Replacing fish meal by mealworm (*Tenebrio molitor*) on the growth performance and immunologic responses of white shrimp (*Litopenaeus vannamei*)

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ABSTRACT. The objective of this study was to determine the effect of replacing FM with mealworm (*Tenebrio molitor*) on the growth performance and immunologic responses of white shrimp. In addition, the toxicity of such replacement to white shrimp was measured. Mealworm was incorporated to partially or totally replace fish meal in diets for white shrimp. Experimental groups of shrimp with an average initial body weight of 2.39 ± 0.49 g were fed each of 4 isonitrogenous diets formulated to include 0, 25, 50 and 100% (control, MW25, MW50 and MW100 respectively) of mealworm substituted for fish meal. After eight weeks of feeding trials, shrimp fed diets MW25, MW50 and MW100 had higher live weight gain (10.05 ± 3.06, 11.41 ± 2.08, and 10.36 ± 1.57, respectively), higher specific growth rate (2.56 ± 0.11, 2.79 ± 0.09, and 2.61 ± 0.07, respectively), and better feed conversion ratio (2.89 ± 0.08, 2.69 ± 0.09, and 2.72 ± 0.19, respectively) compared to those of shrimp fed control diet. Survival rate was 98% in all treatments. No toxicity was found in post-mortem pathophysiologic examinations. The levels of immune markers such as beta-glucan binding protein, prophenoloxidase and crustin associated with shrimp's cellular and humoral immunity were found to be higher in 25 and 50% mealworm replacement groups. These results clearly indicated that replacement of fish meal with 50% mealworm for shrimp diet was optimal in promoting the growth performance of shrimp without any adverse effect.

Keywords: mealworm; nutrition; white shrimp.

Farinha de peixe com larvas do bicho-da-farinha (*Tenebrio molitor*) no desempenho de crescimento e respostas imunológicas do camarão branco (*Litopenaeus vannamei*)

RESUMO. O objetivo deste estudo foi determinar o efeito da substituição do FM por larva de farinha (*Tenebrio molitor*) sobre o desempenho de crescimento e respostas imunológicas do camarão branco. Além disso, a toxicidade dessa substituição ao camarão branco foi medida. As larvas foram inseridas para substituir parcialmente ou totalmente a farinha de peixe em dietas do camarão branco. Grupos experimentais de camarão com a média de peso inicial de 2.39 ± 0.49g, foram alimentados com quatro dietas isonitrogênicas elaboradas para incluir 0, 25, 50 e 100% (control, MW25, MW50 e MW100 respectivamente) de larvas substituído por farinha de peixe. Após oito semanas de experiencia, os camarões alimentados pelas dietas MW25, MW50 e MW100 tiveram um maior ganho de peso (10.05 ± 3.06, 11.41 ± 2.08 e 10.36 ± 1.57, respectivamente), e uma taxa de crescimento específico mais alta (2.56 ± 0.11, 2.79 ± 0.09, e2.61 ± 0.07 respectivamente), comparados a aqueles que tiveram a dieta de controle. A taxa de sobrevivência foi de 98% em todos os tratamentos. Nenhuma toxina foi encontrada após a morte, nas quais foram feitos exames fisiopatológicos. Os níveis de marcadores imunológicos como as ligações de proteína betaglucanos, prophenoloxidase, crustinas associadas à imunidade celular e humoral do camarão, foram encontrados maiores que 25 e 50% dos grupos substituídos pelas larvas. Esses resultados indicaram que a troca da farinha de peixe com 50% de larvas para a dieta dos camarões foi ideal para promover o desempenho de crescimento do camarão sem qualquer efeito adverso.

Palavras-chave: larvas; nutrição; camarão branco.

Introduction

Nutrition is the most crucial factor in shrimp aquaculture because it contributes up to 50% of production costs. Fish meal (FM) has been used as the main dietary protein source for shrimp aquaculture (Tacon & Metian, 2008). Increased

demand of FM and global warming resulted in supply shortage of FM and price increase. Therefore, it is crucial to reduce the use of FM in aquaculture and search for alternative protein sources (Amaya, Davis, & Rouse, 2007). Many researchers have attempted to use other locally available cheap protein sources such as plant proteins, agricultural by- products, fishery by-products, terrestrial animal by -products, grain legumes, and oil seed plants (Davis & Arnold, 2000). Plant protein sources have been used as alternatives of fish meals with some measure of success, particularly grain legumes. However, the issue of cost is still not solved (Bulbul, Koshio, Ishikawa, Yokoyama, & Kader, 2015).

