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Fishmeal replacement by mealworm (*Tenebrio molitor*) in diet of farmed Pacific white shrimp (*Litopenaeus vannamei*): effects on growth performance, serum biochemistry, and immune response

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Abstract – Reducing the use of fishmeal (FM) in shrimp feed means significant savings in the amount of FM consumed globally and subsequently reducing production costs and environmental impacts. Insect meal (IM) is one of the protein sources to replace FM in aquafeeds. In this regard, this study was conducted with the aim of investigating the effect of replacing FM with mealworm (MW, *Tenebrio molitor*) on the growth performance, haemolymph biochemical responses, and innate immunity of *Litopenaeus vannamei*. Shrimps with a mean weight of 7.41 ± 0.13 gram were cultured in 300-liter fiberglass tanks (with a useful drainage volume of 200 liters) with a density of 20 shrimp per tank over a period of 60 days. Dietary treatments, including the control treatment (no mealworm; T0), 15% (T15), 30% (T30), 60% (T60), and 100% (T100) level of replacing FM with mealworm (MW), each with three replications, were investigated in the form of a randomized design. The results of this study showed a significant difference in body weight gain (BWG), feed efficiency (FE), feed conversion ratio (FCR), and hepatopancreas index (HPI) among the treatments ($P < 0.05$). With the increase of the replacement of FM with MW up to 30%, BWG, FE, and HPI were significantly increased then reduced. The levels of cholesterol (Chol), triglycerides (Tg), and glucose (Glu) showed a decreasing trend with increasing replacement of FM with MW and revealed a significant difference with the control treatment at high levels of replacement ($P < 0.05$). Besides, the results showed that replacing FM with MW had a significant effect on the activities of superoxidase dismutase (SOD), phenol oxidase (PO), lysozyme (LZM), acid phosphatase (ACP), alkaline phosphatase (ALP) and the total count of hemocytes (THC) in the practical diets compared to the control group ($P < 0.05$). Overall, the findings suggest that MW is a promising alternative protein source for *L. vannamei*, as it enhances both growth performance and the immune system. The study recommends the use of MW in the diet of farmed species in the aquaculture industry, given its lack of adverse impacts on growth performance and its potential to reduce environmental consequences resulting from its production. The results also underscore the importance of exploring alternative protein sources to reduce dependence on FM and enhance sustainability in the aquaculture industry.

Keywords: *Litopenaeus vannamei* / fishmeal replacement / *tenebrio molitor* / growth performance / biochemical index / immune response

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1 Introduction

Aquaculture is the fastest growing sector of animal feed production, which traditionally uses fishmeal (FM) as its key protein ingredient in commercial aquafeeds formulation (Khanjani and Sharifinia, 2020, 2022). Aquaculture must continue to grow due to the depletion of aquatic stocks and the current peak in wild fisheries catch, as well as to meet the demand for fish for human consumption (FAO, 2018). The current trend, along with regulations, agricultural policies, and limitations on wild fish resources, has led to an exponential increase the price of the raw materials used as feed in aquaculture activities, mainly FM and fish oil (FO), in recent years (FAO, 2018). The increase in global demand and the decrease in the availability of FM have led to a sharp increase in the price of FM and thus the cost of aquaculture production (Olsen and Hasan, 2012; Tacon and Metian, 2008). In fact, reducing the percentage of FM in the shrimp diet means a significant savings in the amount of FM at the global level and also reduces production costs. These reasons have motivated researchers to look for new protein sources with similar nutritional value to FM (Fawole et al., 2020; Khanjani et al., 2022b; Wang et al., 2022).

Considering that the nutritional requirements of farmed aquatic species include a large amount of high-quality protein (Khanjani et al., 2022a), the protein sources used in their diets must therefore meet some essential requirements, including: high protein content, a suitable amino acid profile, high digestibility, palatability, a lack of anti-nutritional factors, and affordability in terms of cost-effectiveness (Sánchez-Muros et al., 2020). As an alternative to FM, many researchers have recommended plant sources (PS) due to their lower price and greater availability (Hardy, 2010; Jannathulla et al., 2019). However, PS have several undesirable characteristics such as imbalance and deficiency of essential and non-essential amino acids, low protein content, high anti-nutritional factors (e.g., saponins, alkaloids, and tannins), non-digestible carbohydrates, and fiber content, which have little palatability (Ferrer Llagostera et al., 2019; Henry et al., 2015). All these unfavorable features limit their percentage of replacement in the diets of aquatic species (Nagappan et al., 2021; Sánchez-Muros et al., 2020).

Due to the environmental problems of FM in the past several years, a lot of effort has been made to use new protein sources for feeding aquatic animals (Ghamkhar and Hicks, 2020). The use of such resources for nutrition can be a viable option for improving growth performance, feed efficiency, and meeting aquatic protein requirements.

Due to increasing costs and concerns about the sustainability of soybean and fishmeal, insects are being viewed as a promising alternative for high-protein animal feed. This is due to their similar nutrient profile and the fact that they can be produced in an environmentally efficient manner (van Huis et al., 2013). In fact, the market for insects as animal feed was estimated to be worth US$688 million in 2018 and is projected to grow to US$1.4 billion by 2024 (Globe Newswire. 2020). The global edible insects market is divided into five major regions: North America, Europe, Asia-Pacific, Latin America, and the Middle East and Africa. During the forecast period, the North American region is expected to experience significant growth in the global edible insects' market. This growth is primarily due to the increasing demand for environmentally friendly high protein diets, a preference for less-processed foods, and a growing concern about the environmental impact of meat production (Research, 2018). Insects have been reported that can be a promising sustainable source of protein (FAO, 2018). Insect meals (IM) are rich in amino acids, lipids, minerals, vitamins, and energy (Barroso et al., 2014; Motte et al., 2019). Another advantage of using IM as a new innovative protein source is their low impact on the environment. Also, the production of insects requires a small space and little water for cultivation (Quang Tran et al., 2022; van Huis, 2022). They have high reproduction rates and production efficiency, and they also produce biomass rich in protein and fat by feeding on organic waste and food (de Carvalho et al., 2020; Makkar et al., 2014). Therefore, insects can also help reduce the environmental issues associated with revaluing food waste (Ferrer Llagostera et al., 2019; Makkar et al., 2014). As a final point, IM has recently been accepted for use as feed for aquaculture (Ferrer Llagostera et al., 2019). It's worth noting that some aquatic species naturally consume the larvae of certain insect species (DiGiacomo and Leury, 2019; Sharifinia, 2015).

In recent years, attention has been significantly increased to study the replacement of FM with IM in aquaculture as an appropriate feed replacement (Belforti et al., 2015; Fawole et al., 2020; Ferrer Llagostera et al., 2019; Gasco et al., 2016; Mastoraki et al., 2020b; Mente et al., 2022; Tubin et al., 2020). Various species of insects are considered for larval meal production, among which mealworm (MW, *Tenebrio molitor*) is one of the most promising (Mente et al., 2022). The larval stage of *T. molitor* can be used as a suitable alternative high-protein food in carnivorous aquatic diets (Belforti et al., 2015; Gasco et al., 2016). Recent studies have reported promising results regarding the use of insect powder in the diet of varying aquatic species (Gasco et al., 2016; Iaconisi et al., 2017; Mastoraki et al., 2020b; Piccolo et al., 2017). Ng et al. (2001) replaced *T. molitor* larvae meal with FM in the African catfish's (*Clarias gariepinus*) diet. Their results showed that with more than 40% replacement, a decrease in growth performance was observed. Feng et al. (2019) examined the effect of different levels of MW on the growth performance and immune system of *Macrobrachium rosenbergii*. They found that the inclusion of 12% of MW had a positive effect on growth performance and immunological parameters and suggested the use of this protein source as a practical replacement in the diet.

