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# The effects of replacing fishmeal by mealworm (*Tenebrio molitor*) on digestive enzymes activity and hepatopancreatic biochemical indices of *Litopenaeus vannamei*

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# The effects of replacing fishmeal by mealworm (*Tenebrio molitor*) on digestive enzymes activity and hepatopancreatic biochemical indices of *Litopenaeus vannamei*

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## Abstract

Reducing the use of fishmeal (FM) in aquafeed means a significant saving in the amount of FM at the global level and reducing environmental impacts. One of the potential protein sources to replace FM in shrimp diet is the use of insects' meal. Therefore, the present study aimed to examine the effects of replacing FM with mealworm (MW; *Tenebrio molitor*) on the growth, digestive enzymes activity and hepatopancreatic biochemical indices of Pacific white shrimp (*Litopenaeus vannamei*). Shrimp were fed for 60 days with a control diet (T0) and 4 practical diets (T15, T30, T60 and T100) where 15, 30, 60 and 100% of the FM was substituted by MW, respectively. Results showed that there were significant differences in weight gain (WG) and hepatopancreatic index (HPI) among treatments and the lowest and highest values for were observed in T0 and T30, respectively. Our findings indicated a significant increase ($P<0.05$) in activity of glutathione peroxidase (GPX), catalase (CAT), superoxide dismutase (SOD), total

nitric oxide (TNO) and total antioxidant capacity (TAC) and a meaningful decrease ($P<0.05$) of malondialdehyde (MDA) in hepatopancreas of *L. vannamei* juveniles fed diets containing MW. The alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the T0 did not show a significant difference ($P>0.05$) with other experimental treatments. Protease and lipase indicated an increasing trend with increasing the amount of MW up to 60%. The protease activity showed a significant difference ($P<0.05$) between the treatments containing MW and the control treatment. These findings indicated that MW could be a feasible candidate for replacing FM in diets of the Pacific white shrimp without any detrimental effects.

Key words: antioxidant capacity, insect meal, hepatopancreas, aquafeed, aquaculture

According to the growing trend of the human population, 23 million tons of seafood are needed to maintain the per capita consumption of seafood until 2030, and since in the past few decades the catches of wild fish have reached a constant level, this shortage must be met through aquaculture (FAO, 2018). In tropical and sub-tropical regions, Penaeid shrimps are one of the valuable resources for aquaculture and fisheries industries (Keshavarzifard et al., 2021). Currently the most cultivated species in the world is Pacific white shrimp (Khanjani et al., 2020, 2023; Khanjani and Sharifinia, 2020, 2021). Most of the farmed aquatic species used in aquaculture are grown with commercial feed, and intensive production of carnivorous species requires the use of high quality fishmeal (FM) as the main ingredient of the diet (Khanjani et al., 2021, 2022a,b; Oliva-Teles et al., 2015). However, the increase in the production of aquafeed has caused a rapid increase in the price of raw materials such as FM (FAO, 2018).

In recent decades, many efforts have been focused on the use of plant proteins in carnivorous species diets (Abasubong et al., 2021; Nasr et al., 2021; Wang et al., 2022a). However, plant protein sources have disadvantages such as relatively low protein content, unfavorable essential amino acid profile, low palatability, presence of anti-nutritional substances and competition with other sectors of the food industry (Gatlin III et al., 2007). This has encouraged researchers to search for new strategies to improve the efficiency of plant protein-based feeds (Gatlin III et al., 2007) as well as the use of other alternatives to FM, such as materials with animal origin, including slaughterhouse by-products or insect meal (Belghit et al., 2019; Motte et al., 2019; Oliva-Teles et al., 2015; Wang et al., 2022b). Several studies have been conducted in the field of using insects as ingredients for aquafeed (Belghit et al., 2019; Henry et al., 2015; Mastoraki et al., 2020a; Mastoraki et al., 2020b; Motte et al., 2019; Panini et al., 2017b; Wang et al., 2022b). In comparison with animal protein sources, insects have many advantages, such as the possibility of breeding on organic by-products, suitable feed conversion ratio, emission of low levels of greenhouse gases and ammonia, simple issues of animal rights and low risk of transmission of infections (Quang et al., 2022). The potential of insects for use in animal feed can also have positive environmental effects; in fact, the production of insects reduces energy consumption and harmful environmental effects (Quang

et al., 2022; van Huis et al., 2013). In addition, the larvae of some species of insects are naturally consumed by aquatic species (DiGiacomo and Leury, 2019; Sharifinia, 2015).

