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ORIGINAL ARTICLE

Effects of substituting fishmeal by mealworm (*Tenebrio molitor*) on growth and composition of Asian seabass in saline ground waters

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Abstract
A six-week trial was conducted to determine the effects of *Tenebrio molitor* larvae meal (TM) on growth performance and body composition of Asian seabass. A total of 240 Juvenile fish (whole body weight 35.42±0.12g) were randomly distributed into four groups with three replicates (tank capacity 300l) with a density of 20 fish per replicate in a recirculating system. Fish were fed with four diets including 0%, 20%, 40% and 60% of TM substitution. At the end of the experiment, there was no significant difference in growth performance and protein efficiency ratio (P>0.05). However, the condition factor and viscerosomatic index showed significant differences among the treatments (P<0.05). Chemical analysis of fish carcasses showed significant differences in moisture and ash among the treatments (P<0.05). There was no significant difference in moisture, ash and crude protein of the hepatopancreas (P>0.05). Nevertheless, the body lipid content increased gradually as the concentration of TM in the fed groups increased. Since there were no adverse effects on growth performance when using TM meal, therefore, it can be adopted as an alternative source of protein in the Asian sea bass diets.

Keywords: Fishmeal replacement, Insect meal, Saline groundwater, Carcass composition.

INTRODUCTION

The fisheries industry is one of the main sources that can be relied upon to protect food security in countries, especially animal protein with a high nutritional value, beside protecting natural stocks and aquatic environments, and creating jobs. Whereas, an increase in the activities of this industry resulted in the raise of many problems, the most important one is the nutrition and provision of animal protein for animal husbandry, represented by fish meal due to its digestible proteins, essential amino acids (EAAs), and palatability (Galkanda-Arachchige et al. 2020; Dawood & Koshio 2020), but fish meals (FM) are limited sources that cannot be produced in sufficient amounts even to sustain the growth trends of fish culture (FAO 2014), so rapid steps are required to find new protein alternatives in fish diets which should be economic, environmentally friendly, safe, sustainable, and palatable for fishes (Dawood et al. 2015; Abdel-Latif et al. 2020). Thus, it is a necessity to find a suitable substitute for fish meal in aquaculture. Among the alternatives, insect larvae meal is tended to provide a relatively suitable protein with high nutritional value (Nogales-Mérida et al. 2019; Abdel-Latif et al. 2021). Insects are part of the fish natural diet (Howe et al. 2014; Whitley & Bollens 2014), with a high protein and lipid content (Barroso et al. 2014) and have many advantages to be used as a substitute for fishmeal, including an abundant EAA, fatty acids, vitamins and minerals contents (Barroso et al. 2014), needless land space for rearing (Wang et al. 2012), fast growth and the ability for using wastes with high feed conversion efficiency (Henry et al. 2015) and simple reproduction system (Khan et al. 2018).

The yellow mealworm (*Tenebrio molitor*) (TM), family Tenebrionidae, is considered one of the best candidates for fish meal replacement in aqua feeds (Sánchez-Muros et al. 2016). In the past decades, researches have shed light on the efficiency of insect dietary meal in aquaculture, including African catfish (*Clarias gariepinus*) (Ng et al. 2001), common catfish (*Ameiurus melas*) (Roncarati et al.

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2015), tilapia (*Oreochromis nilotica*) (Sánchez-Muros et al. 2016), rainbow trout (*Oncorhynchus mykiss*) (Gasco et al. 2014a; Belforti et al. 2015), gilthead sea bream (*Sparus aurata*) (Piccolo et al. 2014, 2017), European sea bass (*Dicentrarchus labrax*) (Gasco et al. 2016; Mastoraki et al. 2020), blackspot seabream (*Pagellus bogaraveo*) (Iaconisi et al. 2017) and mandarin fish (*Siniperca scherzer*) (Sankian et al. 2018).

Asian seabass (*Lates calcarifer*) commonly known as barramundi, is one of the most economically important fish, widely distributed in the East Indian Ocean, Western Pacific region, Sea of Japan, and cultured in several Asian countries (Berra 2001; Anil et al. 2010; Venkatachalam et al. 2018; Froese & Pauly 2022) under an extensive or intensive system in fresh, brackish and marine waters (Glencross 2006; Nandakumar et al. 2013), so it has been identified as a suitable species for inland saline aquaculture.

