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The effects of dietary levels of the sea cucumber (*Bohadschia ocellata* Jaeger, 1833*)* meal on growth performance, blood biochemical parameters, digestive enzymes activity and body composition of Pacific white shrimp (*Penaeus vannamei* Boone, 1931) juveniles

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Abstract

The sea cucumber (*Bohadschia ocellata*) is a species with great density, reproduction rate and growth in the Persian Gulf. A Very few human consumption in Islamic countries makes this species a good choice to be used as a supplementary or stimulant ingredient in cultured aquatic animals. The present study evaluates the effects of dietary levels of *B. ocellata* meal on growth performance, blood biochemical parameters, digestive enzymes activities and body composition of Pacific white shrimp (*Penaeus vannamei*). White shrimp juveniles (6.6±0.1 g) were fed with different levels of the *B. ocellata* meal (0, 2, 4, 6, and 8 % of the diet) during 8-weeks period. According to the results weight gain, specific growth rate, and daily feed intake trends showed significant ($p<0.05$) increase with increasing level of sea cucumber meal from 0% to 4%. The greatest feed conversion ratio and least protein efficiency ratio were recorded in the 8% treatment. In blood biochemical outputs, the least cholesterol and the highest glucose levels were recorded for the 4% and 6% treatments, respectively. Digestive enzymes activity assessments showed that protease activity was significantly increased from 4% to 8% treatments. In general, adding 4-6% of *B. ocellata* meal to commercial diet of *P. vannamei* juveniles clearly improved some body biochemical activities such as protease enzymes activity and blood cholesterol content, and enhanced growth performance of white shrimp.

Keywords: Body composition, Growth performance, *Bohadschia ocellata*, *Penaeus vannamei*, Pacific white shrimp, Sea cucumber

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Introduction

Sea cucumbers are marine invertebrate animals from the class Holothuroidea. They have a spindle-shaped body and their habitat is mainly shallow waters. There are about 1717 recorded species of the sea cucumbers all over the world (Paulay, 2014) with their greatest biodiversity in the Asia Pacific region (Kamarudin *et al*., 2010; Du *et al*., 2012; Sadhukhan and Raghunathan, 2012; Margarita *et al*., 2015; Siddiq *et al*., 2016). Nine species of sea cucumbers belonging to the genus *Holothuria* are reported in Iranian southern coastlines (Afkhami *et al*., 2015). *Bohadschia ocellata* as one of the most abundant species of the *Holothuria* is placed under the genus *Bohadschia* since 2013 (WoRMS, 2020).

Sea cucumbers are remarkable natural sources of novel functional materials which are biologically active and could be used in food and biomedical industries (Pangestuti and Arifin, 2017). Body compositions of these animals have high levels of valuable nutritional components. Including essential amino acids such as Histidine, Leucine, and Lysine, rare elements, essential fatty acids such as ALA, EPA, and DHA, and different proteins (Aminin *et al*., 2015), vitamins such as retinol (A), thiamine (B_1), riboflavin (B_2), niacin (B_3), and also different minerals such as calcium, magnesium, iron, and zinc (Bordbar *et al*., 2011).

Large sizes of the *Bohadschia ocellata* populations provide a good opportunity for the sustainable harvest of such a valuable source of nutrition. *Holothuria ocellata* meal can be a good choice of a supplementary or stimulant ingredient in other cultural aquatic animals like shrimps. It can also be a good alternative for parts of the diet. In this way, beside increase in weight gain, there would be a decrease in cost of feed and the final product (Amaya *et al*., 2007).

The Pacific white shrimp *Penaeus vannamei* (Boone, 1931) is a commercially important penaeid shrimp worldwide, particularly in Asian countries (Wang *et al*., 2015). The natural origin of this species is principally in the eastern Pacific Ocean, from Mexico to as far south as northern Peru, however, during the past few decades it is introduced to numerous locations throughout the world, especially the Asian regions. *P. vannamei* has already become the primary cultural species in Asian countries (Chiu *et al*., 2007) and since 2002 the production of the black tiger shrimp (*Penaeus monodon*) has dramatically declined due to its substitution by *P. vannamei* in many cultural farms (Rezaei Tavabe and Rafiee, 2016). On the other hand, during resent years due to limited availability and increase in the fishmeal value, the cost of *P. vannamei* feed is increased (Tacon and Metian, 2008). Therefore, the shrimp aquaculture industry needs some alternative sources to increase growth rate and reduce the cost of feed simultaneously.