The Pacific white shrimp *Litopenaeus vannamei* is commercially important species throughout the world. As the farming of white shrimp grows, diseases of the species has received increasing attention as pathogens including bacterial and virus infections have affected the production, causing losses totaling hundreds of millions of dollars. Therefore, maintaining the health of *L. vannamei* against infectious diseases is of great importance to resource management (Gross, Bartlett, Browdy, Chapman, & Warr, 2001). Fish nutritionists are seeking alternatives of FM to promote the health of shrimp and strengthen their immune system not only for better profit, but also for better meat quality without any toxicity (Samocha, Davis, Saoud, & DeBault, 2004). To be qualified as FM alternatives, it is very important that the alternative will not only improve the immune system of shrimp, including the expression levels of immune genes and hemocyte viability, but also maintain muscle intactness of shrimp for product quality (Bachère, 2000; Pan & Yeh, 1993).

Insect are now becoming promising alternative protein sources due to the low success rate of replacing FM with plant products or materials from other animals (Van Huis, 2013). The yellow mealworm (*Tenebrio molitor L.*) larvaes are edible. It belongs to the beetle family Tenebrionidae. It is commonly produced on mixed grain diets. Its mass production for human and livestock feed use has been well-documented (Van Broekhoven, Oonincx, Van Huis, & Van Loon, 2015). Currently, insects are being considered as potential alternative protein sources for industrialized feed, such as poultry, pig, and cultivated species of fish like African catfish (Ng, Liew, Ang, & Wong, 2001; Ramos-Elorduy, González, Hernández, & Pino, 2002). However, the effect of replacing FM with mealworm (*Tenebrio molitor*) on the growth performance and immune

status of white shrimp is currently unknown. Therefore, the objective of this study was to determine the effect of replacing FM with mealworm (*Tenebrio molitor*) on the growth performance and immunologic responses of white shrimp. In addition, the toxicity of such replacement to white shrimp was measured.

Material and methods

Experimental diets

Mealworm was obtained from the Laboratory of Applied Entomology Division, National Academy of Agricultural Science (NAAS). They were frozen for 1 h, packed in plastic bags, sealed, placed on oven tray, and oven-dried at -70°C overnight to remove moisture. Dried super worms were milled and stored at 8°C for further use. Four isonitrogenous and isoenergetic diets were formulated (Format NC, Format International™, UK) to contain 38% crude protein (dry weight). A control diet containing FM as the main protein source (FM20%, Diet 1) was made in addition to three experimental diets, in which FM was replaced by mealworm at increasing levels: 25% mealworm (MW25), 50% mealworm (MW50), and 100% mealworm (MW100) as shown in Table 1.

Table 1. Formulation (% dry weight) and proximate composition (%DM) of experimental diets.

Parameter	Control	MW25	MW50	MW100
Ingredient				
Mealworm	0.00	5.00	10.00	20.00
Fishmeal	20.00	15.00	10.00	0.00
Soybean meal	37.62	38.85	40.63	45.10
Squid	10.00	10.00	10.00	10.00
Wheat flour	22.44	23.20	21.80	15.80
Dried Yeast	2.00	2.00	2.00	2.00
Fish oil	1.00	0.45	0.00	0.00
Vitamin and mineral premix	6.94	5.50	5.57	7.10
Proximate composition[1]				
Dry matter	94.50 ± 0.00	95.00 ± 0.20	95.20 ± 0.10	95.40 ± 0.10
Protein	38.01 ± 0.20	37.99 ± 0.20	38.00 ± 0.20	38.03 ± 0.30
Lipid	6.04 ± 0.00	6.00 ± 0.10	7.22 ± 0.10	10.50 ± 0.10
Ash	11.61 ± 0.10	11.28 ± 0.00	9.64 ± 0.00	9.17 ± 0.10
Calculated gross energy(kcal kg^{-1})	4,278	4,306	4,454	4,650

Proximate composition values represent means ± standard deviation of triplicate samples, rounded to the nearest 0.1 g.

All ingredients were ground in a ham mer mill (PLBM10L, Jeonju, South Korea) and thoroughly mixed to ensure the homogeneity of ingredients. A twin-screw extruder equipped with a 5-hp motor and co-rotating intermeshing screws with three pairs of right-handed kneading blocks was used at a constant screw speed of 200 rpm. Three extruder-barrel sections were electrically heated and air-cooled. The temperature profile selected for this

study was 115°C, 80°C, and 115°C, with the first temperature corresponding to the feed barrel section while the last temperature corresponding to the die section. Water was measured into the first barrel section using a variable-stroke piston pump to adjust the moisture content of the mixture-undergoing the extrusion. Pellets were dried in an oven at 70°C for 24h. They were packed in plastic bags, labeled, and kept at 4°C in the laboratory until used for feeding.