Drought has been found as a disaster phenomenon that can significantly affect the agricultural activities, especially in dry regions (e.g., Iran). Drought is also followed by some huge economic, social and environmental costs (Goddard et al. 2003). Scarcity of water, as well as the excessive use of water resources, mainly for industry and traditional agriculture, create negative water balances and changes in plant cover, and accelerate desertification which worsen the situation worldwide (Wang et al. 2013), as well as, for Iran (Jafari & Hasheminasab 2017). Due to severe drought and water shortage in most parts of Iran, as a large part of agricultural lands, cannot be cultivated and the farmers are forced to migrate to the cities. In order to improve the livelihoods, prevent migration and use of saline groundwater, the idea of the inland culture of seabass using saline groundwater was considered. Therefore, the aim of this study was to evaluate the effects of the replacement of TM larvae meal with FM in the Asian seabass diet cultured in a saline groundwater closed recirculating system.

MATERIAL AND METHODS

Fish and experimental conditions: The study was performed for six weeks in an indoor water recirculating system at Isfahan University of Technology Aquaculture Facilities (Isfahan, Iran). Two hundred and forty juvenile of Asian seabass (35.42±0.12g average initial body weight) were obtained from Ramoz Marine Fish Breeding Center (Bushehr Province, Iran). Prior to the experiment, fish were acclimated from seawater (36ppt) to saline groundwater (15ppt) in a 1m^3 circular fiberglass tank, whereas, the chemical composition of groundwater used in the present study includes, Borom (2.5mg/l), Calcium (540.6mg/l), Potassium (54.7mg/l), Magnesium (744.8mg/l), Sodium (3371mg/l) and Strontium (19mg/l). The acclimation process performed by gradual decrease of salinity over 12 days at a rate of 2ppt/day. During the acclimation period, fish were fed a commercial diet (Dorindaneh Co., Shahrekord, Iran; 12% moisture, 42% protein, 18% lipid, 14.8% carbohydrate and 10% ash). For the growth experiment, 240 specimens were randomly distributed in 12 circular fiberglass tanks (300l, 80cm in diameter and 70cm in height), at a stocking density of 20 fish/tank. Water was recirculating into sediment filters, submerged biological filters, and settling sump tanks before returning to the tanks. Aeration was provided via a central blower pump. Fish were fed to apparent satiation three times/day at 8:00, 12:00, and 16:00, during the six-week feeding trial. Water quality parameters measured daily (temperature 27±0.59°C and dissolved oxygen 6.7±0.24mg/l). Weekly measurements for pH and total ammonia nitrogen averaged 7.5±0.08 and 0.02±0.004mg/l, respectively, all values were suitable for Asian seabass. A photoperiod of 12:12 (light:dark) was maintained throughout the experiment.

Diet preparation: The dried mealworm larvae (TM) used in the present study was purchased from a local breeder (Isfahan, Iran) and ground into powder using an electric blender. Four experimental diets were

Table 1. Ingredients and proximate composition of the experimental diets, FM and TM larval meal.

	Experimental diets					
	FM	TM	TM0	TM20	TM40	TM60
Ingredients (g/kg)						
TM larval meal			-	4.34	8.68	13.02
Fish Meal			21.7	17.36	13.02	8.68
Soy bean			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
Yeast			3	3	3	3
Glutamine			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 supplements			1	1	1	1
Vitamin C			0.5	0.5	0.5	0.5
Fish oil			3	3	3	3
Proximate composition (%)						
Dry matter	93.34	97.72	98.32	97.57	98.52	97.5
Crude protein	48.78	48	45.47	45.695	45.83	46.15
Lipid	18.73	33.9	9.96	8.96	9.65	10.56
Moisture	6.56	2.29	1.67	2.42	1.47	2.49
Ash	20.37	3.1	8.42	7.99	6.66	6.72
Carbohydrate	5.56	12.71	34.48	34.94	36.39	33.48
Energy (kcal/kg)*	-	-	4200.13	4170.32	4268.47	4271.51

*Calculate the net energy of the diet based on carbohydrate= 4.1, protein= 4.1, and lipid= 9.3 (Noblet 2007; Tacona 1990; Kellner & Patience 2017).

