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Research Article

Effects of dietary inclusion of mealworm (*Tenebrio molitor*) on the fatty acid compositions of Pacific white shrimp (*Litopenaeus vannamei*)

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Abstract

The increasing demand for sustainable protein resources in aquaculture has prompted the exploration of alternative feed ingredients, such as mealworm (MW; *Tenebrio molitor*). This study evaluates the effects of MW inclusion on the fatty acid composition and proximate analysis of Pacific white shrimp (*Litopenaeus vannamei*) during a 60-day feeding trial involving juvenile shrimp, which were assigned to five experimental diets with varying MW inclusion levels: T_0 (0% MW), T_{15} (15% MW), T_{30} (30% MW), T_{60} (60% MW), and T_{100} (100% MW), all formulated to maintain a protein content of approximately 37%. Proximate analysis indicated that fishmeal (FM) contained a crude protein level of 56.31%, while MW had a comparable content of 53.10% (p>0.05). However, FM demonstrated significantly higher levels of digestible protein, ash, and nitrogen-free extract (NFE) compared to MW, which was characterized by higher lipid and fiber contents. The analysis of shrimp muscle composition revealed that increased MW inclusion (T_{30}-T_{100}) significantly enhanced crude protein levels (p<0.05), while T_0 showed the highest crude lipid content (p<0.05). FA profiling indicated that FM was abundant in essential long-chain polyunsaturated fatty acids (PUFAs), including eicosapentaenoic acid (EPA; 8.54%) and docosahexaenoic acid (DHA; 13.57%). Conversely, MW lacked these essential FAs, leading to a decrease in EPA and DHA levels in shrimp muscle as MW inclusion increased. The predominant FAs in shrimp muscle shifted towards oleic acid (C18:1c), particularly in the T_{100} treatment. The findings of this study indicate that increased inclusion of MW significantly enhances the crude protein levels in Pacific white shrimp while concurrently leading to a decrease in essential fatty acids such as EPA and DHA. This highlights the necessity for dietary supplementation with alternative lipid sources to meet the essential fatty acid requirements of *L. vannamei*.

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Introduction

Aquaculture is becoming increasingly vital for global food production, serving as a key protein source for a rapidly growing population. In 2022, it produced 130.9 million tonnes, surpassing capture fisheries for the first time and accounting for nearly 51% of total fish consumption (FAO, 2024; Sharifinia, 2025). While aquaculture plays a crucial role in enhancing food security, it also faces significant challenges, including disease outbreaks, nutrient deficiencies, and environmental impacts (Emerenciano *et al*., 2025; Sharifinia, 2024; Sharifinia *et al*., 2018, 2025; Yeganeh *et al*., 2020). To address these challenges, future trends in aquaculture are focusing on species diversification, sustainable practices, technological advancements, and an increased emphasis on health and nutrition (FAO, 2024; Sharifinia, 2025).

This growth in aquaculture is essential to meet the rising global demand for fish protein, driven by population growth (Emerenciano *et al*., 2025). Traditionally, fishmeal (FM) has been the primary protein source in aquaculture diets due to its high nutritional quality and digestibility. However, the sustainability of FM is increasingly threatened by overfishing, pollution, and climate change, which have led to substantial declines in wild fish stocks (Sharifinia, 2024). Consequently, there is a pressing need to explore alternative protein sources that can satisfy the nutritional requirements of farmed fish while reducing the pressure on marine ecosystems (Sharifinia *et al*., 2023a, 2023b).

There is a growing interest in utilizing edible insects as a sustainable protein alternative for both human diets and animal feed (Tubin *et al*., 2023; van Huis, 2013). Insects can provide a rich source of proteins, fats, minerals, vitamins, and energy, depending on their species, developmental stage, and diet (Makkar *et al*., 2014; Nowak *et al*., 2016). One notable species is *Tenebrio molitor*, commonly referred to as the mealworm in its larval form. This coleopteran is extensively farmed for pet food and has been evaluated as a substitute for FM in aquaculture diets (Gasco *et al*., 2016; Panini *et al*., 2017b; Piccolo *et al*., 2017). MWs are particularly noteworthy for their high protein content, balanced amino acid profile, fatty acid (FA) composition that is notably rich in n-6 FAs, and ability to convert organic waste into biomass (Agbohessou *et al*., 2021; Basto *et al*., 2020; Fabrikov *et al*., 2021; Iaconisi *et al*., 2019; Mastoraki *et al*., 2020; Panini *et al*., 2017b). Additionally, insects serve as a natural food source for many fish species during their early life stages (Sharifinia, 2015), enhancing their appeal as an ingredient in aquaculture diets (Sharifinia, 2024).