Feeding experiment

Juvenile *Litopenaeus vannamei* were obtained from a private farm, Yongsusan (Dangjin, South Korea). Prior to the experiment, all shrimps were acclimated to laboratory conditions for 3 weeks in a 250 L fiber tank. A commercial diet (DongAOne Co. South Korea) was fed twice daily for the adaptation period. Once the weight of juvenile shrimp reached 2–3 g, they were randomly selected and transferred to each of 50 m³ rearing tanks (a total of 4 tanks, 2,000 heads/tank) with a recirculation water system. Shrimps were bulk-weighed in triplicate (100 heads/replication) at the beginning and the end of feeding trial. The aquarium was cleaned, disinfected, and filled with dechlorinated water. The water from the aquarium was changed periodically at a frequency of three days. For each aquarium, water was changed once, while the quarter portion of the water was left before new water was installed. Each aquarium contained a bottom filter system with aeration using air pump. The experimental diets were fed twice a day at 08:00 and 18:00 with feeding rate of 5-6% of total body weight. Diets were stored in plastic bags at 4°C until every use. The four aquaria were monitored daily for mortality.

Growth performance

During the 56-day feeding trial, 30 shrimps from each tank were randomly selected and individually weighed every week. Weight gain (WG), feed conversion ratio (FCR), specific growth rate (SGR), and survival rate (%) were calculated using the following formula:

$$WG = W2 - W1$$

where W2 was mean final weight and W1 was mean initial weight;

$$FCR = Dry\ feed\ offered/live\ weight\ gain$$

$$SRG = \left(lnW2 - \frac{lnW1}{T} \right) * 100$$

where W2 was final weight of fish, W1 was initial weight of fish, and T was experimental time (day);

$$Survival(\%) = \frac{F2}{F1} * 100$$

where F1 was the number of shrimp at the beginning of experiment, and F2 was number of shrimp at the end of the experiment. All calculations were based on triplicate weighings per treatment.

ATP measurement

At the end of the experiment, 10 juvenile shrimps were randomly removed from the aquaria, sacrificed, and labeled for muscle ATP analysis. After sampling, the tail of the shrimp was cut off. The muscle of shrimp was squeezed out of the exoskeleton as rapidly as possible and stored under liquid nitrogen until analyzed. Half of the frozen tissue was ground to a fine powder in a mortar cooled by liquid nitrogen before analysis. ATP levels in the shrimp muscle tissue were determined using colorimetric assay kit (Biovision, Milpitas, CA, USA) according to the manufacturer's instructions. Samples were prepared using Deproteinizing Sample Preparation Kit (Catalog #K808-200, Biovision, Milpitas, CA, USA). After sample preparation, standard curve was prepared. Sample ATP levels were calculated according to the manufacturer's instructions (Catalog #K354-100, Biovision, Milpitas, CA, USA).

Immune gene expression analysis

For further analysis of immune gene expression, hepatopancreas samples were aseptically collected from each diet group (n = 10 per group). Hepatopancreas tissue samples were collected immediately in liquid nitrogen and stored at −80°C until total RNA isolation. Total RNAs were extracted from haemocytes and the target tissues using Trizol (Invitrogen). RNAs were quantified at 260 and 280 nm using a Multickan GO spectrophotometer (Thermo Scientific, MA, USA). Only RNAs with absorbance ratios of A260:A280 greater than 1.8 were used for further experiments. First-strand cDNAs were synthesized using Maxime RT PreMix (INtRON Biotechnology). PCR was performed using Maxime PCR PreMix i-MAX II (INtRON Biotechnology) on a TaKaRa PCR Thermal Cycler Dice Touch (TaKaRa, Ohtsu, Japan). Primer sequences used for this study were designed based on the sequences of a previous research study (Wang, Chang & Chen, 2007). PCR amplification was carried out with 35 cycles consisting of template

denaturation (1 min at 94°C), primer annealing (2 min at 52°C), and extension (2 min at 68°C). PCR products were subjected to 2% agarose gel electrophoresis and staining with ethidium bromide. The PCR products of beta-glucan binding protein (BGBP), prophenoloxidase (proPO), and Crustin gene fragments had expected sizes of 583, 563, and 539 bp, respectively.

After RT-PCR, mRNA expression levels of target genes in hepatopancreas tissues were determined by quantitative PCR (qPCR). qPCR primer sequences used in this study are also designed based on the sequences of a previous research (Wang, Chang, & Chen, 2007). Applied Biosystems 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) and AccuPower DualStar™qPCR PreMix (Bioneer Co., Ltd., South Korea) were used for qPCR. qPCR amplifications were performed in 96-well plate with thermal profiles of 95°C for 3 min followed by 40 cycles of 95°C for 15 s and 58°C for 1 min. After qPCR amplification, melt-curve analysis was conducted using ABI Software version 1.0 (Applied Biosystems) to determine relative quantities of each marker's mRNA. Sample values were averaged and normalized to β-actin.