One of the most important factors in the juvenile and pre-adult periods of shrimp is access to the highest growth rate in the shortest possible time. On the other hand, in order to operate shrimp culture activities economically, mortality rate should be as low as possible. These two factors ultimately have the greatest impact on the production of shrimp farms. Hematological parameters are considered as important health and growth indicators in shrimp culture (Katya *et al*., 2016). While the product should be of good quality for the market and the consumer health, the total amount of protein, fat and various other substances in the shrimp flesh are also important (Xthe and Pan, 2012). The present investigation was carried out to evaluate the effects of different dietary levels of the sea cucumber (*B. ocellata*) meal on growth performance, blood biochemical parameters, digestive enzymes activity and body composition of the Pacific white shrimp (*P. vannamei*) juveniles.

Materials and methods

Shrimps sources and experimental setup

Juvenile shrimps with an initial weight of 6.6±0.1 g (n=900) for the study were obtained from Razak shrimp breeding center (Shiff county, Bushehr province, Iran). They were moved to the laboratory after checking for normal body structure. The juvenile shrimps were fed twice a day (at 6.00 am and 6.00 pm) for 15 days as the adaptation period with a commercial shrimp feed (Faradaneh Co. aquatic animals feed producer, Shahrekord, Iran) up to 5% of their body biomass. During the experimental period, the average water temperature and water salinity were 29±2°C and 30±2 g L^{-1} respectively. NH_4–N, NO_2–N, and NO_3–N levels were maintained below 0.2, 0.1, and 10.0 mg L^{-1} respectively. The juveniles were fed with 5 experimental diets (Table 1) containing different levels of *B. ocellata* meal including 0% (control), 2%, 4%, 6% and 8% levels three times a day at 6:00, 13:00 and 6:00, about 5% of their body weight during an 8-weeks period. Each treatment was replicated three times and each experimental fiberglass tank (200-L) was stocked with 50 juveniles. In order to measure the amount of feed intake, all feed residuals were siphoned before each feeding time then weighted in comparison to the last time feed amount. All tanks were in an open environment exposed to natural photoperiod**.**

Diet preparation

The sea cucumber (*B. ocellata*) specimens were collected from the Persian Gulf coastline of Bandar Abbas County (Hormozgan province, Iran). After collection, they were washed with fresh water. The collected sea cucumbers were cooked by a steam cooker (FS-12000, Pars Khazar, Iran) for 40 minutes and then were minced by a meat grinder (MK-G1800, Panasonic, Japan).

Table 1: Composition of diets and proximate analysis of the diets and sea cucumber (*Bohadschia ocellata*).

Ingredients (g kg^{-1})	0% (Control)	2%	4%	6%	8%
Fish meal[a]	270	270	270	270	270
Shrimp-head meal[b]	100	100	100	100	100
Soybean meal[c]	250	250	250	250	250
Wheat flour	243	223	203	183	163
Soy lecithin	15	15	15	15	15
Wheat gluten	40	40	40	40	40
Fish oil	20	20	20	20	20
Sunflower oil	15	15	15	15	15
Monocalcium phosphate	10	10	10	10	10
Mineral premix[d]	17.5	17.5	17.5	17.5	17.5
Vitamin premix[e]	17.5	17.5	17.5	17.5	17.5
Vitamin C (ascorbic acid)	2	2	2	2	2
Sea cucumber meal	0	20	40	60	80
Diets proximate analysis (g kg^{-1})					
Crude protein	395.2±12.1	395.7±16.7	395.4±9.8	406.1±11.9	410.1±21.4
Crude lipid	101.3±5.2	102.1±4.7	101.9±4.9	103.1±8.1	101.9±11.5
Moisture	70.3±2.2	70.3±2.5	71.1±1.6	70.6±2	71.3±1.7
Ash	135.5±8	132.9±5.9	134.1±4.2	139.4±7.5	135.5±6.8
***Bohadschia ocellata* proximate analysis (g kg^{-1})**					
Crude protein	313.3±6.3				
Crude lipid	78.1±2.2				
Moisture	218.6±17				
Ash	180±5.4				