Insect meal (IM) can be a sustainable source of protein and good source of amino acids, lipids, minerals, vitamins and energy (Barroso et al., 2014; Motte et al., 2019). The high percentage of protein found in most insect species, both in terms of quality and quantity, makes them a new and promising source for producing food for aquaculture species (Li et al., 2019; van Huis, 2022; van Huis et al., 2013). The amount of protein in insects is on average between 50 and 82% of dry matter, which varies depending on the type of insect and their processing method (Li et al., 2019; Rumpold and Schlüter, 2013). In addition, insects have a favorable amino acid profile because they are rich in essential amino acids, unlike plant proteins, which are deficient in lysine, methionine, and leucine (Gaylord and Barrows, 2009; Gorissen et al., 2018; Li et al., 2019). Furthermore, some amino acids, such as taurine and hydroxyproline, which are abundantly found in FM, are not present in plant sources (Li et al., 2019; Pinto et al., 2013), while many insect species contain acceptable amounts of these amino acids (Li et al., 2019).

The larval stage of the mealworm (MW; *Tenebrio molitor*) can be used as a suitable alternative to be used as a high-protein source in the diet of carnivorous aquatic species (Belforti et al., 2015; Gasco et al., 2016). The primary goal of making food for aquatic animals is to provide a balanced mixture of nutrients to meet the needs such as maintenance energy, growth, reproduction and health. MW contains large amounts of protein, vitamins, and various minerals such as calcium, phosphorus, iron, potassium, etc., and in recent years, its use has been noticed in the world for the purpose of feeding animals, including farmed aquatic animals. So far, the results of research studies have shown that MW can be a suitable substitute for FM in the diet of different aquatic species, including rainbow trout (Belforti et al., 2015), European sea bass (Gasco et al., 2016), blackspot sea bream (Iaconisi et al., 2017), gilthead sea bream (Piccolo et al., 2017), mandarin fish (Sankian et al., 2018), giant freshwater prawn (Feng et al., 2019), Pacific white shrimp (Motte et al., 2019) and largemouth bass (Gu et al., 2022). Nevertheless, there is no comprehensive data on partially or completely replacing FM by MW on digestive enzymes activity and hepatopancreas biochemical indices of Pacific white shrimp (*Litopenaeus vannamei*). The hepatopancreas is one of the main organs of the shrimp digestive system (Liao et al., 2022). Considering that the gastrointestinal tract of crustaceans is continuous, the hepatopancreas, in addition to being an essential organ for digestion and absorption of nutrients, also plays a major role in the innate immunity of invertebrates (Cheung et al., 2015; Liao et al., 2022; Rőszer, 2014). Hence, current study was carried out to investigate the effects of partial or total FM replacement by MW on digestive enzymes activity and hepatopancreas biochemical indices of *L. vannamei*.

## Material and methods

### Experimental 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 were pelleted using a grinder machine and each treatment was placed separately in an oven at 60°C for 6 hours to remove moisture. Then all diets were sealed in vacuum-packed bags and stored in a freezer at 4 °C until used for the feeding trial.

Table1. 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]Fanavaran Darya 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).

**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 with commercial feed (40% protein, 8% lipid; Faradaneh Co. Sharkourd, Iran) 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. All tanks were covered with plastic nets to prevent shrimp from jumping out. All treatments were performed in three replicates. Shrimp fed four times a day at 6:00, 12:00, 18:00, and 24:00. 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.

**Hepatopancreas biochemical analysis**

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. Hepatopancreas samples was taken from 6 shrimp (18 samples of each treatment) in each tank and stored at -80 °C for the analysis of glutathione peroxidase (GPX), superoxide dismutase (SOD), malondialdehyde (MDA), catalase activity (CAT), alkaline phosphatase (ALP), total nitric oxide (TNO), total antioxidant capacity (TAC), lactate dehydrogenase (LDH), alanine aminotransferase (ALT) and aspartate aminotransferase (AST). GPX, SOD, MDA, CAT, TNO, TAC, LDH, ALT and AST activities were measured according to the manufacturer's instruction of Enzyme ZellBio (GmbH, Germany) Test Kits.