The increasing demand for sustainable protein sources in aquaculture has led to significant interest in the potential of insect meal (IM) as a substitute for traditional FM (Sharifinia, 2024). A growing body of research has explored the impacts of this substitution on the growth, nutritional quality, and health indicators of various aquatic species, including fish and shrimp. Panini *et al*. (2017b) conducted a study examining the effects of replacing FM with MW on the muscle quality of farmed shrimp (*Litopenaeus vannamei*). Their findings revealed that while the protein content of shrimp muscle remained stable, lipid levels increased significantly with higher inclusion of mealworm meal. However, this substitution resulted in a linear decline in essential FAs, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are crucial for human nutrition (Erdman *et al*., 2011; Panini *et al*., 2017a). Despite these changes, the overall lipid content of the shrimp was low, and the n-3/n-6 ratio remained within acceptable dietary ranges for human consumption. The following year, Sankian *et al*. (2018) explored the incorporation of *T. molitor* meal into the diets of juvenile mandarin fish (*Siniperca scherzeri*). This research marked the first instance of using MW as an FM alternative for this species. The results indicated that including up to 30% MW meal did not negatively affect fish growth or feed efficiency. Notably, the addition of MW altered the FA profile of the fish fillets, leading to increased levels of saturated and monounsaturated FAs while reducing n-3 polyunsaturated FAs. Moreover, Agbohessou *et al*. (2021) investigated the enrichment capacity of long-chain polyunsaturated FAs (PUFAs) in two dipteran species, *Hermetia illucens* and *Chrysomya putoria*, and their effects on the growth and immune responses of Nile tilapia (*Oreochromis niloticus*). Their study demonstrated that both IMs significantly increased the levels of EPA and DHA in the fish, thereby enhancing their immune status. This highlights the potential of insect-based diets not only to serve as protein sources but also to enrich fish with essential FAs critical for their health. Fabrikov *et al*. (2021) expanded on this concept by examining the impact of IM diets on the FA compositions of sea bream (*Sparus aurata*), tench (*Tinca tinca*), and rainbow trout (*Oncorhynchus mykiss*). Their findings revealed that while the inclusion of IMs reduced valuable long-chain PUFAs in fish fillets, species-specific differences were observed. Notably, tench demonstrated greater resilience to IM inclusion compared to rainbow trout, underscoring the importance of considering species-specific factors when formulating insect-based diets. Further research by Zheng *et al*. (2024) assessed the replacement of FM with defatted black soldier fly (BSF; *H. illucens*) in the diets of Pacific white shrimp (*L. vannamei*). The findings indicated that a 20% substitution of FM with BSF meal resulted in growth performance comparable to the control group. However, higher replacement levels (≥40%) adversely affected growth metrics and flesh quality. In another study, Alvanou *et al*. (2023) examined the effects of substituting FM with BSF meal on the growth performance and chemical composition of juvenile freshwater crayfish (*Pontastacus leptodactylus*). The results indicated that while the control group demonstrated superior feed conversion ratios and specific growth rates, the inclusion of BSF meal enhanced survival rates. The FA composition of the crayfish was significantly altered, with reductions in saturated and PUFAs, indicating a different metabolic utilization compared to conventional FM. The existing literature suggests that IMs, such as those from MWs and BSFs, can effectively replace FM in aquaculture diets. These studies collectively demonstrate that while IMs can improve growth performance and health parameters in aquatic species, careful attention must be paid to their FA profiles and overall nutritional contributions. This ensures that the dietary requirements of both the animals and potential human consumers are satisfied.