L. vannamei, commonly known as Pacific white shrimp, is a highly cultivated species worldwide due to its rapid growth, high reproductive rate, and disease resistance, which makes it a promising candidate for aquaculture and food production (FAO, 2019). It is also a well-established research model for studying various aspects of shrimp biology and ecology (Chen et al., 2019; Liu et al., 2018; Yeganeh et al., 2020). Additionally, *L. vannamei* plays a crucial role in marine ecosystems, making it essential to understand its biology and ecology for effective management and conservation of shrimp populations and their habitats (FAO, 2019). Therefore, this study aims to examine the effects of the dietary replacement of FM by MW on growth performance, serum biochemistry, and innate-immune enzyme activities in *L. vannamei* juveniles.

Table 1. Formulation and proximate composition of the experimental diets for juvenile *Litopenaeus vannamei*.

Ingredients (%)	Experimental diets				
	T0	T15	T30	T60	T100
Fishmeal[1]	24	20.4	16.8	9.6	0
Mealworm meal	0	3.6	7.2	14.4	24
Soybean meal	28	28	28	28	28
Shrimp by-product meal	10	10	10	10	10
Fish oil[2]	2	2	2	2	2
Wheat meal	8	8	8	8	8
Wheat bran	14	13.58	13.73	13.31	12.58
Soybean oil[3]	5	4	3	2	1
Mineral premix[4]	1	1	1	1	1
Vitamin premix[5]	2	2	2	2	2
Cholesterol	0.5	0.5	0.5	0.5	0.5
Soybean lecithin	2	2	2	2	2
DL-Methionine	0.5	0.5	0.5	0.5	0.5
Vitamin C	1	1	1	1	1
Kaolin	0.5	0.5	0.5	0.5	0.5
Binder	1.5	1.65	1.77	2.19	2.65
Filler	0	1	2	3	4
	Proximate composition				
Protein (%)	37.61	37.45	37.31	36.97	36.68
Lipid (%)	8.97	8.93	8.24	9.89	12.98
Ash (%)	21.17	20.84	19.77	18.03	15.78
Moisture (%)	9.65	9.32	9.43	8.77	8.11
NFE[6]	22.60	23.46	25.25	26.07	26.45
Gross Energy (MJ g^{-1})	1.64	1.65	1.65	1.72	1.84

[1] FanavaranDarya Co., Shiraz, Iran (Matota fishmeal; Engraulidae).
[2] Havorash (Bushehr, Iran).
[3] Product of Kesht Va Sanat Shomal Vegetable Oil Factories Complex (Neca, Iran).
[4] Mineral mixture (mg kg^{-1} mixture): Co, 40; I, 220; Se, 300; Zn, 10,000; Fe, 3500; Cu, 4000; Mn, 6000.
[5] Vitamin added to supply the following (per kg diet): vitamin A, 80,000 IU; vitamin D3, 2000 IU; vitamin E, 100 mg; vitamin K, 20 mg; thiamin, 60 mg; riboflavin, 60 mg; pyridoxine, 100 mg; pantothenic acid, 150 mg; niacin, 300 mg; biothin, 2 mg; folic acid, 20 mg; vitamin B12, 0.1 mg; inositol, 300 mg; ascorbic acid, 600 mg; choline chloride, 3000 mg.
[6] Nitrogen-free extracts (NFE) = 100 − (crude protein + crude lipid + fiber + ash).

2 Material and Methods

2.1 Practical diets

The formulation and chemical composition of the practical diets are presented in Table 1. The shrimp were fed with five practical diets (T0, T15, T30, T60, T100) containing FM which was gradually replaced by MW (0, 15, 30, 60, and 100%). The practical diets used in a study were pelleted into 2 mm diameter pellets using a grinder machine and each treatment was placed separately in an oven at 60°C for 6 hours to remove moisture. Employing the standard proximate analysis method prescribed by the Association of Official Analytical Chemists (AOAC) in 2003, the moisture, dry matter, protein, lipid, and fiber content of all experimental diets, underwent analysis. Then all diets were sealed in vacuum-packed bags and stored in a freezer at 4 °C until used for the feeding trial.

2.2 Experimental design

The trial lasted 60 days and was performed at the Persian Gulf SPF Shrimp National Research Center in Bushehr, Bushehr Province, Iran. Juvenile healthy white shrimp, *L. vannamei*, with an average initial weight of 7.41 ± 0.13 g and average initial length of 8.30 ± 0.08 cm were obtained from this center. Before starting the feeding experiment, the shrimps were fed commercial feed (40% protein, 8% lipid) for 7 days to adapt to the experimental conditions. A total of 300 juveniles were randomly distributed in 15 fiber-glass cylindrical tanks (300 L tanks filled with 200 L of seawater) with a density of 20 shrimp in each tank equivalent to 1 shrimp per 0.01 cubic meters (m^3). All tanks were covered with plastic nets to prevent shrimp from jumping out of the tanks. All treatments were performed in three replicates. Shrimp fed four times a day at 6:00, 12:00, 18:00, and 24:00. The daily feed intake ranged from 2-5% of the shrimp body weight, and it was adjusted based on the shrimp body weight to prevent any feed residue from remaining two hours after feeding. The shrimp in each tank were weighed once every 10 days and the daily feed ration was accustomed accordingly. Dead shrimp were immediately removed from the tanks and weighed and recorded. Waste materials accumulated on the bottom of the tanks were cleaned daily by siphoning. The photoperiod was maintained in a natural cycle. Aeration of tanks were provided through air stones, with dissolved oxygen levels

near saturation. During the trial period, parameters of seawater including temperature (26–28 °C), salinity (27–29 g L^{-1}) measured daily. Also, pH (8.1-8.9), and dissolved oxygen level (6.2–6.8 mg L^{-1}) measured weekly.

2.3 Sample collection

At the termination of the experimental trial, in order to reduce the stress level of shrimp, all groups were starving for 24 h before sampling. Haemolymph samples was taken from five shrimp (15 samples of each treatment) in each tank (Shi et al., 2021). Haemolymph was sampled from each shrimp by a 1 mL sterile syringe contained 0.5 mL of precooled (4 °C) anticoagulant (ALSEVER solution 27 mM L^{-1} sodium citrate, 0.34 M L^{-1} NaCl, 9 Mm L^{-1} EDTA, 0.115 M L^{-1} Glucose, pH 7.0). Then the haemolymph samples were divided into two parts for further tests and investigations. One part of haemolymph was stored in ice and transferred to the laboratory for counting the total haemocyte count (THC), and the other aliquot centrifuged (3000 g, 10 min, 4 °C), plasma separated and stored at −80 °C for the analysis of phenoloxidase (PO) activity, lysozyme (LZM), alkaline phosphatase (ALP), acid phosphatase (ACP), and superoxide dismutase (SOD).