**Digestive enzyme analysis**

The total protein content was assayed by bovine serum albumin as a standard based on Bradford method (Bradford, 1976). Protease activity was measured by Folin-phenol reagent according to Anson method (Anson, 1938). Amylase activity was evaluated using iodine solution to reveal non-hydrolyzed starch based on Rick and Stegbauer method (Rick and Stegbauer, 1974). Lipase activity was measured based on the measurement of the release of fatty acids due to the triglycerides enzymatic hydrolysis in the stabilized olive oil emulsion (Borlongan, 1990). The activity of digestive enzymes was expressed as u $mg^{-1}$ protein.

**Calculations equation**

Body weight gain (BWG), body length gain (BLG), and hepatopancreatic index (HPI) as were calculated as follows (Kesselring et al., 2021; Khanjani and Sharifinia, 2022a, b):

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

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

$$\text{HPI (\%)} = \left[\text{Hepatopancreas wet weight (g)} \Big/ \text{Wet body weight (g)}\right] \times 100$$

**Statistical analysis**

All of the statistical analyses were performed using SPSS ver. 23.0 software (Chicago, Illinois, USA). Significant differences between groups were done through one-way analysis of variance (ANOVA). To conduct multiple comparison of means, post hoc test was employed. Results are presented as the mean ± standard deviation. Mean values were considered significantly different at $P < 0.05$ for all statistical tests.

## Results

**Growth performance**

The effects of replacing FM with MW on the growth performance of Pacific white shrimp juveniles are depicted in Table 2. There were no significant differences ($P > 0.05$) in initial body weight (IBW), FBW, initial body length (IBL), final body length (FBL), and length gain (LG) among all groups. Results from ANOVA analysis showed that there were significant differences ($P < 0.05$) in WG and HPI among treatments. The lowest and highest values for WG (6.72 ± 0.38 and 8.25 ± 0.42) and HPI (3.96 ± 0.28 and 5.03 ± 0.39) were observed in T0 and T30 treatments, respectively.

Table 2. Effect of fishmeal replacement by mealworm on the growth performance of Pacific white shrimp juveniles

| Items | Treatments | | | | |
|---|---|---|---|---|---|
| | T0 | T15 | T30 | T60 | T100 |
| IBW (g) | 7.42±0.45 A | 7.40±0.41 A | 7.42±0.43 A | 7.41±0.42 A | 7.40±0.43 A |
| FBW (g) | 14.14±0.38 A | 14.71±0.84 A | 15.47±1.56 A | 14.8±0.69 A | 15.09±0.31 A |
| WG (g) | 6.72±0.38 B | 7.31±0.84 AB | 8.25±0.42 A | 7.41±0.69 AB | 7.68±0.31 AB |
| IBL (cm) | 8.30±0.19 A | 8.29±0.22 A | 8.30±0.22 A | 8.29±0.22 A | 8.34±0.22 A |
| FBL (cm) | 11.70±0.24 A | 11.75±0.13 A | 11.89±0.30 A | 11.71±0.15 A | 11.88±0.14 A |
| LG (cm) | 3.41±0.24 A | 3.47±0.13 A | 3.60±0.30 A | 3.43±0.15 A | 3.54±0.14 A |
| HPI (%) | 3.96±0.28 C | 4.53±0.12 B | 5.03±0.39 A | 4.04±0.23 C | 3.97±0.25 C |

IBW: initial body weight; FBW: final body weight; WG: weight gain; IBL: initial body length; FBL: final body length; LG: length gain; HPI: hepatopancreatic index.
Values with different letters in the same raw are significantly different ($P<0.05$).

**Hepatopancreas biochemical parameters**

*Glutathione peroxidase (GPX) activity*

The results of hepatopancreas GPX in shrimp fed with different replacement levels of FM with MW and its changes are shown in Figure 1 a. The lowest (6.98±0.39) and highest (9.67±0.34) levels of GPX were obtained in control treatments (T0) and treatment with 100% replacement level (T100), respectively. The level of GPX showed an increasing trend with increasing the replacement rate of FM with MW, and a significant difference ($P<0.05$) was observed between the control treatment and the treatments with higher replacement levels.

*Malondialdehyde (MDA)*

The results of the analysis of the activity of MDA in shrimp fed different treatments during a period of 60 days are shown in Figure 1 b. MDA showed a decreasing trend with increasing the replacement rate of fish meal with MW, and a significant difference ($P<0.05$) was observed between the control treatment and the treatments with replacement levels higher than 15%. The highest and lowest amount was obtained in the control (T0) and 100% replacement (T100) treatments, respectively.