Despite these promising insights, challenges remain regarding the integration of IMs into aquaculture diets. A significant concern is the high fat content often found in insect larvae, which can vary considerably depending on the species and developmental stage (Fabrikov *et al*., 2021). While dietary fats are essential for energy, excessive lipid levels can adversely affect shrimp health and growth performance. Additionally, terrestrial insects typically lack long-chain PUFAs such as EPA and DHA, which are vital for the health and development of aquatic species (Sánchez-Muros *et al*., 2014). Therefore, it is crucial to further investigate how the inclusion of IMs affects the FA profiles of shrimp to optimize aquaculture nutrition.

Given the sustainability challenges in aquaculture, this study specifically explores the potential of MW (*Tenebrio molitor*) as an alternative protein source, aiming to provide insights into its effects on the nutritional quality of Pacific white shrimp (*Litopenaeus vannamei*). By elucidating how MW larvae meal influences the nutritional quality of shrimp, this study seeks to provide valuable insights into the potential of insect-based feeds to enhance the FA composition of aquaculture products. Ultimately, this research aspires to contribute to the development of sustainable aquaculture practices that not only meet the growing demand for seafood but also promote environmental stewardship by reducing reliance on traditional fish-based ingredients.

Materials and methods

Experimental diets

The shrimp were fed with five practical diets, labeled T_0, T_{15}, T_{30}, T_{60}, and T_{100}, which incorporated FM that was progressively substituted with MW at varying levels of 0%, 15%, 30%, 60%, and 100% (Table 1). The ingredients were processed into pellets with a diameter of 2 mm using a specialized pellet mill, ensuring uniformity in pellet size and quality. Following the grinding process, each treatment was individually placed in an oven set to 60°C for a duration of six hours. This step was crucial for effectively reducing the moisture content of the pellets, ensuring their stability and longevity. Then, all diets were securely sealed in vacuum-packed bags to preserve their freshness and nutritional integrity. These sealed bags were then stored in a freezer at a temperature of 4°C until they were needed for the feeding trial. This meticulous preparation and storage process ensured that the diets remained in optimal condition for the shrimp during the experimental phase.

Experimental design

The experimental study was conducted over a span of 60 days at the Persian Gulf SPF Shrimp National Research Center in Bushehr, located in Bushehr Province, Iran. The research focused on juvenile Pacific white shrimp, *L. vannamei*, which had an average initial weight of 7.41±0.13 g and an average length of 8.30±0.08 cm. These shrimps were sourced directly from the research facility.

Table 1: Composition and proximate analysis of the experimental diets formulated for juvenile *Litopenaeus vannamei*.

Ingredients (%)	Experimental diets				
	T0	T15	T30	T60	T100
Fishmeal[1]	24.00	20.40	16.80	9.60	0.00
Mealworm meal	0.00	3.60	7.20	14.40	24.00
Soybean meal	28.00	28.00	28.00	28.00	28.00
Shrimp by-product meal	10.00	10.00	10.00	10.00	10.00
Fish oil[2]	2.00	2.00	2.00	2.00	2.00
Wheat meal	8.00	8.00	8.00	8.00	8.00
Wheat bran	14.00	13.58	13.73	13.31	12.58
Soybean oil[3]	5.00	4.00	3.00	2.00	1.00
Mineral premix[4]	1.00	1.00	1.00	1.00	1.00
Vitamin premix[5]	2.00	2.00	2.00	2.00	2.00
Cholesterol	0.50	0.50	0.50	0.50	0.50
Soybean lecithin	2.00	2.00	2.00	2.00	2.00
DL-Methionine	0.50	0.50	0.50	0.50	0.50
Vitamin C	1.00	1.00	1.00	1.00	1.00
Kaolin	0.50	0.50	0.50	0.50	0.50
Binder	1.50	1.65	1.77	2.19	2.65
Filler	0.00	1.00	2.00	3.00	4.00
	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)