[a] fish meal: 52% protein, 12% lipid, 9% moisture, Jonoub fish meal factory, Bandar-abbas, Hormozgan, Iran.
[b] shrimp-head meal: 41% protein, 6% lipid, 12% moisture, Prepared in our laboratory.
[c] soybean meal: 44% protein, 2% lipid, 11% moisture, Behpak Industrial Company, Behshahr, Mazandaran, Iran.
[d] mineral premix (kg^{-1} of diet): Manganese, 8,000 mg; Copper, 2,000 mg; Ferrous, 4,000 mg; Zinc, 8,000 mg; Selenium, 50.0 mg; Iodine, 200.0 mg; Cobalt, 50.0 mg; Choline Chloride, 100,000 mg.
[e] vitamin premix (kg^{-1} of diet): Vitamin A, 2,000,000 IU; Vitamin D3, 400,000 IU; Vitamin E, 40,000 mg; Vitamin K3, 4,000 mg; Vitamin B1 (thiamine mononitrate), 20,000 mg; Vitamin B2 (riboflavin), 9,000 mg; Vitamin B6 (pyridoxine hydrochloride), 10,000 mg; Vitamin B12 (cyanocobalamin), 8.0 mg; Vitamin C, 60,000 mg; Nicotinic acid, 40,000 mg; Calcium d-pantothenate, 20,000 mg; Folic acid, 2,400 mg; d-Biotin, 200.0 mg; Inositol, 60,000 mg; Antioxidant, 5,000 mg.

The obtained materials were dried by a dryer at 60 °C for 6 hours and then they were sifted from a 60-mesh sieve and stored in a freezer until used. To conduct the research, five experimental diets were formulated with different levels of *B. ocellata* meal by eliminating 0, 2, 4, 6 and 8% of wheat flour from feed as a less effective part of diet. Wheat flour was chosen to be omitted due to its low protein content and lack of stimulant and attractive ingredients. Also it has the least effect on growth performance and digestive activity and blood factors of shrimp. On the other hand, wheat flour constituted 24.3% of the control diet and was one of the most consuming components in the diet, so eliminating 2 to 8% of the wheat flour from the diets could have negligible effect on the diets, therefore the effects of different treatments could be considered as the effect of adding different levels of sea cucumber meal. The omitted wheat flour from the diet was replaced by 2, 4, 6, and 8% sea cucumber meal (Yu *et al*., 2016a) (Table 1). To prepare the diets, the raw materials were mixed thoroughly and then oil and water were added to the diets and mixed by a stirrer. The obtained dough was passed through a meat grinder with a 1.4 mm mesh size and then dried in a dryer at 40 ° C for 6 hours. After drying, the obtained strips were broken into particles of 1.8 mm length.

Growth performance and survival rate

At the end of the research period, according to the shrimp initial weight, weight gain (%WG), specific growth rate (SGR), feed conversion ratio (FCR), feed intake (FI), muscle content (MC), protein efficiency ratio (PER) and survival rate were recorded and calculated by the following equations (Ricker, 1975):

Weight gain (%WG)=[(final mean weight (g)−initial mean weight (g))/initial mean weight (g)]×100

Specific growth rate (SGR) (%day^{-1})=100×[Ln final body weight (g)- Ln initial body weight (g)]/t (days)

Feed conversion ratio (FCR)=feed intake (g)/total weight gain (g)

Feed intake (FI)=[dry weight of given feed (g)-dry weight of the sediments from siphon off 10% of tank floor water 30 minutes after feeding (g)]/Number of shrimp

Protein efficiency ratio (PER)=total weight gain (g)/total protein intake (g)

Muscle content (%)=100×muscle weight (g)/whole-body weight (g)

Survival rate (%)=(final number of shrimp (g) / initial number of shrimp (g))×100

Biochemical analyses

Blood biochemical parameters such as total protein (TP), glucose (GL), cholesterol (CHO), alkaline phosphatase enzymes (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assessed in 10 randomly taken shrimps from each tank and blood samples were taken by 2-ml sterile syringes from the abdominal sinus. To measure the biochemical parameters, blood samples were stored in a simple test tube without anticoagulant for 12 hours at 4 °C and after blood clotting, samples were centrifuged (4200×g, 15 min) at 4°C. Following this, the serum was separated from the clot with a sampler and stored at −80°C until analyzed. Separated serum samples were specified using automatic biochemical analyzer (Hitachi 911, Tokyo, Japan) and the attached kits (Pars azmoon, Tehran, Iran; Zist shimi, Tehran, Iran).