*Catalase activity (CAT)*

The results of hepatopancreas CAT activity in shrimp fed with different amounts of MW are presented in Figure 1 c. The results show that there is a significant difference ($P<0.05$) between experimental treatments in terms of catalase activity. The highest level of CAT enzyme activity was observed in the treatment group fed with diet containing 100% MW (T100) and the lowest level was obtained in the control treatment (T0).

*Superoxide dismutase (SOD) activity*

The results of the analysis of the activity of SOD in shrimp tested in different treatments during a period of 60 days are presented in Figure 1 d. The level of SOD activity showed an increasing trend with increasing the replacement rate of FM with MW, and a significant difference ($P<0.05$) was observed between the control treatment and the treatments with higher levels of replacement. The minimum and maximum amount of SOD was obtained in T0 and T60 treatments, respectively.

*Total nitric oxide (TNO) and total antioxidant capacity (TAC)*

The results of replacing FM with MW on the amount of TNO and TAC of the hepatopancreas of Pacific white shrimp (*L. vannamei*) are shown in Figure 1 e and 1 f, respectively. The level of TNO and TAC showed an increasing trend with the increase in the replacement of fish meal with MW, and a significant difference ($P<0.05$) was observed between the control treatment and the treatments with higher replacement levels. The minimum and maximum levels of TNO and TAC were obtained in T0 and T60 treatments, respectively.