formulated to meet the requirements of Asian seabass, as reported in previous researches (Aquacop-Cuzon et al. 1989; Ambasankar et al. 2009). The diets were isoenergetic, isonitrogenic and isolipidic and were prepared using a commercial meat grinder with a 2mm screen, which were air-dried for 24h, ground to adequate size and stored at -2°C in air-tight plastic bags until used. A control diet (TM0) without TM, and tree diets in which fish meal was partially replaced by TM at 20% (TM20), 40% (TM40) and 60% (TM60) were prepared. The diets ingredients and chemical composition of TM, FM, and the diets are presented in Table 1.

Growth performance: All specimens were weighed every two weeks to adjust the amount of feeding rate. At the end, the growth performance indices, including the protein efficiency ratio (PER), protein productive value (PPV), and viscerosomatic index (VSI) were calculated using the following formulas (Wang et al. 2009).

PER= weight gain (g)/total protein given (g)

PPV= $(W_t \times P_1 - W_0 \times p_2)/(I_d \times P)$

VSI%= (weight of viscera/weight of fish)×100

Feed intake as: FI% fish/day=100×total amount of the feed consume (g)/$[(W_0+W_t)/2]/t$

Survival rate as: (final number of fish/initial number of fish)×100

Condition Factor (CF%)= (weight of fish/(length of fish)^3)×100

The body weight gain (BW) as:

BW= final body weight (g)–initial body weight (g)

Specific growth rate (SGR %/day)= [(ln final body weight–ln initial body weight)/number of feeding days]×100

The food conversion rate (FCR) as: FCR= total feed supplied (g)/weight gain (g)

Chemical analyses of feeds, livers and fish carcasses: At the beginning of the trial, the carcasses of three individuals were weighed and kept frozen at –20°C for subsequent initial carcass composition

Table 2. Growth performance, survival rate, and somatic indices of Asian seabass fed the experimental diets.

	control	TM20	TM40	TM60
Growth performance				
Initial body weight (IBW)[2], g	35.21±2.43	35.78±2.13	35.26±1.98	35.44±1.07
Final body weight (FBW)[3], g	99.21±3.87	93.98±6.2	88.02±5.71	94.41±1.02
BWG (g)[4]	63.55 ±1.57[a]	58.11±4.53[ab]	52.76±3.26[b]	58.97±1.33[ab]
SGR (%.day^{-1})[5]	2.44±0.08	2.29±0.11	2.18±0.04	2.33±0.04
FCR[6]	1.1±0.06	1.28±0.22	1.27±0.1	1.18±0.11
FI[7]	2.87±0.05	2.89±0.14	3.13±0.23	2.99±0.17
Survivel[1]	100	100	100	100
Protein utilization				
PER[8]	2.26±0.002	2.24±0.03	1.88±0.02	2.07±0.03
PPV%[9]	13.2±0.52	14±2.95	11.18±1.16	13.11±1.2
Biometric indices				
CF (g.cm^{-3})[10]	1.32±0.01[a]	1.26±0.01[b]	1.17±0.02[c]	1.27±0.02[ab]
HSI (%)[11]	9.72±0.04[b]	10.39±0.18[b]	12.53±0.86[a]	13.29±0.43[a]

Abbreviations: TM0: fish meal group; TM20, TM40 and TM60: *Tenebrio molitor* larvae meal at 20, 40 and 60% level groups, respectively.
1 Survival rate = (final number of fish/initial number of fish) × 100.
2 Initial mean body weight (IBW), (g).
3 Final body weight (FBW), (g).
4 Body weight gain (WG, g)= final body weight (g) - initial body weight (g)
5 Specific growth rate (% /day)= [(ln final body weight−ln initial body weight)/days]×100.
6 Feed conversion ratio = [total feed supplied (g)/weight gain (g).
7 Feed intake (FI,%fish/day)=100× total amount of the feed consumed (g)/[(W_0+W_t)/2]/t
8 Protein efficiency ratio = weight gain (g)/total protein given (g).
9 Productive protein value = [(protein gain (g)/protein intake (g)) × 100].
10 Condition Factor (%)= (weight of fish/(length of fish)^3)×100.
11 Hepatosomatic index (%)= (weight of liver/weight of fish)×100.

