimani *et al*., 2016; Iaconisi *et al*., 2019).

Hematological indices are important indicators for assessing the physiological conditions and health of animals, because these indicators are highly resistant to change (Khadjeh and Peyghan, 2007). For this reason, its results can be used to assess the state of the immune system (Maita, 2007). The results of the present experiment showed that different levels of TM had a statistically significant effect on WBC, MCHC, Hb and Hct in Asian seabass. In agreement with our study, Valipour *et al*. (2019), reported that some indices of hemoglobin, MCH and hematology, such as WBC count and Hct were affected by replacement of fish meal with yellow mealworm. Increased WBC counts may be the result of a protective response of the body during stress (Das *et al*., 2006). Decreased RBC count and a concomitant decrease in Hb and Hct may indicate fish anemia as a result of inhibition of erythrocyte production in hematopoietic organs (Ates *et al*., 2008). The changes of these parameters in relation to different levels of TM in our study did not follow a specific trend and did not indicate a negative effect, although there are not many studies on the effects of insect meal on fish hematological parameters to compare. While the use of insect larvae housefly (*Musca domestica*) as a supplement in diet of African catfish had significant effects on the hematological parameters of this fish (Okore *et al*., 2016). It seems that more studies should be performed to reach a sound conclusion.

Plasma biochemical parameters are important indicators to assess health status and hepatorenal functions (Fazio *et al*., 2019; Abdel-Latif *et al*., 2020) and physiological stress responses of fish fed formulated diets (Dawood *et al*., 2020a, b). In this study, a difference in plasma lipid parameters was observed in Asian seabass which its diet depended on the inclusion of TM in the practical FM-based diet. Whereas, fish fed with diet containing 20%, 40% and 60% TM had significantly higher TG values than fish fed the control diet, while a slight decrease in CHOL was observed in comparison with the control group. Other studies reported that the inclusion of high levels of silkworm pupae (68-90%) in the diets of carp (*Cyprinus carpio*) (Ji *et al*., 2015), TM in the diet of mandarin (*Siniperca scherzeri*) (Sankian *et al*., 2018) and a pre-pupal meal of black soldier fly in

diets of European sea bass (Magalhães *et al*., 2017), causes a significant decrease in plasma CHOL concentrations, which may be attributed to the contribution of chitin that are found in the exoskeletons of insect's meal. In fact, several evidences indicate that chitin and its deacetylated chitosan derivatives can decrease TG in liver and plasma as well as CHOL concentrations. Xia *et al*. (2011) showing significant hypolipidemic and hypocholesterolemic effects in animal and fish models. On the other hand, chitin-chitosan has been suggested to modulate lipid metabolism by interrupting the enterohepatic circulation of bile acid and interfering with digestion and normal absorption of fats in the intestine as well as biosynthesis of fatty acids in hepatocytes (Koide, 1998; Xia *et al*., 2011). In addition, the decreased plasma CHOL level with increased TM levels in Asian seabass diets could be attributed to increased chitin content in those diets, as also demonstrated by previous studies (Li *et al*., 2017; Magalhães *et al*., 2017). Fish plasma glucose levels increase during the stress period, possibly is due to the activity of the hormone catecholamines and the breakdown of glycogen stored in the liver and other tissues (Pottinger, 1998). In the present study, the plasma glucose levels of juveniles were affected by different levels of yellow mealworm. On the other hand, the level of cortisol as a stress hormone was different in treatments and these variations showed a statistically significant difference. According to the results of glucose and cortisol changes, it can be stated that the experimental fish in this study were probably subjected to stress in relation to different diets.