Before initiating the feeding trial, the shrimp were acclimated to the experimental environment by being fed a commercial diet with 40% protein and 8% lipid content for a week. A total of 300 juveniles were then randomly assigned to 15 fiberglass cylindrical tanks, ensuring a representative distribution of shrimp across treatments to minimize bias. This setup resulted in a density of 20 shrimp per tank, which is equivalent to one shrimp per 0.01 cubic meters (m^3). To prevent the shrimp from escaping, all tanks were covered with plastic nets. Each treatment was replicated three times. The shrimp were fed four times a day at 6:00, 12:00, 18:00, and 24:00 hours to optimize nutrient absorption and growth rates in juvenile shrimp. The daily feed intake was adjusted to be between 2% and 5% of the shrimp's body weight, ensuring that no feed residue remained in the tanks two hours after feeding. The shrimp in each tank were weighed every ten days, and the daily feed amounts were adjusted accordingly. Any deceased shrimp were immediately removed from the tanks, weighed, and documented to ensure accurate tracking of shrimp health and survival rates. Additionally, waste materials that settled at the bottom of the tanks were siphoned out daily to maintain optimal water quality. The tanks were kept under a natural light cycle, and aeration was provided using air stones to maintain dissolved oxygen levels close to saturation. Throughout the trial, important seawater parameters were monitored daily, including temperature (maintained between 26 and 28°C) and salinity (ranging from 27 to 29 g L^{-1}). pH levels (between 8.1 and 8.9) and dissolved oxygen levels (ranging from 6.2 to 6.8 mg L^{-1}) were measured weekly to ensure the shrimp were kept in suitable conditions for growth and health.

Sample collection and storage of shrimp muscle

At the end of the feeding trial, all shrimp groups underwent a 24-hour fasting period prior to sampling to standardize their physiological state and reduce variability in muscle composition due to recent feeding. After that, 15 shrimp from each treatment group were gathered. All shrimp were chill-killed and then were washed with clean water. The shells and heads of all the shrimp were then removed and discarded. A freeze-dryer set to -40°C was employed to freeze-dry the muscle samples for a duration of three days in a dark environment. Once dried, the muscle tissues were ground using a grinder and stored at -20°C for subsequent FA analysis (Sharifinia *et al*., 2023a, 2023b).

Proximate analyses of practical diets, fishmeal, insect meal, and shrimp muscle

The chemical composition of the FM, IM, practical diets, and shrimp muscle samples was analyzed using standard procedures established by the Association of Official Analytical Chemists (AOAC, 2006), specifically referencing the methods for moisture, ash, crude protein, and crude lipid determinations. Moisture levels were assessed by drying the samples in an oven at 105°C until a constant weight was achieved, while ash content was determined through combustion in a Thermolyne™ muffle furnace (Thermo Scientific, Asheville, NC, USA) at 600°C for 4 hours. Crude protein was measured using the Kjeldahl method with an automatic Kjeldahl System (Buchi, Flawil, Switzerland). The crude lipid content was extracted using petroleum ether via a Soxhlet extractor (VELP Scientifica, Milano, Italy).

Fatty acids analysis

The analysis of FA composition involved the extraction of total lipids using a chloroform: methanol solvent system in a 2:1 volume ratio, following the methodology outlined by AOAC (2006). To prepare FA methyl esters (FAMEs), the lipid extracts underwent acidic methanolysis utilizing BF3 in methanol. This process converts the FAs into their corresponding methyl esters, which are more suitable for analysis. Following the methanolysis, the FAMEs were recovered using n-hexane, as FAMEs are preferred for their stability and ease of analysis in gas chromatography, facilitating accurate quantification of FAs (Metcalfe and Schmitz, 1961). The recovered methyl esters were then subjected to analysis using a gas chromatograph (model: CP3800 Varian), which was equipped with a flame ionization detector (FID) and a capillary column (BPX70 SGM) measuring 60 meters in length, with an internal diameter of 0.32 mm and a film thickness of 0.25 µm. For the gas chromatography analysis, the injector and detector were maintained at temperatures of 210°C and 250°C, respectively. The temperature of the column was programmed to increase from 160°C to 180°C at a rate of 2°C per minute. Helium served as the carrier gas throughout the process, and the total run time for each sample was set at 85 minutes. The peaks corresponding to the FAs were integrated using Varian Star Chromatography software (version 6.41), facilitating the quantification of the FAs present in the samples. Identification of the FAs was accomplished by comparing the retention times of the sample peaks with those of known standards obtained from Sigma-Aldrich.

Statistical analysis

The impact of incorporating MW into the diet on the proximate composition of shrimp muscle was evaluated using a one-way analysis of variance (ANOVA) with the SPSS software version 23 (SPSS Inc., Chicago, IL, USA). Assumptions of normality and homogeneity of variances were checked before conducting the ANOVA to ensure the validity of the statistical results. To identify statistically significant differences (p<0.05) among the mean responses, Tukey's multiple range test was applied. Results are presented as mean ± standard deviation (SD) for the triplicate groups.