In order to assess the intestinal digestive enzymes activity, 5 shrimps were taken randomly from each tank and placed in a solution of 40 g L^{-1} clove extract for 10 minutes. After anesthetizing the shrimps, intestinal tissues were removed from the bodies and homogenized with sufficient amount of sterile 0.8% saline solution to achieve 10% (W: V) homogenates based on the findings of Miandare *et al*. (2017). Homogenates were centrifuged at 168×g for 8 minutes at 4°C. Immediately after the end of centrifuge operation, assessment of digestive enzymes activity was performed using spectrophotometer (SP-VIS100, Analytik, Germany). The activity of each digestive enzyme such as protease enzymes, amylase enzymes and lipase enzymes were performed by using their substratescasein, pure starch and olive oil, respectively, and also their special commercial kits (Sigma-Aldrich Co, USA) following the manufacturer's instruction (Bio-RAD, USA). Finally, the activity level of all three digestive enzymes was expressed in units of U per gram protein.

Approximate analysis of diets, whole-body and muscle of the shrimp and sea cucumbers were performed according to the standard methods (AOAC, 2019). Shrimp feeding was stopped 12 hours prior to the enzymatic activity assessments to ensure that the digestive system is empty, then 5 shrimps from each tank were taken randomly and sent to the lab. To measure moisture, samples of diet, shrimp and sea cucumber were dried at 110°C for 7 hours until they reached a constant weight, then protein was calculated by estimating total nitrogen (N×6.25) using the Kjeldahl method and crude lipid was calculated by the Soxhlet method using chloroform solvent. Also, ash content was determined by burning in a furnace at 550ºC for 16 h.

Statistical analysis

The data were normalized by the Kolmogorov-Smirnov test prior to further statistical analyses. And then, analysis of variance (One-way ANOVA) and significant differences among the means (Mean±SD) were found ($p<0.05$) by Duncan's test in SPSS version 21 (IBM, USA).

Results

Growth performance and survival

Growth performance and survival rate results are expressed in Table 2. Survival rate was more than 95% indicating not significant difference among treatments. FW, WG, SGR and

DFI parameters increased significantly ($p<0.05$) from 0% (control) to 4% treatment, but there were no significant difference ($p>0.05$) among the 4, 6 and 8% treatments. Fig. 1 shows the weekly growth trend of the studied shrimps during the 8-week research period. From the fourth week, weight gain in 4, 6, and 8% treatments were 7.33, 7.13 and 6.73g respectively and significantly increased compared to the control and 2% treatments that were 4.87 and 5.59g.

Table 2: Effect of different *Bohadschia ocellata* meal levels dietary on growth performance and survival of *Penaeus vannamei*.

	0% (Control)	2%	4%	6%	8%
IW[1] (g)	6.63 ± 0.27	6.59 ± 0.31	6.69 ± 0.23	6.66 ± 0.31	6.58 ± 0.39
FW[2] (g)	19.33 ± 0.34[a]	21.21 ± 0.41[b]	22.99 ± 0.31[c]	23.07 ± 0.2[c]	22.86 ± 0.08[c]
WG (%)	191.55 ± 5.26[a]	221.85 ± 6.49[b]	243.64 ± 5.06[c]	246.39 ± 3.12[c]	247.41 ± 0.33[c]
SGR (% day^{-1})	1.78 ± 0.36[a]	1.94 ± 0.44[b]	2.05 ± 0.33[c]	2.07 ± 0.24[c]	2.07 ± 0.18[c]
FCR	1.70 ± 0.17[b]	1.71. ± 0.24[b]	1.69 ± 0.15[b]	1.67 ± 0.09[b]	1.79 ± 0.04[a]
PER	1.48 ± 0.03[c]	1.45 ± 0.03[c]	1.37 ± 0.08[cb]	1.31 ± 0.04[ba]	1.25 ± 0.06[a]
DFI [3] (g)	0.36 ± 0.02[a]	0.43 ± 0.01[b]	0.51 ± 0.01[c]	0.51 ± 0.04[c]	0.53 ± 0.01[c]
MC[4] (%)	49.23 ± 0.39	49.01 ± 0.88	49.20 ± 0.40	49.66 ± 1.03	49.30 ± 0.51
Survival (%)	98.33 ± 2.88	96.66 ± 5.77	100.00 ± 0.00	98.33 ± 2.88	98.33 ± 2.88