WSSV challenge test

WSSV infected shrimps with prominent white spots were collected from shrimp farm located near Dangjin, South Korea. Haemolymph samples were drawn from these infected shrimps using sterile syringes followed by centrifugation at 3000 × g for 20 min at 4°C. The supernatant was then re-centrifuged at 8000 × g for 30 min at 4°C. The final supernatant was then filtered through a 0.4-μm filter. The filtrate was then stored at −20°C for WSSV challenge study.

The challenge test was prepared on day 56 of the feeding trial. After the end of feeding experiment, 10 shrimps from each dietary group were separated and maintained for 7 days. After 7 days acclimation to the new atmosphere, each shrimp was injected intramuscularly with WSSV solution. The WSSV filtrate prepared from infected shrimps as described above was intramuscularly injected to the second abdominal segment. After injection, WSSV challenged shrimps were placed in separate visible glass-tanks with continuous flow-through system. The number of dead juveniles was recorded every 8 – 10h for 10 days.

Total hemolymph counting and histolopathological analysis

On day 56 of feeding trial, 10 shrimps were randomly selected from each diet group. About 0.8 mL of haemolymph were withdrawn from the ventral sinus in the first abdominal segment using a 26-gauge hypodermic needle with 1-mL syringe. Each syringe was pre-filled with 0.2 mL of anticoagulant (10 mM Tris–HCl, 250 mM sucrose, 100 mM sodium citrate, pH 7.6). More anticoagulant was added to make an equal volume ratio of haemolymph to anticoagulant. Ten haemolymph samples from each treatment were analyzed individually. A volume of 50 μl anticoagulated haemolymph was fixed with an equal volume of neutral buffered formalin (10%) for 30 min to measure the total haemocyte count (THC). Differential haemocyte count (DHC) was conducted after PAS staining.

Statistical analysis

ANOVA and Student's t-tests at 0.1, 1, or 5% confidence levels were used to comparative data. All data were expressed as mean ± standard deviation (S.D.).

Results

Growth performance results are summarized in Table 2.

Table 2. Formulation (% dry weight) and proximate composition (%DM) of experimental diets.

Parameter	Control	MW25	MW50	MW100
Initial weight, g	2.39±0.49	2.39±0.49	2.39±0.49	2.39±0.49
Final weight, g	9.06±2.05	10.05±3.06	11.41±2.08	10.36±1.57
WG (Weight Gain), g	6.67±1.56	7.66±2.57	9.02±1.59	7.97±1.08
SGR (Specific Growth Rate)	2.37±0.09	2.56±0.11	2.79±0.09	2.61±0.07
FCR (Feed Conversion Ratio)	3.21±0.13	2.89±0.08	2.69±0.09	2.72±0.19
Survival, %	>98.00±0.0	>98.00±0.0	>98.00±0.0	>98.00±0.0
t-test vs Diet1	-	p < 0.05	p < 0.05	p < 0.05

$WG = W2 - W1$, where W2 = mean final weight, W1 = mean initial weight. FCR = Feed offered / live weight gain. $SRG = \left(lnW2 - \frac{lnW1}{T}\right) * 100$; where W2 = final weight of fish, W1 = initial weight of fish, T = experimental period (day). $Survival (\%) = \frac{F2}{F1} * 100$; F1 = number of fish at the beginning of experiment, and F2 = number of fish at the end of the experiment. All calculations were based on the triplicate tank treatment (x, inter variance exists).

There were statistically significant (p < 0.05) differences in growth performance of shrimps. The mean final weight, WG, and SGR of fish fed mealworm diets at ratio of 25, 50, and 100% were significantly (p < 0.05) higher than those fed 0% mealworm diet (control). The growth of white shrimp was significantly improved by dietary mealworm incorporation (Figure 1). During the experimental period, weekly growth rate of shrimps

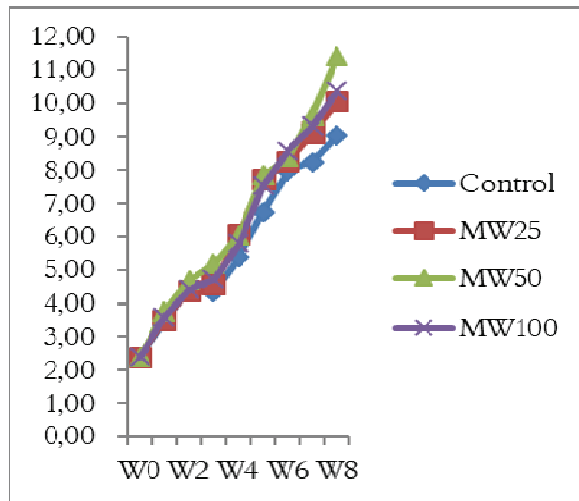fed mealworm diets was more than 10% higher than that of control group.

Figure 1. Growth performance of FM replacement feed groups.