analysis. At the end of the trial, all fish were starved for 24h (to empty the digestive tract). After the biometry, the carcasses of three individuals per treatment were randomly collected for composition analysis. Proximate composition of the carcasses of the fish, TM larval meal, fish meal and the four experimental diets (Table 1), were determined according to AOAC (2005). Briefly speaking, they were dried in an oven at 105°C for 24h to reach a constant weight. Crude protein was estimated by analysis of nitrogen content (N×6.25) and the Kjeldahl method (Kejeltec V40 auto analyzer, Bakhshi, Iran), crude lipid by petroleum ether extraction (at 40-60°C) using the Soxhlet extraction method (model 6XI Extraction Unit, Bakhshi, Iran), and ash by incinerating dried samples at 550°C for 4h, using a Nabertherm muffle furnace (Model: K, Germany).

Statistical analysis: The data were tested for normality and homogeneity of variances using the Kolmogorov-Smirnov test, then, One-way ANOVA was used to compare means. The differences among means was determined by the Duncan ad-hock test at 95% confidence level. SPSS 25.0 software was used for the statistical analysis and Excel software for drawing the charts.

RESULTS

Growth performance and somatic indices: The results of the effects of experimental diets containing TM as an alternative to FM on growth performance and biological parameters are presented in (Table 2). It seems that the experimental diets were well accepted by the fish and no mortality was observed during the entire growth trial. There were no significant differences in initial body weight (IBW), final body weight (FBW), specific growth rate (SGR), feed conversion ratio (FCR), feed intake (FI), body weight gain (BWG), protein efficiency ratio (PER) and productive protein value (PPV). While the body weight gain (BWG), condition factor (CF) and Viscerosomatic index (VSI) showed significant differences among the treatments (P<0.05).

Table 3. Effect of TM meal on proximate composition in tissues of Asian seabass.

	control	TM20	TM40	TM60
		Fish carcasses		
Moisture	71.64±0.06[a]	71.31±0.25[a]	70.14±0.52[b]	70.5±0.34[b]
Dry matter	28.35±0.06[b]	28.69±0.25[b]	29.85±0.52[a]	29.49±0.34[a]
Protein	69.98±1.62	71.19±0.59	70.57±0.54	70.19±1.08
lipids	17.8±1.09	19.43±0.41	20.98±1.93	20.34±0.95
Ash	12.49±0.42[a]	13.11±0.29[a]	11.95±0.24[ab]	11.16±0.15[b]
		Hepatopancreas		
Moisture	39.25±1.09	37.95±2.2	43.31±2.71	44.43±2.67
Dry matter	60.74±1.09	62.04±2.2	52.68±2.71	55.56±2.67
Protein	20.88±0.48	20.36±0.06	21.66±3.11	20.52±1.44
lipids	68.66±2.09[b]	72.04±1.18[ab]	74.77±2.22[ab]	78.45±2.71[a]

Abbreviations: TM0: fish meal group; TM20, TM40 and TM60: *Tenebrio molitor* larvae meal at 20, 40 and 60% inclusion level groups, respectively.

Proximate compositions: The results of the hepatopancreas and carcass proximate composition of the fish feeding with TM are shown in Table 3. Chemical analysis of fish carcasses showed significant differences in moisture, dry matter and ash among the treatments ($P<0.05$). In the hepatopancreas, there were no significant differences in moisture, dry matter and crude protein. While the lipid content increased gradually with increase in the concentration of TM in the treatments.

DISCUSSION

The influence of partial replacement of FM with TM as a new protein source in Asian seabass feeding was tested. Despite the growing interest in the use of insect meal for fish, comparison with other researches is difficult due to the lack of available studies on the use of insect meal in the production of Asian seabass in brackish waters. However, the results showed that TM can be a valuable source of protein for inclusion in Asian seabass feed at 20, 40, and 60% as a substitute for FM. The different levels of TM meal did not lead to negative or significant effects on FBW, SGR, FCR, FI, PER and PPV, since the diets were formulated to be isonitrogenous and isoenergetic, and did not produce any different response by the fish. Observed similar growth performance and feed intake, compared to the control group, suggest a good palatability of the diets for the fish.