[1] Initial weight, [2] Final weight, [3] Daily feed intake (g day^{-1} shrimp^{-1}), [4] percentage of muscle content. Values are represented as means±SD (n=3). Means in the same row with different superscript show significant differences ($p<0.05$).

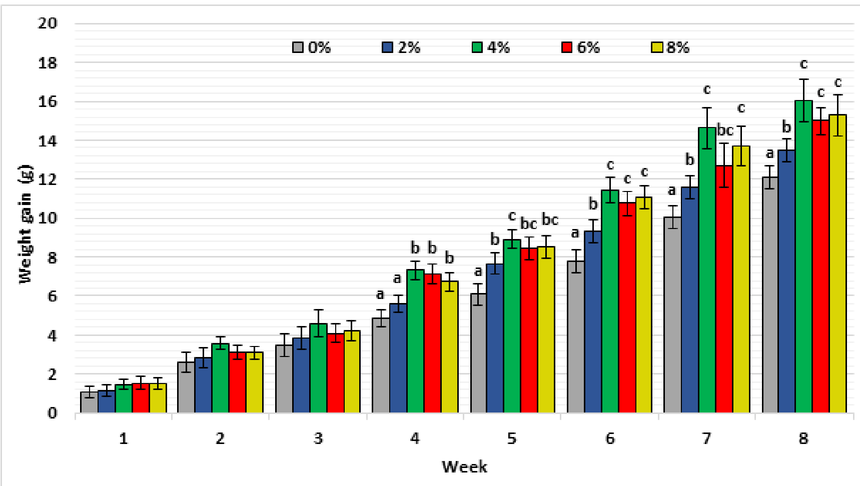

Figure 1: Process of weight gain during the trial period, in *Penaeus vannamei* affected by different dietary levels of *Bohadschia ocellata* meal. Each bar represents the mean value±SD (n=3). Values with different letters differ significantly ($p<0.05$) among treatments of each week.

Even though the greatest FCR was recorded for the 8% treatment, the difference was not significant. MC differences among the treatments were not significant. PER for the shrimps fed with 0, 2 and 4% *B. ocellata* diets were significantly greater than PER for the other treatments.

Blood biochemical parameters

At the end of 8-weeks experimental period, some changes were observed in the blood biochemical parameters of treatments (Table 3). There were no significant differences in GL levels among 0, 2, and 4% treatments. The highest and the least CHO levels were recorded in 4 and 0% treatments, respectively. Other parameters such as ALT, ALP, AST, and TP did not show significant difference among treatments during the research period.

Table 3: Effect of different dietary *Bohadschia ocellata* meal levels on blood serum biochemical parameters of the *Penaeus vannamei*.

	0% (Control)	2%	4%	6%	8%
TP (g L^{-1})	74.23 ± 7.02	78.10 ± 6.13	74.00 ± 3.76	72.81 ± 3.08	75.59 ± 4.99
GL (mg L^{-1})	13.38 ± 0.34^{b}	12.93 ± 0.41^{b}	13.19 ± 0.41^{b}	16.86 ± 0.26^{a}	16.56 ± 0.08^{a}
CHO (mmol L^{-1})	1.82 ± 0.19^{a}	1.21 ± 0.07^{b}	1.03 ± 0.11^{c}	1.31 ± 0.03^{b}	1.29 ± 0.11^{b}
ALP (U L^{-1})	29.93 ± 0.61	28.19 ± 2.11	33.14 ± 1.03	31.51 ± 0.41	30.05 ± 1.83
AST (U L^{-1})	191.49 ± 14.29	193.27 ± 6.04	189.71 ± 18.19	197.30 ± 8.70	202.42 ± 21.27
ALT (U L^{-1})	251.64 ± 28.19	243.70 ± 50.17	255.71 ± 39.71	260.13 ± 12.96	254.22 ± 52.34

Values are represented as means±SD (n=3). Means in the same row with different superscript show significant differences (p<0.05).