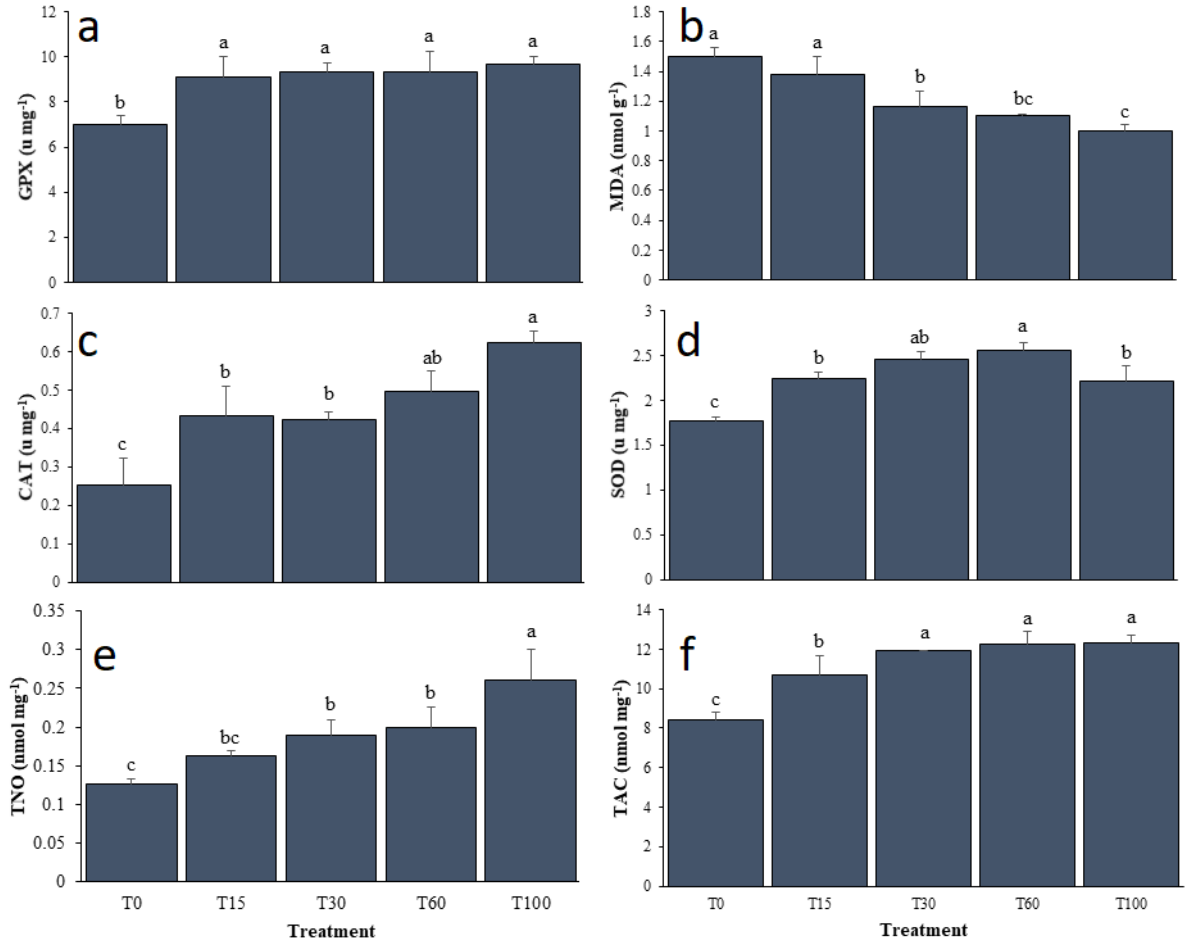



Figure 1. The effect of different levels of replacing fishmeal with mealworm (*T. molitor*) on the activity of the antioxidant enzymes in the hepatopancreas of Pacific white shrimp (*L. vannamei*). (a) GPX: Glutathione peroxidase; (b) MDA: Malondialdehyde; (c) CAT: Catalase; (d) SOD: Superoxide dismutase; (e) TNO: Total nitric oxide; (f) TAC: Total antioxidant capacity. Values are representing as means ± standard deviations. Means with different letters are significantly different ($P<0.05$) from each other.

*Alkaline phosphatase (ALP)*

The results of replacing fish meal with MW on the level of ALP enzyme activity in the hepatopancreas of Pacific white shrimp (*L. vannamei*) are presented in Figure 2 a. The amount of ALP activity in all treatments containing MW was higher than the control treatment. A significant difference ($P<0.05$) was observed between the control treatment with the treatments with 15 and 30% replacement level, but it did not show any significant difference with the treatments with 60 and 100% replacement level. The minimum and maximum levels of ALP activity were observed in the control treatments and the treatment containing 30% substitution, respectively.

*Lactate dehydrogenase (LDH)*

The effects of FM replacement by MW on LDH (mean ± SD) of *L. vannamei* hepatopancreas during a 60 days feeding trial are depicted in Figure 2 b. The mean values of

LDH varied from 1.85±0.11 to 2.94±0.21 u $mg^{-1}$ protein and the lowest and highest values were observed in treatments T0 and T30, respectively. In general, the LDH in the treatments containing MW was higher than the control treatment, and a significant difference ($P<0.05$) was observed between different treatments.

*Alanine aminotransferase (ALT) and aspartate aminotransferase (AST)*

Figures 2 c and 2 d are shown the alanine (ALT) and aspartate aminotransferase (AST) activity of shrimp hepatopancreas in different diets, respectively. The mean values of ALT and AST were varied from 1.38±0.16 to 2.35±0.47 and 1.28±0.24 to 1.66±0.18 u $mg^{-1}$ protein, respectively. The ALT and AST in the control treatment did not show a significant difference ($P>0.05$) with other experimental treatments.