Ng et al. (2001) reported the highest FBW, WG%, SGR, and PER in African catfish fed diets with 20% of FM substituted with TM powder. Meanwhile, a slight reduction in the FBW, SGR, FER, and PER was seen in catfish groups fed solely on live TM. Also, Belforti et al. (2015) showed that the inclusion of TM in rainbow trout diets did not influence the final fish weight and weight gain, but significantly improved some parameters such as CF, PPV, and PER. Iaconisi et al. (2017), as in our trial, reported that there were no considerable changes in FI, FCR, and SGR in blackspot seabream (*Pagelluse bogaraveo*) fed TM-based diets with 25% and 50% of the FM food compared to those fed FM-based diets. Consistent with our findings, Redman et al. (2019) revealed a significant elevation in FBW, WG, FI, and SGR in black seabass fed diets with 25% of FM substituted with TM. Moreover, no noticeable differences were reported in FI, survival rate percentage, HSI, and CF among all the treatments. On the other hand, Józefiak et al. (2019) demonstrated no differences in the FBW, WG, FCR, SGR, PER, and SR% in Siberian sturgeon fed diets with 15% of FM substituted with TM larvae. However, Su et al. (2020) reported no substantial differences in FI, SGR, and feed conversion efficiency among yellow catfish fed TM-based diets with up to 75% FM replacement, compared to those fed FM-based diets. Rema et al. (2019) reported a significant increase in FBW, FI, SGR, PER, and improved feed conversion rate (FCR) in rainbow trout fed diets with replacement of 100% of FM with defatted TM larvae. However, we observed a significant negative effect on the BWG in the TM40 compared to the control group. On the contrary, Gasco et al. (2016) found that the 50% inclusion of TM in the diet of European seabass caused a worsening of final body weight, weight gain, specific growth rate, and feeding rate compared to the control, while no negative effects were obtained at 25% inclusion.

The higher values of the HSI (higher than the standard values (1-2%) showed that feeding TM causes some troubles in fish, especially in the fat and carbohydrate metabolism, the existence of oxidized feed ingredient in the diet, and extra carbohydrate and vitamin deficiency (Munshi & Dutta 1996). In our trial, TM60 and TM40 groups had a higher HSI than control, this aspect needs further investigation as could indicate metabolic trouble in fish. On the other hand, for the TM20 group, the HSI fall in the physiological range even if it is higher than that of the control group. Similar results were obtained by Piccolo et al. (2017) in an experiment with 25 and 50% TM in gilthead seabream diets, where showed a significant increase in HSI with the increase in the concentration of mealworm diets. Opposite results were obtained for HSI in rainbow trout with a decrease in this index value at the increase of TM levels in the diets (Belforti et al. 2015). However, the highest HSI values was recorded in fish groups fed a diet in which FM was completely replaced by partially defatted TM larvae. Hoffmann et al. (2020) detected the highest HSI and VSI in sea trout (*Salmo trutta*) fed diets supplemented with 20% enzymatically hydrolyzed and non-processed full-fat TM larvae compared to the FM group. However, the highest FBW and WG were noticed in the FM group.

The proximate composition of *Lates calcarifer* carcass was not affected by TM inclusion. Dry matter contents were significantly increased while the moisture and ash decreased with an increase in the mealworm larvae added in the diets. However, protein and lipid contents slightly increased when larval meal was added to the diets, although these variations were not statistically significant. It also had no significant effects on Hepatopancreas dry mater, moisture, and protein, but significantly increased the lipid contents of the hepatopancreas in TM60. Ng et al. (2001) reported the highest crud protein content in the bodies of African catfish fed diets with FM replaced with 20% of TM powder. However, a higher whole-body crud lipid content was recorded in catfish fed solely live TM. Gasco et al. (2014b) found no noticeable differences in the whole-body proximate analysis of *Dicentrarchus labrax* fed diets supplemented with 25% and 50% TM larvae. Piccolo et al. (2014) also demonstrated no changes in the whole-body composition of gilthead seabream fed diets with 25% and 50% FM replaced with TM larvae. Similarly, a study carried out using TM larval meal in diets of blackspot seabream (*Pagellus bogaraveo*) showed an increase of fat in filleted muscles in fish fed TM larval meal (Iaconici et al. 2017).