2.4 Growth performance

At the end of the experiment, the shrimp in each tank were fasted for 24 h before sampling. All shrimp in each tank were counted and weighed to calculate body weight gain (BWG), body length gain (BLG), specific growth rate (SGR), feed conversion ratio (FCR) and survival rate (SR). Meanwhile, the weight of hepatopancreas were measured to calculate the hepatopancratic index (HPI) as follows. All calculations carried out using following formulas:

$$\mathrm{BWG\,(g)} = \mathrm{Final\ weight} - \mathrm{Initial\ weight}$$

$$\mathrm{BLG(cm)} = \mathrm{Final\ length} - \mathrm{Initial\ length}$$

$$\mathrm{FCR} = \frac{\mathrm{Feed\ given\,(g)}}{\mathrm{Alive\ weigh\ gain\,(g)}}$$

$$\mathrm{SR}(\%) = \left[\frac{\mathrm{Final\ number\ of\ shrimp}}{\mathrm{Initial\ number\ of\ shrimp}}\right] \times 100$$

$$\mathrm{HPI}(\%) = \left[\frac{\mathrm{Hepatopancreas\ wet\ weight\,(g)}}{\mathrm{Wet\ body\ weight\,(g)}}\right] \times 100$$

$$\mathrm{CF}\left(\mathrm{gcm}^{-3}\right) = \left[\frac{\mathrm{Body\ weight(g)}}{\mathrm{Body\ length}^3(\mathrm{cm}^3)}\right] \times 100$$

$$\mathrm{FE}(\%) = \left(\frac{\mathrm{BWG}}{\mathrm{Feed\ consumed}}\right) \times 100.$$

2.5 Haemolymph biochemical parameters

For the biochemical analysis of the haemolymph factors, the samples were collected in small microtubes containing 0.4% Alsever's solution (115 mM glucose, 336 mM NaCl, 27 mM sodium citrate, and 9 mM EDTA with a pH of 7) (Jones et al., 2010). The haemolymph samples were centrifuged at 12000 rpm for 15 min at 5 °C using a centrifuge machine. The supernatant was collected and analyzed using quantitative assay kits on an autoanalyzer to measure the biochemical indices.). The haemolymph samples were centrifuged at 12000 rpm for 15 min at 5 °C using a centrifuge machine. To measure the cholesterol (Chol), triglyceride (Tg), total protein (TP), and glucose (Glu) parameters, the supernatant was collected and determined spectrophotometrically (ERMA Inc., Tokyo, Japan) by the use of commercial diagnostic kits (Pars Azmoon Co., Tehran, Iran).

2.6 Immunological parameters analysis

For the cell counts, a drop of the haemolymph–anticoagulant mixture (diluted haemolymph) was placed on a haemocytometer (Neubauer, Germany) to measure THC using a light microscope (Cetti, triton II, England) at 100×magnification. PO activity was measured by spectrophotometric method (490 nm) with the formation of DOPA-chromium red pigment after the oxidation of the enzyme substrate L-dihydroxyphenylalanine (L-DOPA) as described by Perazzolo and Barracco (1997). The LZM activity (U ml^{-1}) was measured by turbidimetric method according to Ellis (1990). The SOD activity was measured according to the manufacturer's instruction of Enzyme ZellBio (GmbH, Germany) Test Kits. For analysis of SOD, the hepatopancreas samples were homogenized briefly in the phosphate buffer (0.025 m KH2PO4, 0.025 m Na2PO4.12H2O, pH 6.4). Total SOD activity (U mg^{-1}) in supernatant samples was measured following the method described by Bolann and Ulvik (1991). The ACP and ALP activities were determined photometrically according to the method of Burtis and Bruns (2014). Crude enzyme solutions were incubated with ρ-nitrophenyl phosphate substrate in diethanolamine-MgCl buffer (Pars Azmoon Co., Tehran, Iran). After that, nitrophenol phosphate was added and the absorbance was measured photometrically at 405 nm and the activity was expressed as units L^{-1}.

2.7 Statistical analysis

Statistical analyses were performed using SPSS ver. 23.0 software (Chicago, Illinois, USA). One-way analysis of variance (ANOVA) was used to determine significant differences between groups. Post hoc tests were employed for multiple comparisons of means. Results are presented as mean ± standard deviation. Statistical significance was set at $P < 0.05$ for all tests.

3 Results

3.1 Growth performance

The effects of different levels of FM replacement by MW on the growth performance of *L. vannamei* are presented in

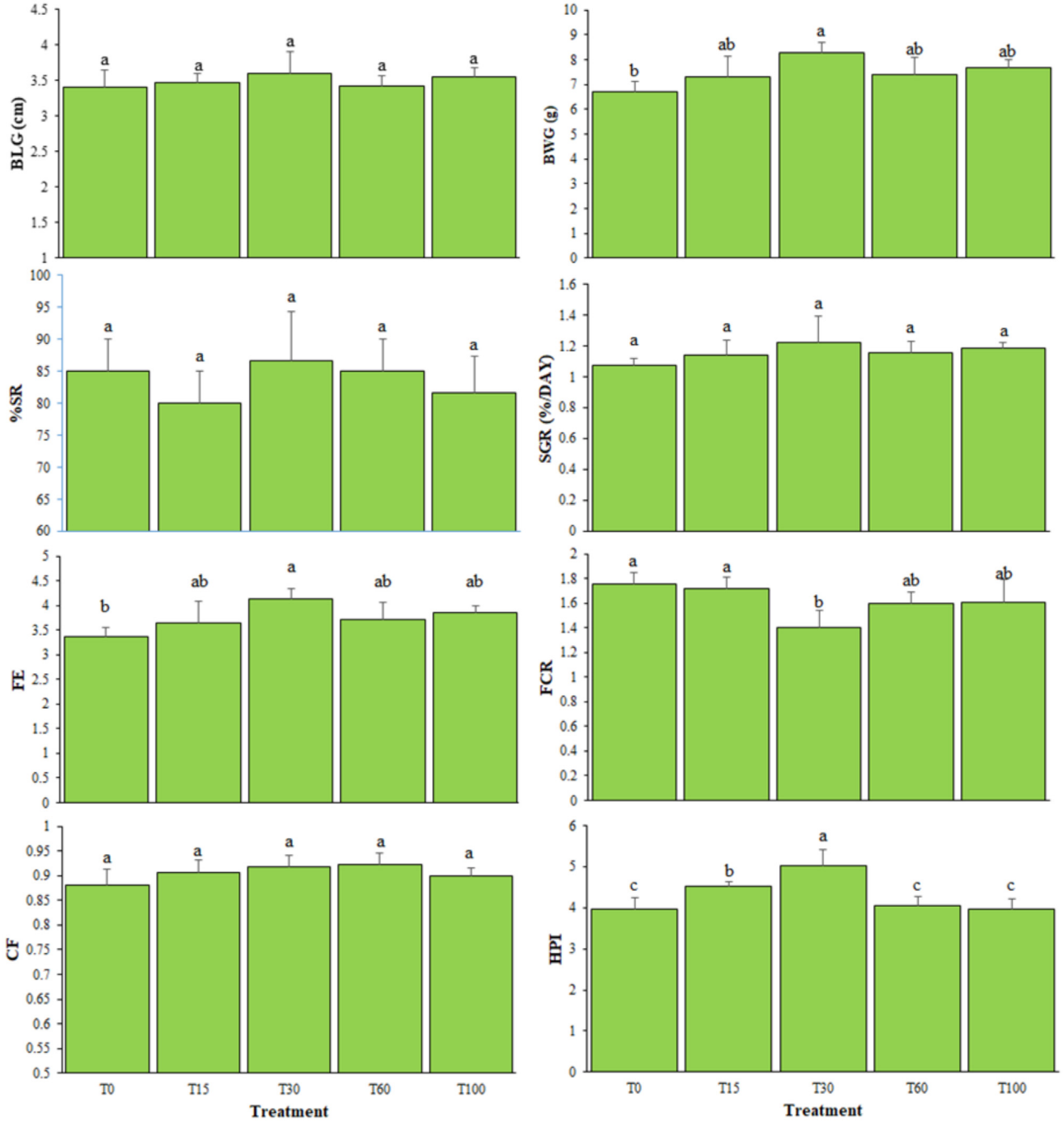

Fig. 1. Growth performance and feed utilization parameters of *Litopenaeus vannamei* fed the experimental diets. Values with different letters are significantly different ($P < 0.05$). BLG, body length gain; BWG, body weight gain; SR, survival rate; SGR, specific growth rate; FE, feed efficiency; FCR, feed conversion ratio; CF, condition factor; HPI, hepatopancratic index. T0 = diet without mealworm (MW) inclusion; T15, T30, T60, and T100 =15, 30, 60, and 100% replacement level of FM with MW, respectively.