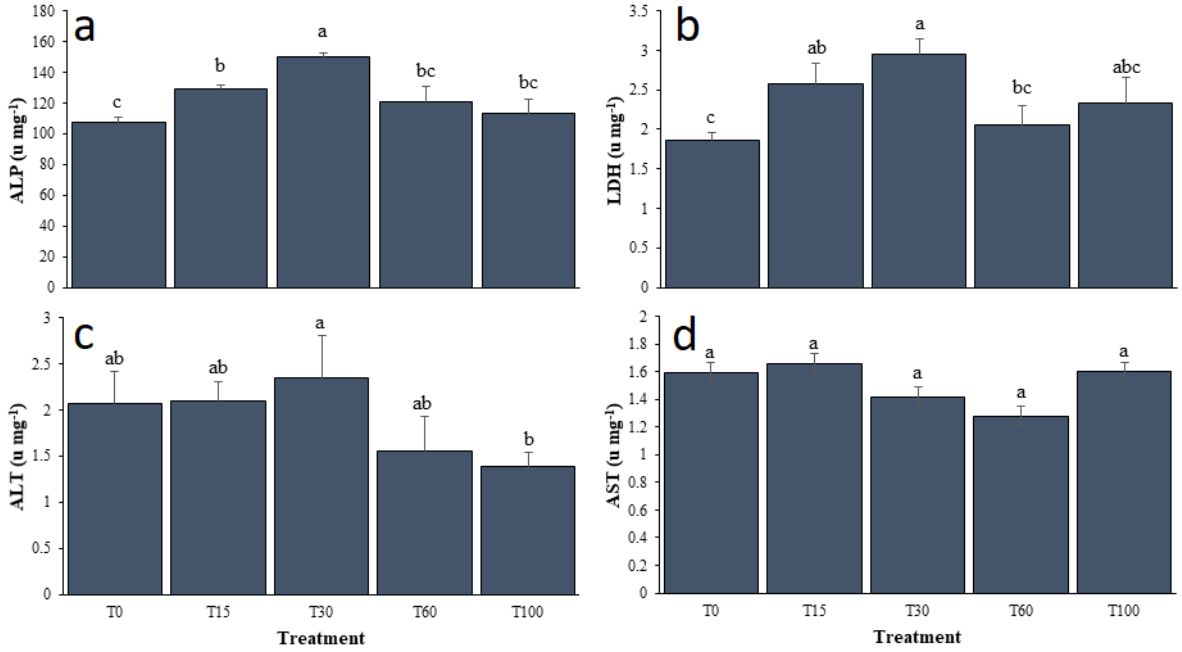



Figure 2. The effect of replacing fishmeal with different levels of mealworm on (a) alkaline phosphatase (ALP), (b) lactate dehydrogenase (LDH), (c) alanine aminotransferase (ALT) and (d) aspartate aminotransferase (AST) of Pacific white shrimp during a 60-day feeding trial. Values are representing as means ± standard deviations. Means with different letters are significantly different ($P<0.05$) from each other

**Activity of digestive enzymes**

The mean (±SD) values of total protein and activity of digestive enzymes (protease, amylase, and lipase) in intestine of *L. vannamei* fed with different levels of MW replacement over a period of 60 days are presented in Figure 3. The amount of total protein (Figure 3 a) varied from 27.66±0.57 to 30.34±0.58 mg $g^{-1}$ tissue and showed a significant difference among treatments ($P<0.05$). Its minimum and maximum amount was obtained in treatments T0 and T60, respectively. In terms of digestive enzyme activities, protease (Figure 3 b) and lipase (Figure 3 d) enzymes showed an increasing trend with increasing the amount of MW up to 60%. The protease activity showed a significant difference ($P<0.05$) between the treatments containing MW and the control treatment. The amylase activity (Figure 3 c) exhibited a

significant difference (P<0.05) between the T30 and T60 treatments with T0 (control treatment). The amount of lipase activity varied from 2.68±0.06 to 3.37±0.02 u $mg^{-1}$. Its lowest and highest values were obtained in T0 and T30, respectively. There was also a significant difference between treatments T15–T60 with the control treatment (T0).