Hepatic enzymes including ALK, AST and ALT, are important indicators for assessing liver disorders in living organisms (Ayalogu *et al*., 2001). Whereas, an increase in its concentrations in the blood plasma indicates damage and necrosis of hepatocytes (Sheikhzadeh *et al*., 2012; Hyder *et al*., 2013; Song *et al*. 2014; Wang *et al*. 2014). The results of the current study showed an increase in the activity of ALT, AST and ALk serums when feeding the Asian seabass on diets containing different levels of TM meal, and therefore, TM meal may cause negative effects on the health of the liver. However, in terms of statistical analysis, AST and ALT were not significantly affected by different levels of TM meal, while ALK showed significant differences in the experimental groups. This result was in agreement with the results of Valipour *et al*. (2019), when feeding juvenile rainbow trout on different levels of yellow mealworm larval meal. Also, increase AST activity was observed in juvenile largemouth bass fed with 66%TM (Gu *et al*., 2022). This is contrary to the study of Li *et al*. (2017), which indicated no effect on the activities of ALT and AST in the serum when feeding juvenile carp with defatted black soldier fly larval meal. Increased serum TP concentrations, including both Alb and Glu are

evidence of immune disorders, impaired kidney activity and liver dysfunction (Banaee *et al*., 2011; John, 2007; El-Kamary *et al*., 2009). In this study, no difference in TP was seen when seabass was fed on diets containing different levels of TM, while a decrease in Alp was found. In agreement with the findings of the present research, the study by Gu *et al*. (2022) showed that the use of 55% and 66% TM in the diet of juvenile Largemouth bass led to a decrease in Alp. Sankian *et al*. (2018) reported no significant differences in TP and Alb in the serum of mandarin fish when using different levels of mealworm diets.

Oxidative stress occurs because of the over-production of reactive oxygen species (ROS), which leads to serious effects on the body of fish such as damage to the DNA in the nucleus of the cell; disruption of the cell membrane, cell wall and cellular proteins; and lipid peroxidation within cell membranes (Pamplona and Costantini, 2011). Radical scavenging enzymes, such as SOD, CAT, and GPX play a role in protecting cells from damage. CAT and GPX scavenge hydrogen peroxide and convert it into the water, while biological function of SOD is the detoxification of the superoxide anions created by partial reduction of O_2 and its transformation to hydrogen peroxide (H_2O_2) (Nordberg and Arnér, 2001). MDA is one of the breakdown products of lipid metabolism and is used as a reliable marker of lipid peroxidation. In our current study, we found the significantly higher activity of CAT in the serum of Asian seabass fed TM20 diet and GPX in serum of fish fed TM40 and TM60. This situation was consistent with research that found high CAT activity in the serum of carp fed with diets with 75% and 100% of defatted black soldier fly larval meal (Li *et al*., 2017) and also in serum of African catfish liver when including riccket meal in their diets (Taufek *et al*., 2016). whereas many of studies confirmed that CAT and SOD activity in juvenile largemouth bass (Gu *et al*. 2022), and GPX activity in mandarin fish (Sankian *et al*. 2018) increased when including different levels of TM meal in their diets. The reason for these results may be attributed to the presence of chitin in the exoskeletons of insects, where sources reported that chitin and its derivatives have antioxidant, antimicrobial and immune-stimulating properties which can positively affect the health of animals (Zhao *et al*., 2010; Ravi *et al*. 2011; Rumpold and Schlüter, 2013; Makkar *et al*., 2014) and prevent harmful effects in various diseases (Khoushab and Yamabhai, 2010; Ngo and Kim, 2014).

Lysozyme is a mucolytic enzyme characterized by the ability to break down the bacterial cell wall by cleaving the 1-4-β-linkages between N-acetylmuramic acid and N-acetylglucosamine in the bacterial cell wall (Saurabh and Sahoo, 2008). In the current study, an increase in serum lysozyme activity was observed in fish fed on graded levels of TM diets with significantly higher values than fish fed

a TM-free diet. The results of a similar study by Sankian *et al*. (2018), showed that TM meal inclusion in mandarin fish feed had positively influenced their humoral immune response and antioxidant defense status, whereas it showed increased activity of lysozyme when TM30 was fed. However, these findings are in contrast to Henry *et al*. (2018), who reported that there were no significant differences in lysozyme activity when European seabass were fed a TM25 meal.

Based on our results, Asian seabass fed on TM diets grew faster compared to those fed a TM-free diet. Therefore, we can consider *Tenebrio molitor* larval meal as a promising candidate for use as an alternative protein source for partial replacement in the diet of Asian seabass. The replacing of 40% of the FM protein with TM did not lead to adverse effects on the growth performance, proximate composition, and antioxidant enzyme activities of Asian seabass**.**

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