Figure 1. Briefly, T30 treatment exhibited meaningfully ($P < 0.05$) higher BWG, FE, and HPI; and significantly ($P < 0.05$) lower FCR with respect to T0 treatment. BLG, SGR, and CF gradually showed an increasing trend by replacing FM with MW up to 30% level (T30), but did not show any significant difference with other treatments ($P > 0.05$). Moreover, in terms of survival rate, no significant difference was observed between different treatments ($P > 0.05$), and the lowest level of mortality was observed in T30.

3.2 Haemolymph biochemical parameters

Shrimp fed different diets shown significant differences in their haemolymph biochemical parameters ($P < 0.05$; Fig. 2). Trends of reduction in Cho, TG, and Glu with the reduction in FM content were seen between different treatments (Fig. 2). The highest and lowest values of Cho and Tg were observed in T0 and T100 treatments. Shrimp fed with 60% replacement of FM (T60) gained significantly more protein in their

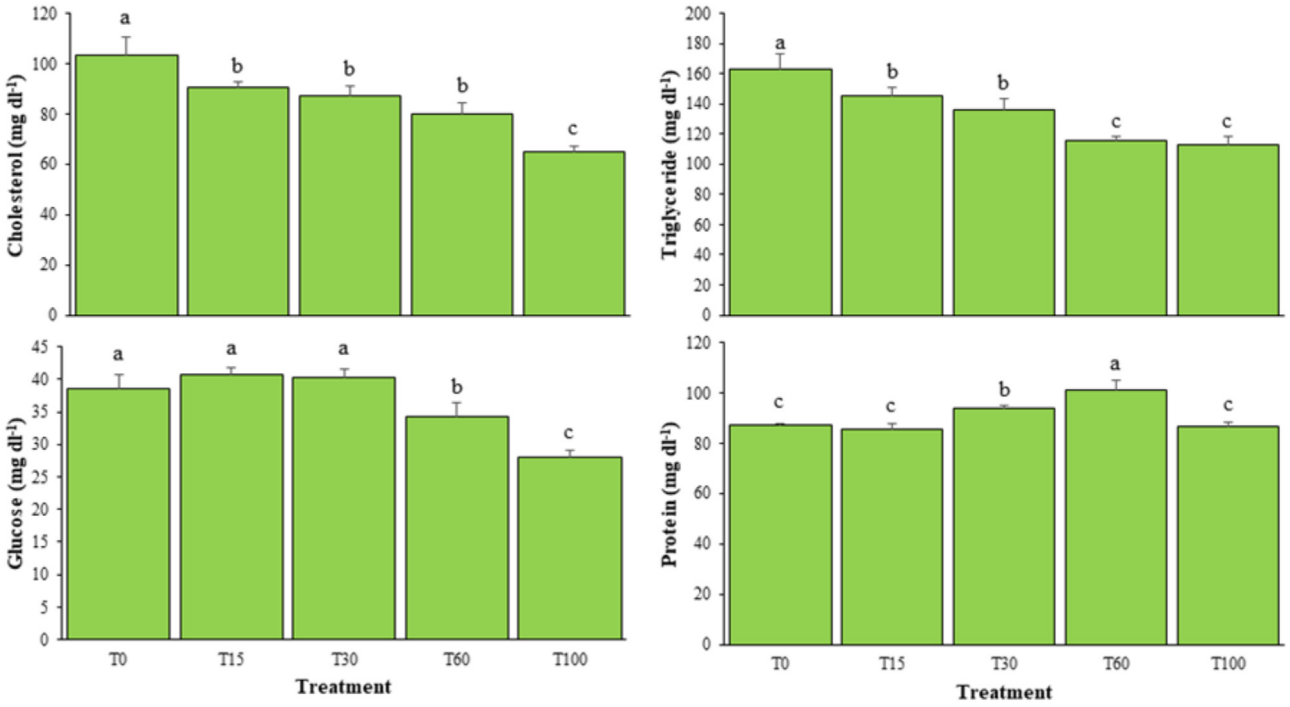

Fig. 2. Hematological and biochemical parameters of juvenile *Litopenaeus vannamei* fed the experimental diets for 60 days. Values are presented as the mean ± standard deviation. Different letters indicate statistically significant differences between different experimental groups ($P < 0.05$). T0 = diet without mealworm (MW) inclusion; T15, T30, T60, and T100 = 15, 30, 60, and 100% replacement level of FM with MW, respectively.

haemolymph and indicated a significant difference ($P < 0.05$) with T0 treatment.

3.3 Innate immunity enzyme activities

Effects of FM replacement with MW on immunity enzyme activities of the haemolymph of *L. vannamei* juveniles are shown in Figure 3. The lowest and highest values of the haemolymph SOD activity was obtained in T100 and T30 treatments, respectively. Significant differences were found among different treatments ($P < 0.05$). The activity of PO was meaningfully higher in the T15, T30, and T60 groups compared with other groups ($P < 0.05$). The level of LZM enzyme activity revealed an increasing trend with increasing the amount of replacing FM with MW up to the level of 60%. Its highest value was observed in T60 and had a significant difference with other experimental treatments ($P < 0.05$). ALP and ACP activities in shrimp fed with control diet (T0) were significantly ($P < 0.05$) lower than other treatments. Furthermore, the highest levels of ALP and ACP activities were observed in T30 treatment. The highest THC was observed in T30 and showed no significant difference ($P > 0.05$) with other treatments except T100.

4 Discussion

With mounting environmental pressures and limited marine resources, the demand and price of FM are on the rise. This has prompted researchers to explore more sustainable sources of protein, such as IM (Feng et al., 2019; Gu et al., 2022; Mente et al., 2022). Over the past few years, several efforts have been made to investigate the potential of IM as an alternative to FM in the diets of aquaculture species (Gasco et al., 2016; Henry et al., 2018a; Song et al., 2018; Su et al., 2017). Therefore, the results of this study could provide valuable and practical information for researchers and aquafeed manufacturers seeking to develop more sustainable and efficient aquaculture practices.