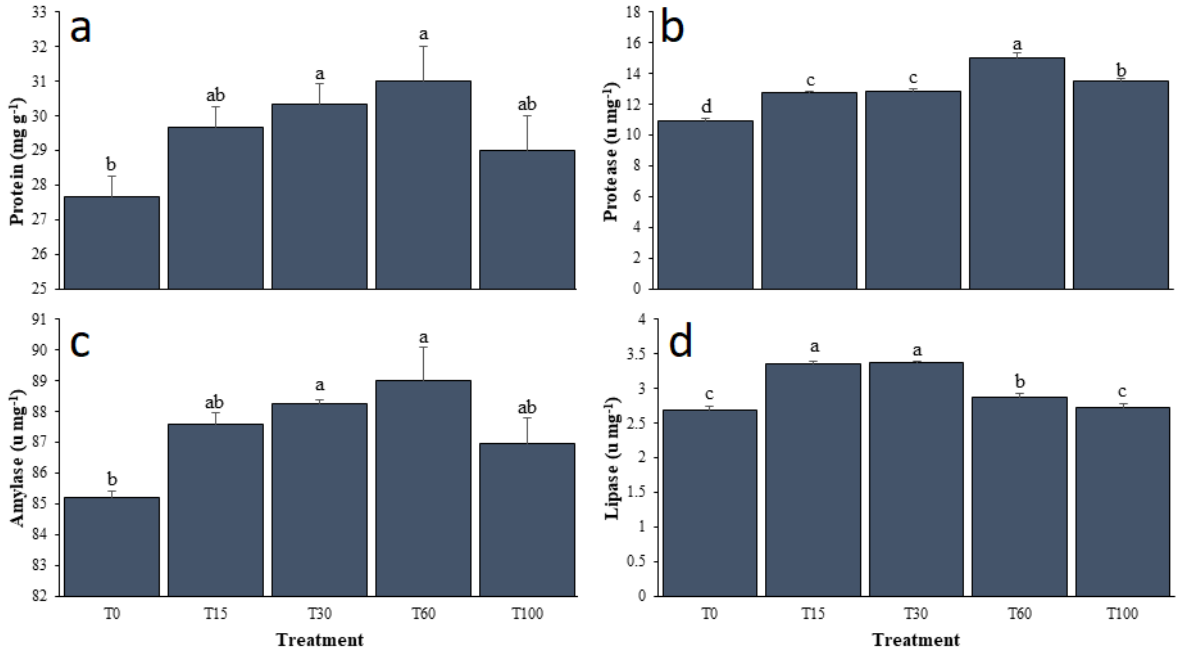



Figure 3. The effect of replacing fishmeal with different levels of mealworm on total protein content (a) and digestive enzymes activity (b: protease; c: amylase; d: lipase) of *L. vannamei* during a 60-day feeding trial. Values are representing as means ± standard deviations. Means with different letters are significantly different (P<0.05) from each other (n = 3)

## Discussion

Considering the importance of the use of IM as a high quality protein source, which has been discussed in various studies and in the diet of different species (Belforti et al., 2015; Belghit et al., 2019; Chaklader et al., 2019; Elia et al., 2018; Fawole et al., 2020; Gasco et al., 2016; Gu et al., 2022; Mente et al., 2022; Rahimnejad et al., 2019; Roncarati et al., 2015), but research on IM as a substitute to FM in Pacific white shrimp diet is still scarce and requires more comprehensive research to finding a suitable result to apply this protein source in shrimp diet.

### Growth performance

The results of the present study showed that not only replacing FM with MW does not have a negative effect on growth performance, it can even improve weight gain in Pacific white shrimp juveniles. In line with the results of the present study, Panini et al. (2017 a) and Choi et

al. (2018) reported that it is possible to completely replacement of FM with *T. molitor* meal in the diet of *L. vannamei* without negative effects on growth performance. Rahimnejad et al. (2019) evaluated the effects of FM replacement with of silkworm pupa meal (*Bombyx mori*) in Pacific white shrimp (*L. vannamei*), and found that complete replacement of FM has no negative effect on growth performance and can even have positive effects on molting time, antioxidant capacity and food digestibility. They have also suggested a maximum replacement of 75% in order to prevent damage to hepatocytes. Moreover, Tippayadara et al. (2021) reported that replacing FM with *Hermetia illucens* meal did not have a negative effect on survival rate and growth performance of Nile Tilapia, *Oreochromis niloticus*. Nevertheless, some studies have shown that the growth performance of aquatic species were significantly reduced when FM replaced with IM in their diets. Cai et al. (2022) found that replacing FM by MW decreased the growth performance of *L. vannamei*. These different results may be due to the differences in the species of aquatic animals, their life stage, the type of insects and the breeding substrate of the insects, as well as the way the diet is processed (He et al., 2022; Tschirner and Simon, 2015). As in the present study, the replacement of FM by MW did not affect the growth performance of juvenile white shrimp.

**Antioxidant enzymes activity**

Despite various studies on the effect of replacing FM with MW in the diet of different aquatic species on the activity of antioxidant enzymes of the intestine, hemolymph and liver or hepatopancreas (Choi et al., 2018; Feng et al., 2019; Gu et al., 2022; Henry et al., 2018; Sankian et al., 2018; Song et al., 2018; Su et al., 2017), comprehensive research in this case on *L. vannamei*, which has a high production rate in the world is still scarce. Antioxidant enzymes play a key role in maintaining oxidative balance, which are necessary for optimal response and efficient functioning of the immune system. Therefore, changes in the activity of antioxidant enzymes are often considered as useful indices to assess health status in response to external stimuli, such as dietary manipulation (Jeong et al., 2021). Our findings indicated a significant increase in activity of GPX, CAT, SOD, TNO and TAC antioxidant enzymes and a meaningful decrease of MDA (a suitable index for lipid peroxidation) in hepatopancreas of *L. vannamei* juveniles fed diets containing MW. These results confirmed that MW has the capacity to improve the antioxidant capacity of *L. vannamei*. Based on the results of current study, GPX showed an increasing trend with increasing the replacement rate of FM with MW, and a significant difference was observed between the control treatment with treatments containing MW. These results are in agreement with the findings of Su et al. (2017), Sankian et al. (2018) and Jeong et al. (2021) who reported a significant increase in GPX activity as a result of increasing the replacement of FM with MW in yellow catfish (*Pelteobagrus fulvidraco*), mandarin fish (*Siniperca scherzeri*) and juvenile olive flounder (*Paralichthys olivaceus*) diets, respectively. Regarding TAC, CAT, SOD and MDA, similar results were previously reported in other studies on the replacement of different insect species meal with FM in fish and shrimp. Wang et al. (2021) investigated the effects of FM replacement by defatted *H. illucens* larvae meal in practical diets of *L. vannamei*, and reported that by increasing the amount of replacing FM with IM, the activity of the TAC, CAT and SOD increased while the activity of the MDA decreased. Similarly, Su et al. (2017) reported that yellow catfish (*Pelteobagrus fulvidraco*) fed diets containing MW increased SOD activity and decreased MDA compared to diets