ATP quantities positively increased in mealworm feeding groups (Figure 2).
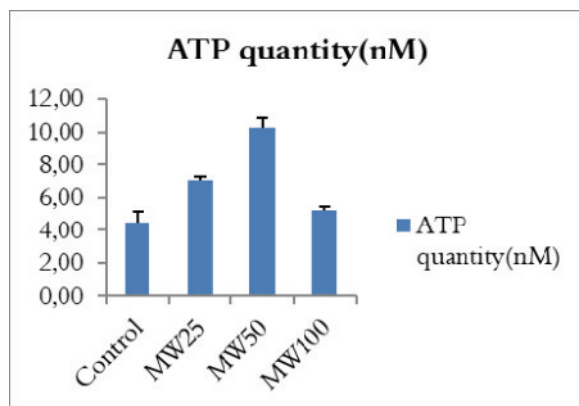

Figure 2. Tissue ATP levels in the abdominal muscle of the shrimp compared to the control group.

Compared to the ATP concentration of control group at 4.44 ± 0.70 nM, those of MW25 and MW50 groups showed a significant increase to 7.00 ± 0.24 (63.4%) and 10.24 ± 0.66 nm (230%), respectively. However, the ATP concentration of MW100 group was not significantly ($p > 0.05$) different compared to that of control group (Table 3).

Table 3. Tissue ATP levels in the abdominal muscle of the shrimp and t-test results compared to the control group.

Parameter	Control	MW25	MW50	MW100
ATP quantity(nM)	4.44±0.70	7.00±0.24	10.24±0.66	5.20±0.25
t-test vs Diet1	-	$p < 0.05$	$p < 0.01$	$p = 0.27$

The transcriptional levels of BGBP, proPO, and crustin mRNA appeared to be increased in shrimps in mealworm containing groups compared to those of control group (Figure 3).
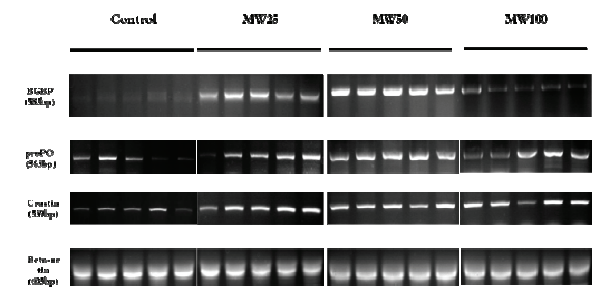

Figure 3. BGBP, proPO and crustin expression in RT-PCR.

The highest expression levels of BGBP, proPO, and crustin mRNA in hepatopancreas were found in shrimps of MW50 group. Hemocyanin, PEN, SOD, and lysozyme expression were not detected by RT-PCR (data not shown). Therefore, gene expression levels were studied with qPCR. Dietary inclusion of mealworm significantly ($p < 0.05$) enhanced the mRNA expression of BGBP, proPO, and crustin in the hepatopancreas of *L. vannamei* after 8 weeks of feeding trial (Table 4).

Table 4. Relative mRNA expression of BGBP, proPO and Crustin mRNA in Pacific white shrimp Litopenaeus vannamei when studied by qPCR analyses and t-test results compared to the control group.

Relative mRNA expression level (%)	Control	MW25	MW50	MW100
BGBP	100.00	465.97	593.48	164.87
proPO	100.00	233.58	1396.79	366.17
Crustin	100.00	204.61	170.33	88.40
p-value by t-test vs Diet1	Diet 1	Diet 2	Diet 3	Diet 4
BGBP	-	0.00	0.00	0.03
proPO	-	0.00	0.01	0.00
Crustin	-	0.052	0.042	0.392

The expression of BGBP was gradually enhanced. All mealworm groups showed statistically significant ($p < 0.05$) increase in the level of mRNA (Figure 4). The expression of proPO showed a similar increasing pattern to BGBP in a statistically significant manner (Figure 5). The expression of crustin was significantly ($p < 0.05$) enhanced only in MW25 group (Figure 6).

Cumulative mortalities of *L. vannamei* challenged with WSSV after 8 weeks of feeding trial were checked for 10 days. In all treatment groups, dead shrimps were recorded at one day post challenge. The survival rate of shrimps fed MW50 was ($p < 0.05$) higher than that of the control group at day 5 after viral challenge. At day 5 post WSSV challenge, the survival rate of shrimps fed MW25 was 40% (mortality 60%), while the survival rate of shrimps fed control diet was 0% (mortality 100%). Shrimps fed control all died by day 2 post WSSV challenge.