Results

Proximate composition of fishmeal and insect meal

The outcomes of the comparative chemical analysis between FM and MW are illustrated in Figure 1. The results revealed that the protein content in FM was higher than MW; however, this difference was not statistically significant (p>0.05). In contrast, regarding nutritional quality, FM exhibited significantly elevated levels of digestible protein, ash, and nitrogen-free extract (NFE) in comparison to MW (p<0.05).

Proximate composition of shrimp muscle

Figure 2 displays the findings of the shrimp muscle proximate composition. The proximate analysis results indicated that there were significant changes in muscle composition (p<0.05). The replacement of FM with MW at higher levels (T30-T100) resulted in a significantly increased crude protein percentage in the shrimp muscle compared to the control treatment (T0). In contrast, T_0 treatment exhibited a significantly higher percentage of crude lipid in the shrimp muscle than all other treatments (p<0.05). Furthermore, the highest ash content in the shrimp muscle was observed in the T_0, which showed a significant difference from the T_{30}-T_{100} treatments (p<0.05).

Fatty acids composition of fishmeal, mealworm, and shrimp muscle

Table 2 presents the FA composition of FM, MW (*T. molitor*), and shrimp muscle when FM is partially substituted with MW. The analysis revealed that the primary FAs found in FM were palmitic acid (C16:0), docosahexaenoic acid (C22:6), dihomo-linoleic acid (C20:3), and oleic acid (C18:1c). In contrast, FM exhibited a low concentration (<1%) of several other FAs, including arachidic acid (C20:0), heptadecanoic acid (C17:0), pentadecanoic acid (C15:0), heneicosylic acid (C21:0), and linolenic acid (C18:3).

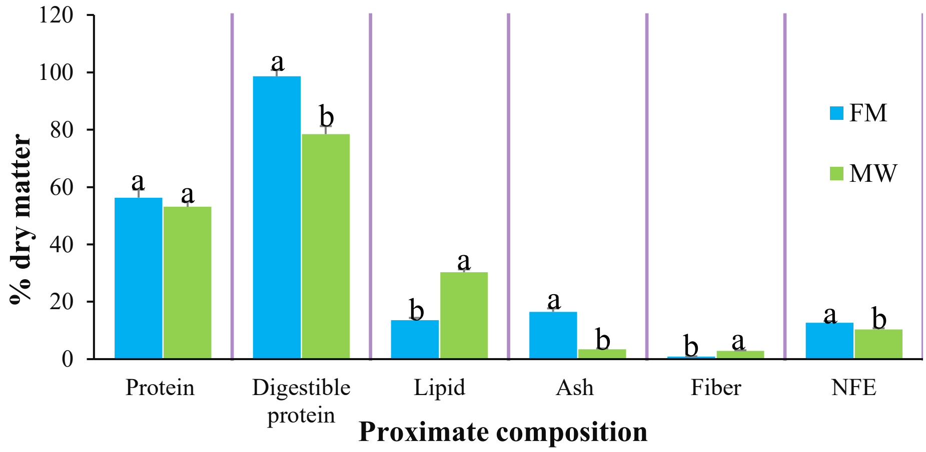

Figure 1: Proximate composition (% dry matter) of fishmeal (FM) and mealworm (MW). Nitrogen-free extracts (NFE) = 100 - (crude protein + crude lipid +fiber + ash).