without IM. The increased GPX, CAT, SOD and TAC antioxidant enzymes and a meaningful decrease of MDA as dietary MW increasing suggesting that inclusion of MW may improve the antioxidant capacity in hepatopancreas of *L. vannamei* juveniles. The improvement of antioxidant status in shrimp hepatopancreas with increasing level of MW inclusion in current study is probably due to the antioxidant property of chitin. Ngo and Kim (2014) and Henry et al. (2018) stated that IM has antioxidant properties due to the presence of polysaccharide chitin in its exoskeleton and other bioactive compounds.

The activity of transaminase enzymes (AST and ALT) can be affected by nutritional status, stress and general health and their increase can primarily be a sign of liver damage as well as gill damage (Coz-Rakovac et al., 2005; Mastoraki et al., 2020 a; Peres et al., 2014). In this study, the replacing FM with MW did not effect on the levels of ALT and AST in hepatopancreas of *L. vannamei* juveniles. Results of no effect on activities of ALT and AST by replacing FM with MW indicated that MW may not have negative effects on health of hepatopancreas. These results are in agreement with those conducted on Jian carp (Li et al., 2017) and European sea bass (Mastoraki et al., 2020 a).

**Digestive enzymes activity**

The activity of digestive enzymes, especially amylase, lipase, and protease, which play a significant role in the digestive process, have a potential effect on feed utilization and thus growth performance (Li et al., 2017). In current study, protease and lipase indicated an increasing trend with increasing the amount of MW up to 60%. The protease activity showed a significant difference between the treatments containing MW and the control treatment. The amylase activity exhibited a significant difference between the T30 and T60 treatments with T0 (control treatment). The lowest and highest values were of lipase activity obtained in T0 and T30, respectively. There was also a significant difference between treatments T15–T60 with the control treatment (T0). These results indicated that inclusion of MW in *L. vannamei* diet could affect the activity of digestive enzymes and thus the feed utilization ability in the intestine of shrimp. Likewise, Li et al. (2019) indicated a quadratic increase of digestive enzymes activities (amylase, lipase and protease) in intestine of *Rana* (*Lithobates*) *catesbeiana* when 50–75% of FM substituted by housefly (Musca domestica) maggot meal. In addition, the findings reported in the replacement of FM with Mopane worm (*Imbrasia belina*) in Mozambique tilapia (*Oreochromis mossambicus*) exhibited that the amount of FM replacement up to 50% can relatively increase the activity of protease and lipase digestive enzymes (Rapatsa and Moyo, 2017). However, the results of Li et al. (2017) on *Cyprinus carpio* var. Jian and Belghit et al. (2018) on *Salmo salar* to investigate the replacement of FM with *H. illucens* larvae and fly meal, respectively, did not show significant differences on the activity of digestive enzymes between different treatments. The results of this study can indicate that the increase in the activity of digestive enzymes in response to the increase in the replacement of FM with MW could be considered as a form of adaptation to the decrease in the availability of nutrients as a result of the increase in chitin content in the diet, which can act as a strategy to compensate for the deficiency of pancreatic enzymes (Corring, 1980; Karlsen et al., 2017; Li et al., 2019).

In conclusion, this study indicates that FM can be successfully replaced with MW larvae (*T. molitor*) in diets of *L. vannamei* juveniles, without any adverse effect on growth performance, digestive enzymes activity and hepatopancratic biochemical indices. Further research to economic evaluate of its incorporation in the diets, are needed to better assess the potential of MW inclusion rates in commercial aquafeeds.

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