This study provides compelling evidence to suggest that *T. molitor* larvae have the potential to serve as a viable alternative protein source to FM in the diet of *L. vannamei* juveniles. The growth performance indices clearly demonstrate that the inclusion of MW in the diet does not have a negative effect on the growth performance of *L. vannamei*. These findings have significant implications for the aquaculture industry, as they offer a sustainable and cost-effective protein source option for feed manufacturers, while maintaining the growth performance of *L. vannamei*. By offering a partial or complete replacement for FM, MW could help mitigate the overreliance on this dwindling resource, and promote a more sustainable and environmentally-friendly approach to aquaculture. The cost of IM as a replacement for fishmeal varies depending on the specific insect used and the production process. Generally, IM is more expensive than traditional protein sources such as soybean or FM. However, the cost of IM has been decreasing as production technology improves and economies of scale are achieved (van Huis et al., 2013). Additionally, the cost of IM may be offset by the potential benefits of using a more sustainable protein source that has a lower environmental impact. It's worth noting that the cost of IM may also depend on the region and market

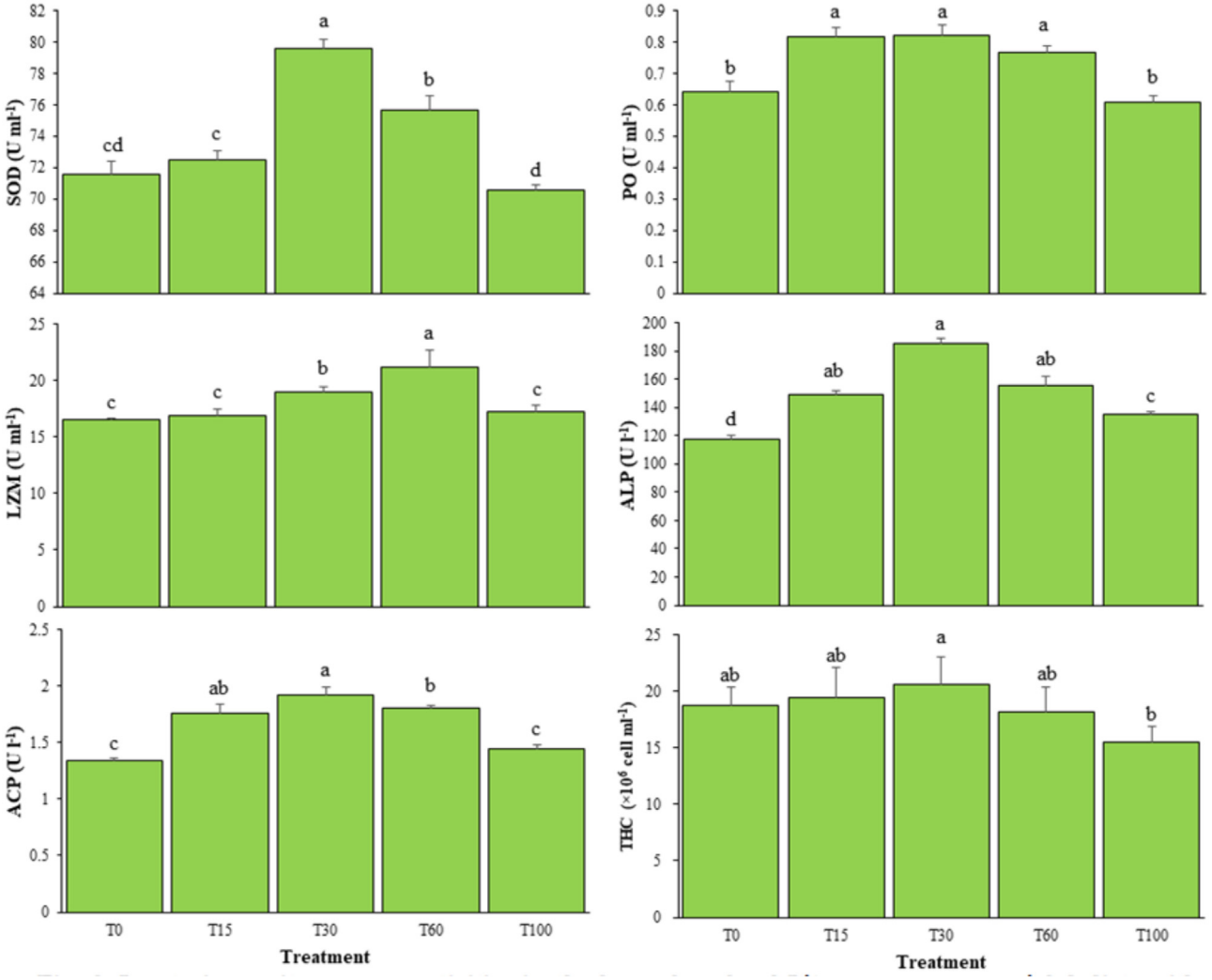

Fig. 3. Innate immunity enzyme activities in the haemolymph of *Litopenaeus vannamei* fed diets with increasing replacement of fish meal with mealworm. Data are expressed as the mean of triplication of each group ± SD ($n = 3$). Significant differences ($P < 0.05$) are marked with different letters. SOD, superoxide dismutase; PO, phenoloxidase; LZM, lysozyme; ALP, alkaline phosphatase; ACP, acid phosphatase; THC, total haemocyte count. T0 = diet without mealworm (MW) inclusion; T15, T30, T60, and T100 = 15, 30, 60, and 100% replacement level of FM with MW, respectively.

demand. In some regions, such as Africa and Asia, IM may already be a commonly used protein source that is relatively inexpensive (van Huis et al., 2013). In other regions, such as North America and Europe, insect meal may be more expensive and less widely available. Overall, the cost of using IM as a replacement for FM in animal feed is an important factor that needs to be carefully considered in conjunction with the potential benefits and limitations of this alternative protein source.

The findings of this study align with previous research that has explored the possibility of substituting FM with IM in the diets of various aquatic species. These earlier studies have consistently reported no negative impact on growth performance resulting from the replacement of FM with insect meal, further supporting the potential of insect meal as a sustainable and cost-effective protein source for aquaculture (Belghit et al., 2019; Dumas et al., 2018; Motte et al., 2019; Panini et al., 2017).

Numerous studies have explored the potential of using IM as a substitute for FM in the diets of various aquatic species. While some experiments have shown that partial or complete replacement of FM with MW did not negatively impact the growth performance of species such as *Pagellus bogaraveo* and *Lates calcarifer* (Iaconisi et al., 2017; Lin and Mui, 2017), other studies have reported mixed results in species like *L. vannamei* and *Micropterus salmoides* (Cai et al., 2022; Gu et al., 2022). For instance, replacing FM with MW did not adversely affect the survival rate or body weight of *L. vannamei*, but it did increase the FCR and decrease the PRE, which could potentially have negative effects on growth performance (Cai et al., 2022). Factors such as insect substrate, tolerance levels of insect compounds between different species, and different life stages of the aquatic species used in the experiments could account for these mixed results (Belghit et al., 2018; Cai et al., 2022; Sealey et al., 2011).

Overall, these findings underscore the importance of further research to optimize the use of IM as a sustainable and cost-effective protein source in aquaculture.

Although plasma blood parameters lack standard values and are not commonly used, they can provide reliable indicators of the physiological status and health of aquatic organisms in different nutritional conditions (Kader et al., 2010; Mastoraki et al., 2020a; Roque et al., 2010). The present study found that increasing the replacement of FM with MW decreased the amount of Cho, TG, and Glu in the haemolymph of *L. vannamei*. Shrimp fed with 60% FM replacement obtained more protein in their haemolymph and showed a significant difference from the control treatment. Other studies suggest that chitin in insect meal could be responsible for reducing serum Cho, TG, and Glu concentrations (Gu et al., 2022; Khosravi et al., 2018)., which is consistent with the present results. It appears that the nutritional performance of MW on haemolymph Cho and TG concentration in L. vannamei may be mainly influenced by chitin content. Tharanathan and Kittur (2003) stated that the capacity for protein binding and chitin hydrophilicity affect fat absorption in fish and mammals, thereby reducing Cho and TG. IM chitin prevents the absorption of cholesterol and lipid by binding to anionic carboxyl groups of fat and bile acids in the intestine and connecting them to neutral lipids with hydrophobic bonds. Additionally, chitin increases the excretion of cholesterol and lipids in feces by inhibiting the reabsorption of bile cholesterol (Šimunek and Bartonová, 2005; Yildirim et al., 2020).