Additionally, De Haro et al. (2016) observed a slight increase in the fillets fat in fish fed larvae meal of *Lucilia sericata* in diets for gilthead sea bream. Iaconisi et al. (2017) reported the same findings in blackspot seabream-fed diets with 25% and 50% FM replaced with full-fat TM larvae. There were no significant differences in the whole-body composition of rainbow trout fed TM-based diets compared to those fed FM-based diets (Rema et al. 2019; Jeong et al. 2020). The results of mentioned studies are conflicting with those obtained by other researchers that showed a significant decrease in fat and an increase in the protein of rainbow trout fillets as a consequence of the dietary inclusion of full-fat TM larval meal in the diet (Belforti et al. 2015). Moreover, Harsij et al. (2019) found the highest crude protein and crude lipid in fillets of rainbow trout fed diets supplemented with up to 60% TM.

The contradictions in the growth performance and whole-body composition of fish fed with the replacement of FM by graded levels of TM can be attributed to several factors, some of which are related to TM factors such as quality, processing, and approximate chemical composition; fish factors such as size, age, stage of growth, and ability to digest TM larval meal; dietary factors (TM-compensated FM level and approximate composition of diets); and experimental conditions such as water temperature, salinity, breeding system, and experimental settings, etc. Thus, optimal FM substitution levels could vary among fishes by insect species, rearing conditions, processing methods of insect meal (IM), and duration of the experimental trials, thus, making the comparisons difficult (Osimani et al. 2016; Iaconisi et al. 2019).

Asian seabass is one of the most important species that are widely cultivated in many countries of the world due to its high nutritional and commercial value. In our study, MT larvae meal appeared to be a promising candidate as an alternative protein source for partial replacement of fish meal (40 to 60%) in the diet of Asian seabass. Since there were no adverse effects on growth performance and proximate composition of Asian seabass when using mealworm, therefore, it can be used as an alternative source of protein in the Asian seabass diets. More research should be conducted to find out the effect of mealworm on the amino acids and fatty acids profile, as well as, its effects on organoleptic characteristics of filleted muscles of Asian seabass.

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مقاله کامل

اثرات جایگزینی پودر ماهی با پودر *Tenebrio molitor* بر رشد و ترکیب باس دریایی آسیایی در آب‌های شور زیرزمینی

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چکیده: یک آزمایش شش هفته‌ای برای تعیین اثرات پودر لارو *Tenebrio molitor* (TM) بر عملکرد رشد و ترکیب بدن باس دریایی آسیایی انجام شد. تعداد ۲۴۰ بچه ماهی (وزن کل بدن ۰/۱۲±۳۵/۴۲ گرم) به‌طور تصادفی در چهار گروه با سه تکرار (ظرفیت مخزن ۳۰۰ لیتر) با تراکم ۲۰ قطعه ماهی در هر تکرار در سیستم مدار بسته توزیع شدند. ماهی‌ها با چهار جیره شامل ۰، ۲۰، ۴۰ و ۶۰ درصد جایگزینیTM تغذیه شدند. در پایان آزمایش، تفاوت معنی‌داری در عملکرد رشد و نسبت کارایی پروتئین مشاهده نشد (*P*>۰/۰۵) اما فاکتور وضعیت و شاخص احشایی تفاوت معنی‌داری بین تیمارها نشان داد (*P*<۰/۰۵). آنالیز شیمیایی لاشه ماهی تفاوت معنی‌داری را از نظر رطوبت و خاکستر در بین تیمارها نشان داد (*P*<۰/۰۵). تفاوت معنی‌داری در رطوبت، خاکستر و پروتئین خام هپاتوپانکراس وجود نداشت (*P*>۰/۰۵) با این وجود، با افزایش غلظت TM در گروه‌های تغذیه شده، محتوای چربی بدن به تدریج افزایش یافت. از آنجا که هنگام استفاده از پودر TM هیچ اثر نامطلوبی بر عملکرد رشد وجود نداشت، بنابراین، می‌توان آن را به‌عنوان منبع جایگزین پروتئین در رژیم غذایی باس دریایی آسیایی استفاده کرد.

کلمات‌کلیدی: جایگزینی پودر ماهی، پودر حشرات، آب‌های زیرزمینی شور، ترکیب لاشه.