Intestinal digestive activity

The trend of protease enzyme activity at the end of the experimental period showed a significant increase from 4 to 8% treatments. Figure 2 shows the final values for protease, it was the smallest value for the 2% treatment (344 U g^{-1}), and for the 8% treatment it was the greatest value (431 U g-1). Lipase and Amylase enzymes activities did not show any significant change among treatments.

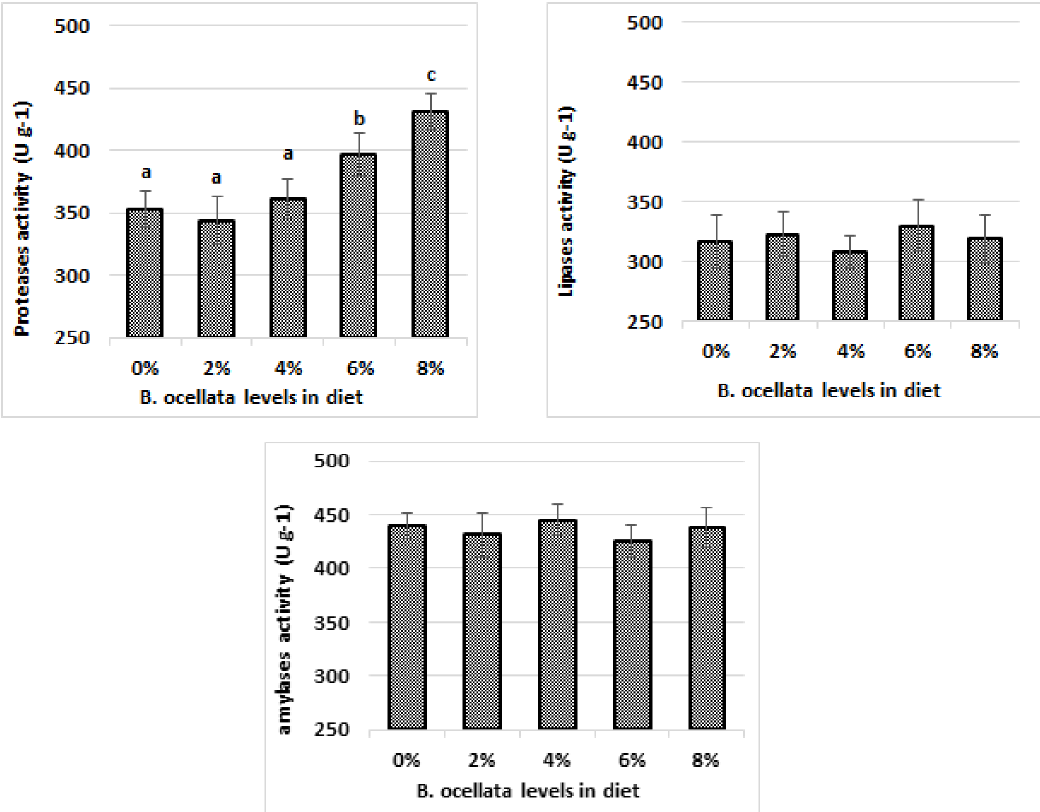

Figure 2: Intestinal digestive activity of *Penaeus vannamei* affected by different *Bohadschia ocellata* meal levels dietary. Each bar represents the mean value±SD (n=3). Values with different letters differ significantly (*p*<0.05) among treatments.

Diet, muscle and whole-body composition

After preparation of the experimental diets with different levels of sea cucumber (*B. ocellata*) meal, the approximate content analysis of the diets was determined (Table 1). The data depicted that difference in the levels of *B. ocellata* meal had no significant effect on crude protein, crude lipid, moisture and ash content of the diets (*p*>0.05). The results also showed that feeding of *P. vannamei* with the diet containing 6% *B. ocellata* significantly increased the moisture content in both the muscle and the whole-body (Table 4). Also, the ash content in both the muscle and the whole-body for the 8% treatment was greater than other treatments, other parameters such as crude protein and crude lipid did not show any significant difference among treatments (*p*>0.05).