Encapsulation, phagocytosis, and accumulation of nodules are processes by which circulating hemocytes remove foreign particles from the haemolymph (Feng et al., 2019; Iwasaki and Medzhitov, 2010). This study found that replacing FM with MW up to 30% in the diet of *L. vannamei* increased the counts of THCs, although this increase was not significant. These findings were accordance with those described by Feng et al. (2019) who observed a significant increase of *Macrobrachium rosenbergii* THC fed diets containing *T. molitor* protein in comparison with control group. Likewise, Su et al. (2017) reported that the levels of THCs, GCs, and HCs, as well as mitotic cells and the mitotic index of hematopoietic tissues were increased in yellow catfish fed MW-supplemented diets. These results revealed that the supplementation of MW in the *L. vannamei* diet could cause the hemocytes proliferation in the hematopoietic tissue and increase the mobilization of shrimp THC.

Nutrients and feed ingredients can greatly affect the aquatic animals immune system (Feng et al., 2019; Rauta et al., 2012; Zhu et al., 2013). In the present study, the results of immune parameters showed that the replacement of FM with MW in the diet of *L. vannamei* juveniles could significantly improve immune responses. LZM is a low molecular weight alkaline protein involved in the innate immune system against invading bacterial pathogens (Saurabh and Sahoo, 2008; Wu et al., 2019). According to the results of this research, the level of LZM enzyme activity showed an increasing trend with increasing the amount of replacing FM with MW up to the level of 60%, which showed that dietary MW could improve the response of innate immune system of *L. vannamei* juveniles. In agreement with our findings, the results of other studies reported that the replacement of FM with MW in experimental diets of *Oncorhynchus mykiss* (Henry et al., 2018a), European seabass (Henry et al., 2018b), and *Siniperca scherzeri* (Sankian et al., 2018) could significantly increase the serum LZM activity. Similarly, an increase in the serum LZM activity has been reported as a result of adding meal of other insect species (e.g. *Hermetia illucens*) to the diet of aquatic species (Chaklader et al., 2019; Foysal et al., 2019; Xiao et al., 2018).

SOD and PO are among other available enzyme activities that can be also considered as a parameter for the immune response (Feng et al., 2019; Fridovich, 1995; Saurabh and Sahoo, 2008). As an important antioxidant enzyme, SOD plays a facilitating role in converting superoxide anions into harmless hydrogen peroxide (Feng et al., 2019). As an important regulatory enzyme in all living organisms, ALP is associated with various essential functions (Pinoni and Mañanes, 2004). Results from present study showed that the activities of SOD, PO, ACP, and ALP of *L. vannamei* juveniles fed diets containing MW up to 30% replacement level were meaningfully higher than those of the control. These results consistent with the previous studies by Ng et al. (2001), Su et al. (2017), Iaconisi et al. (2017), and Feng et al. (2019), who reported that feeding an MW-based diet could increase their SOD, PO, and ALP activities. These findings show that supplementation of MW in *L. vannamei* diet can increase the activities of these protective enzymes and strengthen the function of the immune system.

5 Conclusion

In conclusion, this study provides strong evidence that MW (*T. molitor*) can effectively replace FM in the diet of *L. vannamei*, resulting in enhanced growth, health, and immunity of the shrimp. This sustainable protein source can significantly reduce production costs and environmental impacts in the aquaculture industry. The study highlights the importance of utilizing plasma blood parameters and hemocyte counts as accurate indicators of aquatic organism health and recommends the use of mealworms in farmed species without adverse environmental consequences.

Further research is necessary to optimize the use of MW in aquafeeds and gain a better understanding of their mechanisms of action on shrimp health and immune response. Overall, the findings underscore the potential of MW as a valuable and sustainable alternative protein source in aquaculture, emphasizing the need for continued exploration of alternative protein sources to reduce the industry's reliance on FM.

Ethical approval and consent to participate

The research undertaken complies with the current animal welfare laws in Iran. *Litopenaeus vannamei* used in this experimental work does not need approval from the Ethics Committee for Animal Use in Iran. All the authors agree to participate in this experiment.

Human and animal ethics

The authors followed international and institutional animal management guidelines for the experiments.

Consent for publication

All the authors of this article agree to the publication.

Availability of supporting data

The data that support the findings of this study are available on request from the corresponding author.

Competing interests

The authors declare no competing interests.

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Authors' contributions

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References

Barroso FG, de Haro C, Sánchez-Muros M-J, Venegas E, Martínez-Sánchez A, Pérez-Bañón C. 2014. The potential of various insect species for use as food for fish. *Aquaculture* 422: 193–201.

Belforti M, Gai F, Lussiana C, Renna M, Malfatto V, Rotolo L, De Marco M, Dabbou S, Schiavone A, Zoccarato I. 2015. *Tenebrio molitor* meal in rainbow trout (*Oncorhynchus mykiss*) diets: effects on animal performance, nutrient digestibility and chemical composition of fillets. *Ital J Anim Sci* 14, 4170.

Belghit I, Liland NS, Gjesdal P, Biancarosa I, Menchetti E, Li Y, Waagbø, R., Krogdahl Å, Lock E-J. 2019. Black soldier fly larvae meal can replace fish meal in diets of sea-water phase Atlantic salmon (*Salmo salar*). *Aquaculture* 503: 609–619.

Belghit I, Liland NS, Waagbø R, Biancarosa I, Pelusio N, Li Y, Krogdahl Å, Lock EJ. 2018. Potential of insect-based diets for Atlantic salmon (*Salmo salar*). *Aquaculture* 491: 72–81.

Bolann B, Ulvik R. 1991. Improvement of a direct spectrophotometric assay for routine determination of superoxide dismutase activity. *Clin Chem* 37: 1993–1999.

Burtis CA, Bruns DE. 2014. Tietz fundamentals of clinical chemistry and molecular diagnostics-e-book. *Elsevier Health Sciences*.

Cai Y, Huang H, Yao W, Yang H, Xue M, Li X, Leng X. 2022. Effects of fish meal replacement by three protein sources on physical pellet quality and growth performance of Pacific white shrimp (*Litopenaeus vannamei*). *Aquac Rep* 25: 101210.

Chaklader MR, Siddik MA, Fotedar R, Howieson J. 2019. Insect larvae, Hermetia illucens in poultry by-product meal for barramundi, *Lates calcarifer modulates histomorphology, immunity and resistance to Vibrio harveyi. Sci Rep* 9: 1–15.

Chen B, Li E, Liu Q, Liu Y, Zhang J. 2019. Comparative transcriptome analysis of *Litopenaeus vannamei* hepatopancreas in response to low salinity stress. *Genomics* 111: 81–90.

de Carvalho NM, Madureira AR, Pintado ME. 2020. The potential of insects as food sources – a review. *Crit Rev Food Sci Nutr* 60: 3642–3652.

DiGiacomo K, Leury B. 2019. Insect meal: a future source of protein feed for pigs? *Animal* 13: 3022–3030.

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

Ellis AI. 1990. Lysozyme assays. *Tech Fish Immunol* 1: 101–103.

FAO. 2018. The State of World Fisheries and Aquaculture. *Meeting the sustainable development goals. Food & Agriculture Org*.