At day 3 post WSSV challenge, the mortalities of shrimps fed control, MW25, MW50 and MW100 were 100, 40, 30, and 70%, respectively. Although there were substantial mortalities in all WSSV challenged groups, no shrimp in MW50 group died until day 2 (Figure 7).
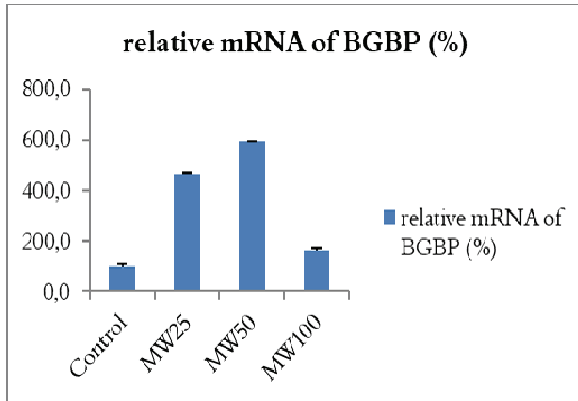

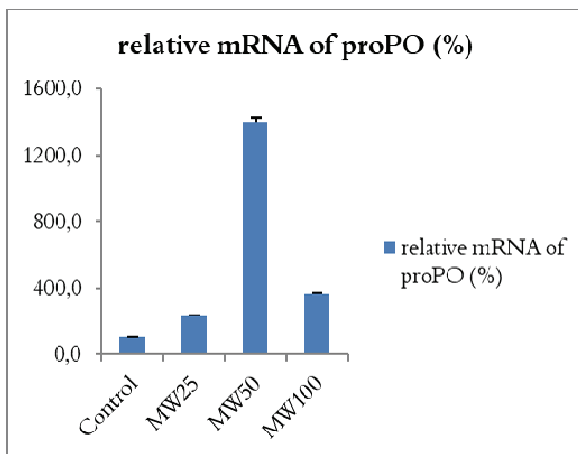**Figure 4.** Relative mRNA expression of BGBP.
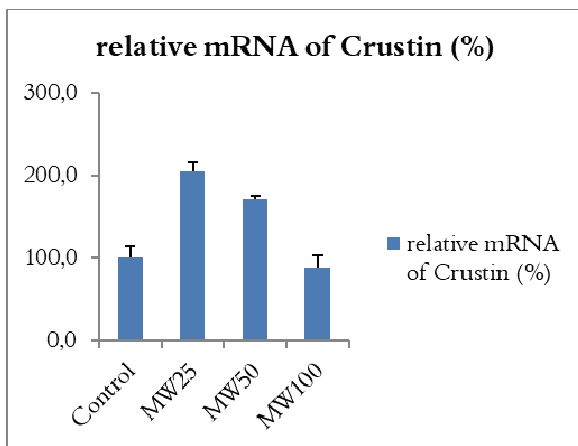

Figure 5. Relative mRNA expression of proPO.

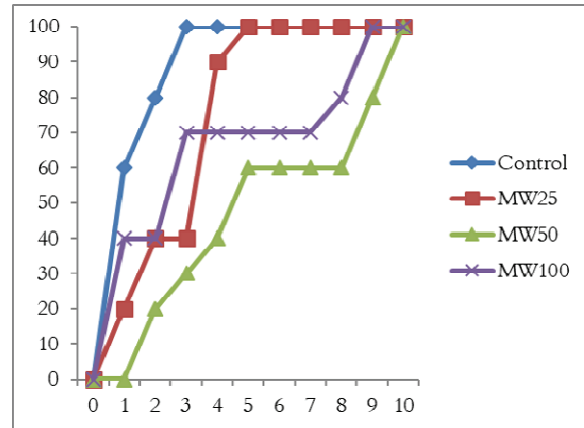**Figure 6.** Relative mRNA expression of Crustin.

Figure 7. Mortality of diet 1, 2, 3 and 4 by WSSV challenge test.

Total hemolymph counting and histolopathological analysis

The haemolymph was drawn directly from experimental and control animal groups using prewashed syringe with anticoagulant (sodium citrate) to avoid clotting at the end of the 8-week feeding trial. The THC of mealworm diet group shrimps were significantly ($p < 0.05$) increased (13% increase in MW25 group, 69% increase in MW50 group, and 32% increase in MW100 group) compared to that of control group (Table 5 and Figure 8).

Table 5. THC (total haemolymph count) analysis and t-test vs Diet1 group results of Diet1, Diet2, Diet3 and Diet4 feed Pacific white shrimp, Litopenaeus vannamei after 8-week feeding trial. Each value is a mean ± S.D.