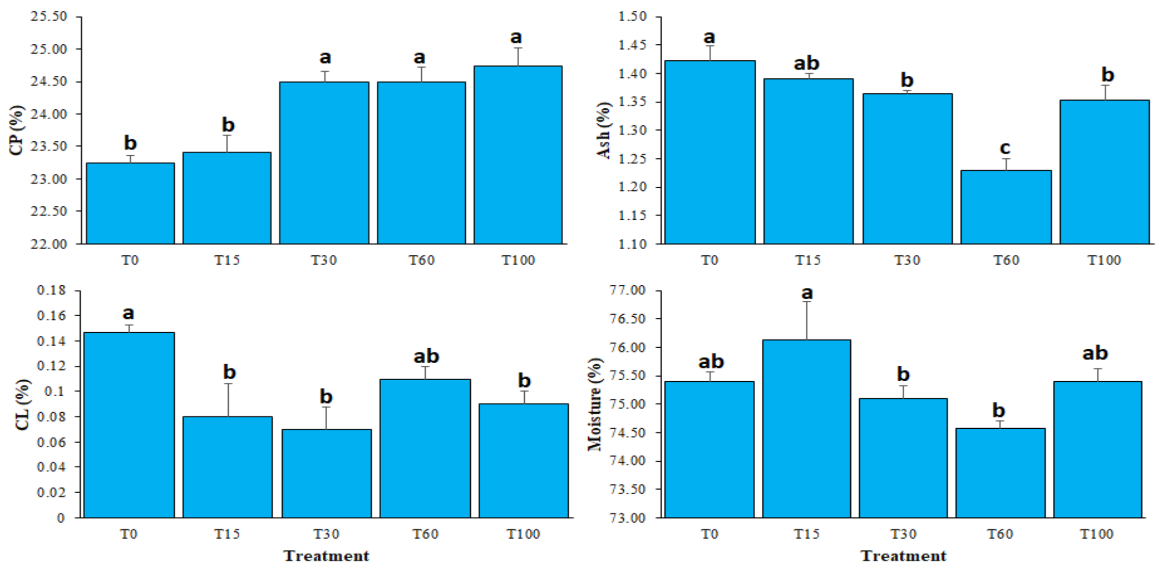

Figure 2: Proximate composition of muscle in Pacific white shrimp (*Litopenaeus vannamei*) that were fed five experimental diets for a duration of 60 days (% wet weight). The values represent the mean of three replicates and are expressed as mean ± standard deviation (SD). Bars marked with different letters indicate statistically significant differences (p<0.05). T_0, T_{15}, T_{30}, T_{60}, and T_{100}, which incorporated FM progressively substituted with MW at varying levels of 0%, 15%, 30%, 60%, and 100%.

Similarly, the predominant FAs identified in MW included palmitic acid (C16:0), oleic acid (C18:1c), linoleic acid (C18:2c), and tricosylic acid (C23:0). Conversely, MW also contained a low percentage (<1%) of various FAs, such as myristoleic acid (C14:1), pentadecanoic acid (C15:0), heptadecanoic acid (C17:0), arachidic acid (C20:0), linolenic acid (C18:3), and eicosenoic acid (C20:1). Notably, MW was devoid of long-chain PUFAs, including EPA (C20:5) and DHA (C22:6). Overall, while both FM and MW share some common FAs, their profiles differ significantly, particularly in the presence of long-chain PUFAs. A total of 16 FAs were detected in the muscle of *L. vannamei* shrimp that were fed five experimental diets, encompassing both essential and non-essential FAs. The contents of EPA and DHA in shrimp muscle exhibited a declining trend as the proportion of FM replaced by MW in the experimental diets increased. The predominant FAs found in shrimp muscle, listed in decreasing order, were palmitic (C16:0), linoleic (C18:2c), oleic (C18:1c), stearic (C18:0), tricosylic (C23:0), and docosahexaenoic (DHA) acids. However, as the proportion of FM substituted with MW increased, the FA composition altered, leading to oleic acid emerging as the predominant FA. The total saturated fatty acid (SFA) content was found to be lowest in the T_{100} treatment and highest in the T_0. The main SFAs identified were palmitic (C16:0), stearic (C18:0), and tricosylic (C23:0) acids, while myristic (C14:0), pentadecanoic (C15:0), and arachidic (C20:0) acids accounted for less than 1% of the total FAs across all groups. In terms of total mono-unsaturated FAs (MUFA), the lowest levels were recorded in T_0, whereas the highest levels were in T_{100}. Oleic acid (C18:1c) was the most abundant MUFA in all groups, while myristoleic acid (C14:1) was only detected in the T_{60} group. The total poly-unsaturated FA (PUFA) content was lowest in T_{100} and highest in T_0. Linoleic acid (C18:2c) and docosahexaenoic acid (C22:6) were the predominant PUFAs across all groups, with the highest relative levels observed in T_0.