FAO. 2019. Cultured Aquatic Species Information Programme: *Litopenaeus vannamei*. *Food and Agriculture Organization of the United Nations*. http://www.fao.org/fishery/culturedspecies/Litopenaeus_vannamei/en/

Fawole FJ, Adeoye AA, Tiamiyu LO, Ajala KI, Obadara SO, Ganiyu IO. 2020. Substituting fishmeal with Hermetia illucens in the diets of African catfish (*Clarias gariepinus*): effects on growth, nutrient utilization, haemato-physiological response, and oxidative stress biomarker. *Aquaculture* 518: 734849.

Feng P, He J, Lv M, Huang G, Chen X, Yang Q, Wang J, Wang D, Ma H. 2019. Effect of dietary *Tenebrio molitor* protein on growth performance and immunological parameters in *Macrobrachium rosenbergii*. *Aquaculture* 511: 734247.

Ferrer Llagostera P, Kallas Z, Reig L, Amores de Gea D. 2019. The use of insect meal as a sustainable feeding alternative in aquaculture: Current situation, *Spanish consumers' perceptions and willingness to pay. J Clean Prod* 229: 10–21.

Foysal MJ, Fotedar R, Tay C-Y., Gupta SK. 2019. Dietary supplementation of black soldier fly (*Hermetica illucens*) meal modulates gut microbiota, innate immune response and health status of marron (*Cherax cainii*, *Austin 2002) fed poultry-by-product and fishmeal based diets. PeerJ* 7: e 6891.

Fridovich I. 1995. Superoxide radical and superoxide dismutases. *Annu Rev Biochem* 64: 97–112.

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

Ghamkhar R, Hicks A. 2020. Comparative environmental impact assessment of aquafeed production: sustainability implications of forage fish meal and oil free diets. *Resour Conserv Recycl* 161: 104849.

Globe Newswire. 2020. Global $1.39Bn Insect Feed Market, 2024: Insights into Growth Trends & Opportunities. 9 March 2020. *Globe Newswire*.

Gu J, Liang H, Ge X, Xia D, Pan L, Mi H, Ren M. 2022. A study of the potential effect of yellow mealworm (*Tenebrio molitor*) substitution for fish meal on growth, immune and antioxidant capacity in juvenile largemouth bass (*Micropterus salmoides*). *Fish Shellfish Immunol* 120: 214–221.

Hardy RW. 2010. Utilization of plant proteins in fish diets: effects of global demand and supplies of fishmeal. *Aquacult Res* 41: 770–776.

Henry M, Gasco L, Piccolo G, Fountoulaki E. 2015. Review on the use of insects in the diet of farmed fish: past and future. *Animal Feed Sci Technol* 203: 1–22.

Henry MA, Gai F, Enes P, Peréz-Jiménez A, Gasco L. 2018a. Effect of partial dietary replacement of fishmeal by yellow mealworm (*Tenebrio molitor*) larvae meal on the innate immune response and intestinal antioxidant enzymes of rainbow trout (*Oncorhynchus mykiss*). *Fish Shellfish Immunol* 83: 308–313.

Henry MA, Gasco L, Chatzifotis S, Piccolo G. 2018b. Does dietary insect meal affect the fish immune system? The case of mealworm, *Tenebrio molitor* on European sea bass, *Dicentrarchus labrax*. *Dev Compar Immunol* 81: 204–209.

Iaconisi V, Marono S, Parisi G, Gasco L, Genovese L, Maricchiolo G, Bovera F, Piccolo G. 2017. Dietary inclusion of *Tenebrio molitor* larvae meal: effects on growth performance and final quality treats of blackspot sea bream (*Pagellus bogaraveo*). *Aquaculture* 476: 49–58.

Iwasaki A, Medzhitov R. 2010. Regulation of adaptive immunity by the innate immune system. *Science* 327: 291–295.

Jannathulla R, Rajaram V, Kalanjiam R, Ambasankar K, Muralidhar M, Dayal JS. 2019. Fishmeal availability in the scenarios of climate change: inevitability of fishmeal replacement in aquafeeds and approaches for the utilization of plant protein sources. *Aquac Res* 50: 3493–3506.

Jones CM, Ng WK, King M. 2010. Alsever's solution: a review of its history, chemistry, and production. *Transfus Med Rev* 24: 259–267.

Kader MA, Koshio S, Ishikawa M, Yokoyama S, Bulbul M. 2010. Supplemental effects of some crude ingredients in improving nutritive values of low fishmeal diets for red sea bream, *Pagrus major. Aquaculture* 308: 136–144.

Khanjani MH, Mozanzadeh MT, Sharifinia M, Emerenciano MGC. 2022a. Biofloc: a sustainable dietary supplement, nutritional value and functional properties. *Aquaculture* 738757.

Khanjani MH, Sharifinia M. 2020. Biofloc technology as a promising tool to improve aquaculture production. *Rev Aquacult* 12: 1836–1850.

Khanjani MH, Sharifinia M. 2022. Biofloc technology with addition molasses as carbon sources applied to *Litopenaeus vannamei* juvenile production under the effects of different C/N ratios. *Aquac Int* 30: 383–397.

Khanjani MH, Sharifinia M, Hajirezaee S. 2022b. Recent progress towards the application of biofloc technology for tilapia farming. *Aquaculture* 738021.

Khosravi S, Kim E, Lee YS, Lee SM. 2018. Dietary inclusion of mealworm (*Tenebrio molitor*) meal as an alternative protein source in practical diets for juvenile rockfish (*Sebastes schlegeli*). *Entomolog Res* 48: 214–221.

Lin YH, Mui JJ. 2017. Evaluation of dietary inclusion of housefly maggot (*Musca domestica*) meal on growth, fillet composition and physiological responses for barramundi, *Lates calcarifer*. Aquac Res 48: 2478–2485.

Liu X, Tan B, Huang X, Li X, Qin JG. 2018. The immune system and bacterial disease resistance in shrimp: a review. *Aquac Res* 49: 1750–1760.

Makkar HP, Tran G, Heuzé V, Ankers P. 2014. State-of-the-art on use of insects as animal feed. *Animal Feed Sci Technol* 197: 1–33.

Mastoraki M, Mollá Ferrándiz P, Vardali SC, Kontodimas DC, Kotzamanis YP, Gasco L, Chatzifotis S,Antonopoulou E. 2020a. 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.

Mastoraki M, Vlahos N, Patsea E, Chatzifotis S, Mente E, Antonopoulou E. 2020b. The effect of insect meal as a feed ingredient on survival, growth, and metabolic and antioxidant response of juvenile prawn *Palaemon adspersus* (Rathke, 1837). *Aquac Res* 51: 3551–3562.

Mente E, Bousdras T, Feidantsis K, Panteli N, Mastoraki M, Kormas KA, Chatzifotis S, Piccolo G, Gasco L, Gai F, Martin SAM, Antonopoulou E. 2022. *Tenebrio molitor* larvae meal inclusion affects hepatic proteome and apoptosis and/or autophagy of three farmed fish species. *Sci Rep* 12: 121.

Motte C, Rios A, Lefebvre T, Do H, Henry M, Jintasataporn O. 2019. Replacing fish meal with defatted insect meal (Yellow Mealworm *Tenebrio molitor*) improves the growth and immunity of pacific white shrimp (*Litopenaeus vannamei*). *Animals* 9: 258.