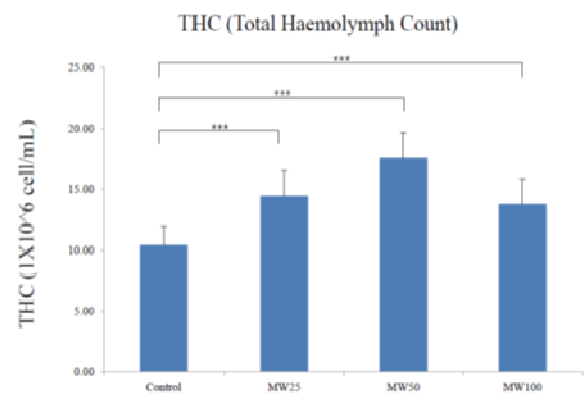| Parameter	Control	MW25	MW50	MW100
THC ($\times 10^6$ cell mL^{-1})	10.40 ± 1.52	14.40 ± 2.07	17.60 ± 2.07	13.80 ± 2.04
t-test vs Diet1	-	$p < 0.01$	$p < 0.01$	$p < 0.01$

Figure 8. THC (Total Haemocyte Counting) changes in diet 1, 2, 3 and 4.

The count of hemocytes of *L. vannamei* after 8 weeks of feeding trial showed the most and statistically significant increase in MW50 group. In

the group, agglutination of hyaline cells and small granulocytes were found with large granulocytes representing haemocyte phagocytic activity (Figure 9).
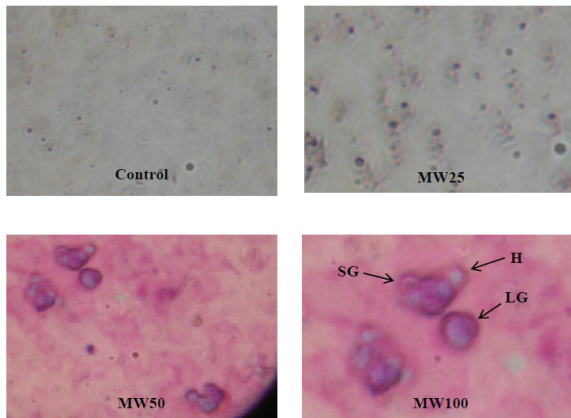
Figure 9. Differential count of hemocytes from L. vannamei after WSSV challenge in diet 1 (left) and diet 2 (right). Agglutination of hyaline cells(H) and small granulocytes(SG) and large granulocytes(LG) are shown. PAS stain. x200.

The hyalinocyte and granulocyte counts in control group were relatively fewer than those in the other groups. The haemocytes in MW50 group were the most abundant.

Histopathological toxicity evaluation for insect-based feed

Histopathologic analyses of hepatopancreas, intestine, and muscle were conducted for *L vannamei* after feeding the experimental diets. No pathologic finding of apoptosis, inflammatory cell infiltration, or others was found in shrimps of any diet groups (Figure 10).

Discussion

Results of this study clearly indicated that the growth performance and immune-enhancing effect of Pacific white shrimp were affected by dietary composition. Experimental diets were designed to replace fish meal with mealworm (*Tenebrio molitor*) at ratio of 25%, 50, and 100% for Pacific white shrimp, *Litopenaeus vannamei*. The final weight, weight gain, and SGR values of fish fed mealworm diets (25, 50, and 100% mealworm replacement, respectively) were significantly improved when compared to those of shrimps fed control diet (FM20). The highest growth performance of shrimps was obtained in MW50 group, indicating that 50% inclusion of mealworm could be optimal to replace fish meal in diet of *L. vannamei*. Lower weight gain was observed when mealworm content in diet was

higher than 50%, suggesting that higher mealworm level in the diet can lead to growth reduction.
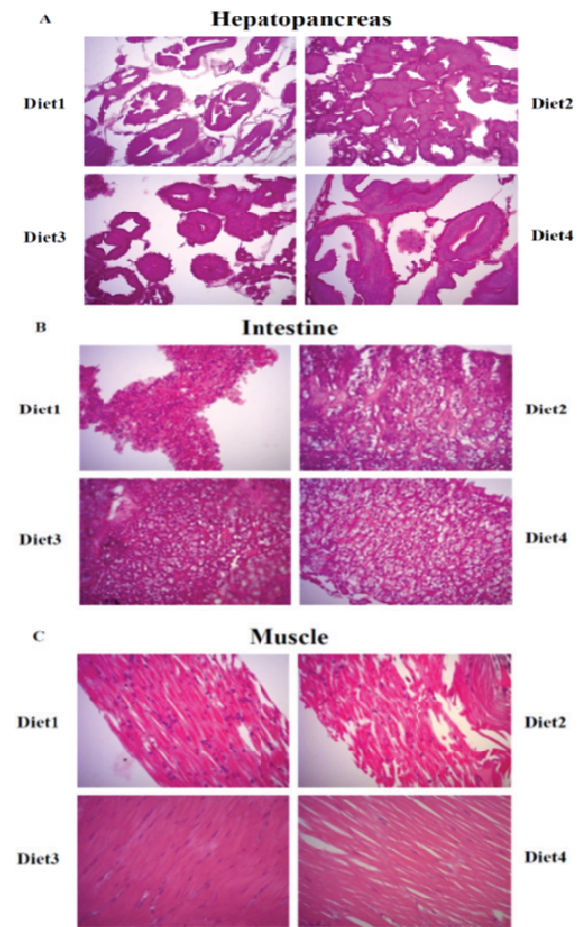
Figure 10. Histopatholgic analyses of hepatopancreas (A), intestine (B) and muscle (C) from L. vannamei after feeding diet 1, diet 2, diet 3 and diet 4.