Discussion

The present study revealed that changes of the *B. ocellata* meal levels in the *P. vannamei* diet caused significant changes in the shrimp blood biochemical factors, digestive enzymes activities, growth performance, and body composition. There are some reports claiming that addition or substitution of plant sources in *P. vannamei* diet as a replacement of fish meal or other diet items, can: reduce the feeding costs (Amaya *et al*., 2007); increase feed quality and efficiency (Samocha *et al*., 2004); improve immunity (Nonwachai *et al*., 2010); improve growth performance (Davis and Arnold, 2000) and improve muscle quality (Liu *et al*., 2012). Also, some marine cucumber species have anti-microbial effects (Ebrahimi *et al*., 2018). Despite these findings, Shao *et al*. (2017) showed that replacing 15% of fish meal with biofloc meal in *P. vannamei* diet did not make any difference on shrimp growth performance. Also, replacing 100% of fish meal with a mixture of microbial floc meal and soy protein in diets did not reduce the growth rate of the shrimp (Bauer *et al*., 2012). As Lim and Dominy (1990) reported, diets containing 14% soybean meal as a replacement of 20% animal protein increased the weight gain of *P. vannamei* after 56 days feeding period. Addition of low value and cheap protein sources to the shrimp feed can be beneficial if it does not reduce the growth and disrupt the body functions.

Providing 30% protein in a diet using co-extruded consisting of a 35:65 proportion mixture of tuna fish viscera and whole corn flour increased weight gain of *P. vannamei* juveniles during 41 days feeding period (Hernández *et al*., 2004). The results of this study also indicated that adding an aquatic animal source to improve the diet of *P. vannamei* has strong effects on the shrimp growth and some body biochemical performances.

In the present study, even though there were significant differences in weight gain, SGR, daily feed intake and PER parameters among the treatments, muscle content and survival rate did not show any significant change. Weight gain in shrimp is mainly influenced by the protein source rather than the amount of protein in the diet (Sudaryono *et al*., 1995; Ahmadi *et al*., 2019). This increase in weight gain could be due to new protein source palatability and subsequently an increase of feed intake or the amino acids balance of the used protein in the diet. Dominy and Lim (1991) indicated that a mixture of fresh wet squid viscera by-product with soybean improved shrimp growth performance and decreased final diet cost in comparison to fish meal use. On the other hand, Wang *et al*. (2016) showed that usage of the Orpin rose (*Rhodiola rosea*) as a supplement in the shrimp diet had no significant impact on the growth rate, while replacement of fish meal by soy protein and corn gluten meal in *P. vannamei* diet, improved the shrimp weight gain during the feeding period (de Carvalho *et al*., 2016). Also, Yu *et al*. (2016b) depicted that final body weight and weight gain of the shrimps fed by diets containing 2 and 3% *Gracilariopsis lemaneiformis*, were significantly greater than the shrimps fed by a controlled (0%) diet. *P. vannamei*, like other crustaceans, requires minerals such as calcium, magnesium, phosphorus, zinc etc. for normal body function and it seems that its ability to absorb these elements from marine animal sources is more efficient

(Cheng *et al*., 2006; Rezaei Tavabe *et al*., 2013; Rezaei Tavabe *et al*., 2015; Bharadwaj *et al*., 2017). For example, using krill meal in Pacific white shrimp diet can improve the growth performance and feed intake (Derby *et al*., 2016). Generally, since body composition of marine animals are closer to the body compassion of *P. vannamei*, adding sources from marine animals to *P. vannamei* diet improves its growth performance more than adding other sources based on plants or terrestrial animals. This is probably because various feeding stimulants, minerals, and vitamins at these sources provide the shrimp's nutritional requirements. Also, plant protein sources such as soybean meal which is mostly used in shrimp diets; contain four main anti-nutritional factors including trypsin inhibitors, saponins, non-starch polysaccharides and phytic acid (Francis *et al*., 2001; Xie *et al*., 2016).