Nagappan S, Das P, AbdulQuadir M, Thaher M, Khan S, Mahata C, Al-Jabri H, Vatland AK, Kumar G. 2021. Potential of microalgae as a sustainable feed ingredient for aquaculture. *J Biotechnol* 341: 1–20.

Ng WK, Liew FL, Ang LP, Wong KW. 2001. Potential of mealworm (*Tenebrio molitor*) as an alternative protein source in practical diets for African catfish, *Clarias gariepinus*. *Aquac Res* 32: 273–280.

Olsen RL, Hasan MR. 2012. A limited supply of fishmeal: Impact on future increases in global aquaculture production. *Trends Food Sci Technol* 27: 120–128.

Panini RL, Freitas LEL, Guimarães AM, Rios C, da Silva MFO, Vieira FN, Fracalossi DM, Samuels RI, Prudêncio ES, Silva CP. 2017. Potential use of mealworms as an alternative protein source for Pacific white shrimp: digestibility and performance. *Aquaculture* 473: 115–120.

Perazzolo LM, Barracco MA. 1997. The prophenoloxidase activating system of the shrimp Penaeus paulensis and associated factors. *Dev Comparat Immunol* 21: 385–395.

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

Pinoni S, Mañanes AL. 2004. Alkaline phosphatase activity sensitive to environmental salinity and dopamine in muscle of the euryhaline crab *Cyrtograpsus angulatus*. *J Exp Mar Biol Ecol* 307: 35–46.

Quang Tran H, Van Doan H, Stejskal V. 2022. Environmental consequences of using insect meal as an ingredient in aquafeeds: a systematic view. *Rev Aquac* 14: 237–251..

Rauta PR, Nayak B, Das S. 2012. Immune system and immune responses in fish and their role in comparative immunity study: a model for higher organisms. *Immunol Lett* 148: 23–33.

Research M. 2018. Edible insects market by type (edible crickets, mealworms, black soldier fly, grasshopper), product (whole insects, insect flour, cricket protein bars, insect snacks, insect in chocolate)-global opportunity analysis and industry forecast (2018–2023). *Report ID MRFB*- 10448.

Roque A, Yildiz HY, Carazo I, Duncan N. 2010. Physiological stress responses of sea bass (*Dicentrarchus labrax*) to hydrogen peroxide (H2O2) exposure. *Aquaculture* 304: 104–107.

Sánchez-Muros MJ, Renteria P, Vizcaino A, Barroso FG. 2020. Innovative protein sources in shrimp (*Litopenaeus vannamei*) feeding. *Rev Aquac* 12: 186–203.

Sankian Z, Khosravi S, Kim Y-O, Lee S-M. 2018. Effects of dietary inclusion of yellow mealworm (*Tenebrio molitor*) meal on growth performance, feed utilization, body composition, plasma biochemical indices, selected immune parameters and antioxidant enzyme activities of mandarin fish (*Siniperca scherzeri*) juveniles. *Aquaculture* 496: 79–87.

Saurabh S, Sahoo P. 2008. Lysozyme: an important defence molecule of fish innate immune system. *Aquac Res* 39: 223–239.

Sealey WM, Gaylord TG, Barrows FT, Tomberlin JK, McGuire MA, Ross C, St-Hilaire S. 2011. Sensory analysis of rainbow trout, *Oncorhynchus mykiss*, fed enriched black soldier fly prepupae, *Hermetia illucens*. J World Aquac Soc 42: 34–45.

Sharifinia M. 2015. Macroinvertebrates of the Iranian running waters: a review. *Acta Limnolog Bras* 27: 356–369.

Shi B, Xu F, Zhou Q, Regan MK, Betancor MB, Tocher DR, Sun M, Meng F, Jiao L, Jin M. 2021. Dietary organic zinc promotes growth, immune response and antioxidant capacity by modulating zinc signaling in juvenile Pacific white shrimp (*Litopenaeus vannamei*). *Aquac Rep* 19: 100638.

Šimunek J, Bartonová H. 2005. Effect of dietary chitin and chitosan on cholesterolemia of rats. *Acta Veterinaria Brno* 74: 491–499.

Song SG, Chi SY, Tan BP, Liang GL, Lu BQ, Dong XH, Yang QH, Liu HY, Zhang S. 2018. Effects of fishmeal replacement by *Tenebrio molitor* meal on growth performance, antioxidant enzyme activities and disease resistance of the juvenile pearl gentian grouper (*Epinephelus lanceolatus*♂× *Epinephelus fuscoguttatus*♀). *Aquac Res* 49: 2210–2217.

Su J, Gong Y, Cao S, Lu F, Han D, Liu H, Jin J, Yang Y, Zhu X, Xie S. 2017. Effects of dietary *Tenebrio molitor* meal on the growth performance, immune response and disease resistance of yellow catfish (*Pelteobagrus fulvidraco*). *Fish Shellfish Immunol* 69: 59–66.

Tacon AG, Metian M. 2008. Global overview on the use of fish meal and fish oil in industrially compounded aquafe eds trends and future prospects. *Aquaculture* 285: 146–158.

Tharanathan RN, Kittur FS. 2003. *Chitin-the undisputed biomolecule of great potential*.

Tubin JSB, Paiano D, Hashimoto GSdO, Furtado WE, Martins ML, Durigon E, Emerenciano MGC. 2020. *Tenebrio molitor* meal in diets for Nile tilapia juveniles reared in biofloc system. *Aquaculture* 519: 734763.

van Huis A. 2022. Edible insects: challenges and prospects. *Entomolog Res* 52: 161–177.

van Huis A, van Itterbeeck J, Klunder H, Mertens E, Halloran A, Muir G, Vantomme P. 2013. Edible insects: future prospects for food and feed security. *Food and agriculture organization of the United Nations*.

Wang T, Wang X, Shehata AI, Wang R, Yang H, Wang Y, Wang J, Zhang Z. 2022. Growth performance, physiological and antioxidant capacity responses to dietary fish meal replacement with insect meals for aquaculture: a case study in red claw crayfish (*Cherax quadricarinatus*). *Aquac Res* 53: 3853–3864.

Wu C, Shan J, Feng J, Wang J, Qin C, Nie G, Ding C. 2019. Effects of dietary Radix Rehmanniae Preparata polysaccharides on the growth performance, immune response and disease resistance of *Luciobarbus capito. Fish Shellfish Immunol* 89: 641–646.

Xiao X, Jin P, Zheng L, Cai M, Yu Z, Yu J, Zhang J. 2018. Effects of black soldier fly (*Hermetia illucens*) larvae meal protein as a fishmeal replacement on the growth and immune index of yellow catfish (*Pelteobagrus fulvidraco*). *Aquac Res* 49: 1569–1577.

Yeganeh V, Sharifinia M, Mobaraki S, Dashtiannasab A, Aeinjamshid K, Borazjani JM, Maghsoudloo T. 2020. Survey of survival rate and histological alterations of gills and hepatopancreas of the *Litopenaeus vannamei* juveniles caused by exposure of *Margalefidinium / Cochlodinium* polykrikoides isolated from the Persian Gulf. *Harmful Algae* 97: 101856.

Yildirim U, Sarica S, Kanoglu B. 2020. Defatted yellow mealworm larvae (*Tenebrio molitor*) meal as possible alternative to fish meal in quail diets. *South Afr J Animal Sci* 50: 481–491.

Zhu LY, Nie L, Zhu G, Xiang LX, Shao JZ. 2013. Advances in research of fish immune-relevant genes: a comparative overview of innate and adaptive immunity in teleosts. *Dev Compar Immunol* 39: 39–62.

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