The nutritional value of mealworm as animal feed has been recognized in the past. Many studies have demonstrated that insect-based diets are potentially cheaper than those based on fish meal. Numerous studies on the use of insects as alternative to fish meal have been conducted. In the present study, we demonstrated that *T. molitor* larvae had higher nutritional value for white shrimp. Replacing fish meal with mealworm at 50% resulted in a 35% increase in the growth performance of shrimps compared to the 100% fish meal diet without adding any mealworm. Replacement of 50% mealworm group resulted better performance than the others. Therefore, mealworms might be able to provide essential amino acids to shrimps for the optimum growth.

The innate immunity system of shrimp is mainly consisted of prophenoloxidase (proPO) activating

system, clotting system, phagocytosis, encapsulation and nodule formation, antimicrobial peptides (AMPs) formation, and cell agglutination. β-1, 3-glucan binding proteins (BGBP) is a constitutive plasma protein which is vital component of the immune system in shrimp. Activated haemocytes are also engaged in phagocytosis by eliminating microbes or foreign particles. Stimulation of the proPO activating system plays a very prominent role in non-self-recognition, haemocyte communication, and the production of melanin (Vargas-Albores & Yepiz-Plascencia, 2000). Activated haemocytes are also engaged in phagocytosis by eliminating microbes or foreign particles. Stimulation of the proPO activating system plays a very prominent role in non-self-recognition, haemocyte communication, and the production of melanin (Bachère, 2000). Activated haemocytes can also be induced to release other proteins related to defense responses such as AMPs, including crustin (Wang et al., 2007).

In the present study, white shrimps receiving *T. molitor* – based diets exhibited enhanced immune modulation, resulting in increased expression levels of BGBP, proPO, and crustin, representing resistance against pathogen. These diets also showed protective effect against WSSV infection. When challenged with WSSV, shrimps in MW25 group had 40% survival rate at 8 days post WSSV challenge, while shrimps in the control group all died within 3 days post WSSV challenge. Increased total haemolymph count (THC) indicates that mealworm based feed could improve the immunity of shrimps. The THCs of shrimps fed diets containing mealworm as protein source were significantly higher than those of shrimp fed control diet. These facts suggest that the activation of BGBP, proPO, and crustin might be caused by the increase of circulating haemocyte induced by mealworm-based high-performance diet. After two months of feeding with mealworm-based diets, upregulation of BGBP, proPO, and crustin mRNA in shrimps resulted in enhanced resistance against pathogen WSSV. Crustaceans have mechanisms to recognize cell wall components of bacteria and fungi such as β-1,3-glucans, lipopolysaccharides, and peptidoglycan. Several pattern recognition proteins such as β-1,3-glucan binding protein (BGBP), LPS (lipopolysaccharide)-binding protein (LBP), PG-binding protein (PGBP), lipopolysaccharide β-1,3-glucan binding protein (LGBP), and lipoprotein can recognize and respond to microbial intruders. They are involved in activating the proPO (prophenoloxidase) system (Cheng, Liu, Tsai, & Chen, 2005). Invertebrates lack antibodies. Therefore, they have efficient innate immune

system to defend themselves against invading foreign particles. BGBP is known to activate the proPO cascade. We found that dietary inclusion of 50% mealworm was optimal for shrimp. Crustin, the most important antimicrobial peptides in shrimp, is known to be required for cellular defense. High expression levels of BGBP, proPO, and crustin might have provided shrimps a ready-to-defend status. Present results suggest that *T. molitor* based feed might be effective for improving not only shrimp's growth performance, but also their immunity. Maintaining high intracellular ATP levels is also an indicator of healthy status.

Conclusion

T. molitor could be an excellent protein source for shrimp. It has beneficial effect for white shrimps in terms of better growth performance, better pathogen-resisting ability, and enhanced immunity (higher expression in immune genes such as BGBP, proPO, and crustin) without any toxicity. This is the first report about the efficacy and toxicity of mealworm (*Tenebrio molitor*) for the growth performance and immune status of white shrimp (*Litopenaeus vannamei*). *T. molitor* based feed at 50% to replace FM can offer an economic advantage. It can provide higher growth performance to white shrimps with a more efficient way to fight pathogens without any toxicity.

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