The present research finding showed that different levels of the sea cucumber meal in *P. vannamei* diet had no significant effect on total protein, alkaline phosphatase enzymes, aspartate aminotransferase and alanine aminotransferase parameters in the shrimp blood serum. Although knowledge about nutritional effects on shrimp's blood biochemical parameters is very essential, there is a general lack of information on this topic. Sun *et al*. (2016) showed that replacing fish meal by fermented cottonseed meal in different levels to substitute 25, 50, 75 and 100% of the fish meal did not show any significant difference in the total protein and aspartate aminotransferase values in blood serum. Even, adding different levels of aflatoxin B_1 (0, 25, 50, 100, 500, 1000 μg kg^{-1}) as a lethal toxin to shrimp diet did not show a significant change on the amount of total protein and aspartate aminotransferase parameters in shrimp blood serum (Zeng *et al*., 2016). Considering the findings of previous researches and the present study, it seems that adding low levels of plants and aquatic animal sources and even some natural toxins to shrimp diet does not alter shrimp hepatopancreas function to increase or decrease the total protein levels, aspartate aminotransferase and alanine aminotransferase in the serum, however it is reported that synthetic toxins, such as t-2 toxin, often alter blood indices and disrupt the hepatopancreas function (Qiu *et al*., 2016). Serum alanine aminotransferase level, aspartate aminotransferase level and their ratio (AST/ALT ratio) are commonly measured clinically as biomarkers for hepatopancreas health (Rezaei Tavabe and Rafiee, 2016). Therefore, considering these parameters no significant difference among treatments indicate that usage of different levels of sea cucumber meal in the shrimp diet had no detrimental effect on hepatopancreas function. In contrast, glucose parameter showed significant difference among treatments and the highest value was recorded for 6 and 8% treatments of *B. ocellata* meal. Apún-Molina *et al*. (2015) also reported that adding a probiotic mixture composed of four lactic acid bacteria and one yeast strain to shrimp diet in a polyculture system of shrimp (*P. vannamei*) and Nile tilapia (*Oreochromis niloticus*) increased the glucose level of shrimp serum. It can be concluded that new and unfamiliar feed in the shrimp diet may cause stress and increase glucose level by breaking down stored glycogen in the hepatopancreas to glucose and releasing it to the shrimp's blood.

Liu *et al*. (2009) indicated that adding *Bacillus subtilis* E20 to *P. vannamei* diet (108 CFU kg^{-1} of diet) as a protease-producing probiotic can improve the growth performance by improving the protease activity in the digestive tract. In the current study, protease enzyme activity showed an increasing trend with a direct relationship to *B. ocellata* meal proportion in the shrimp diet. Therefore, the use of *B. ocellata* in the diet of *P. vannamei* increased weight gain of the shrimp, not only through increasing palatability and enhancing feed intake but also by raising protease activities in the digestive tract. In contrast, different levels of *B. ocellata* meal in the diet did not show any significant effect on amylase and lipase enzymes activity. Similarly, replacement of fishmeal by marine microalgae (*Spirulina platensis*) in the Pacific white shrimp (*P. vannamei*) diet did not show any significant effect on these two digestive enzymes activities (Pakravan *et al*., 2017). The researcher showed that most of sea cucumber species of Holothuroidea class have high protein and low-fat content (Wen *et al*., 2010; Aydın *et al*., 2011). Our findings showed that the addition of different levels of sea cucumber meal to the shrimp diet did not significantly change crude protein and crude lipid contents in the shrimp body while ash and moisture values were significantly different.

This study highlights that adding 4-6% of the *B. ocellata* meal to a commercial diet of *P. vannamei* juveniles can clearly enhance its daily feed intake, growth performance, and improve some body biochemical parameters during the cultural period. Also, there was no significant increase in serum glucose content up to 6% of *B. ocellata* meal in the diet, which could be due to the normal stress condition for the shrimp. The protease digestive enzyme activity increased significantly from 4 to 8% *B. ocellata* meal treatments. Finally, we recommend that in areas where there is enough access to the sea cucumber, adding 4-6% of the *B. ocellata* meal to the *P. vannamei* in the cultural period diet can clearly enhance output and production of the shrimp.

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