Discussion

This study investigates the proximate composition and fatty acid profiles of FM and MW (*T. molitor*), providing essential insights into their potential roles as dietary components for *L. vannamei* shrimp. The findings elucidate the nutritional implications of substituting FM with MW in aquaculture feeds, emphasizing both the benefits and limitations of using IM as an alternative protein source. The comparative analysis of FM and MW revealed notable differences in their nutritional profiles. Although FM exhibited a higher protein content than MW, this difference was not statistically significant (p>0.05). This finding is consistent with previous studies that highlight variability in protein levels among IMs compared to traditional FMs (Huis *et al*., 2013; Makkar *et al*., 2014). While FM is often regarded as a superior protein source due to its high digestibility and amino acid profile, the nutritional potential of MW should not be underestimated, especially in regions where FM is either scarce or economically unfeasible.

In terms of digestibility, FM demonstrated significantly higher levels of digestible protein, ash, and NFE compared to MW ($p<0.05$). The enhanced digestibility of FM can be attributed to its balanced amino acid profile, which is particularly advantageous for aquatic species (Khanjani *et al*., 2024; Naylor *et al*., 2009). Moreover, the increased ash content in FM indicates a higher availability of essential minerals, which are crucial for the growth and health of aquatic organisms (Tacon and Metian, 2008). Minerals such as calcium, phosphorus, and trace elements play vital roles in physiological processes, including bone formation, enzyme function, and immune response (Sharifinia, 2025).

Conversely, MW exhibited significantly higher lipid and fiber contents compared to FM ($p<0.05$). The elevated lipid levels in MW are particularly noteworthy, as they provide essential FAs and energy vital for the growth and reproduction of aquatic species (Syahrulawal *et al*., 2023). Essential FAs, such as omega-3 and omega-6, are crucial for maintaining cellular integrity and supporting metabolic functions (Sargent *et al*., 1993; Tocher *et al*., 2008). Furthermore, the higher fiber content in MW may positively influence gut health and digestion in fish, potentially enhancing nutrient absorption and overall well-being (Davies, 2019). Fiber can stimulate gut motility and promote the growth of beneficial gut microbiota, which are essential for optimal digestion and nutrient utilization (Davies, 2019). However, the increased fiber levels may also pose challenges regarding digestibility, which could limit the overall nutritional effectiveness of MW when used as a primary feed ingredient (Syahrulawal *et al*., 2023).

The incorporation of MW into shrimp diets resulted in significant changes in the proximate composition of shrimp muscle. Notably, the inclusion of MW at higher levels (T_{30}-T_{100}) resulted in a statistically significant increase in crude protein content in the shrimp muscle compared to the T_0 treatment. This enhancement in protein levels aligns with previous research that highlights the potential of IMs, such as MW, to serve as effective protein sources in aquaculture diets (Khosravi *et al*., 2018; Sánchez-Muros *et al*., 2016). In contrast, the T_0 treatment exhibited a significantly higher percentage of crude lipid in the shrimp muscle than all other treatments. This finding indicates that the traditional FM diet provides a greater lipid content, which is critical for various physiological functions in shrimp, including energy metabolism and cellular integrity (González-Félix *et al*., 2002). The elevated lipid levels observed in T_0 may be attributed to the inherent fat content of FM, which is typically richer in lipids compared to insect-based diets (Gasco *et al*., 2016).

Furthermore, the highest ash content was recorded in the T_0 treatment, which significantly differed from the T_{30}-T_{100} treatments. This result suggests that the mineral composition of shrimp muscle is influenced by the dietary source, with FM contributing to a higher mineral content compared to MW. The variations in ash content may have implications for the overall nutritional profile of the shrimp, as minerals are essential for numerous metabolic processes (Tocher, 2015). These findings highlight the complexities involved in formulating aquaculture diets that balance protein, lipid, and mineral content. While the replacement of FM with MW can effectively enhance protein levels in shrimp muscle, it may also lead to reductions in lipid and ash content. This raises important considerations regarding the nutritional adequacy of diets high in MW, suggesting a potential need for additional lipid sources or mineral supplementation to ensure optimal growth and health in shrimp (Bell *et al*., 2003; Liland *et al*., 2017).

The present study examined the FA composition of *L. vannamei* shrimp muscle in response to varying dietary inclusion levels of MW as a substitute for FM. A total of 16 FAs were detected, encompassing both essential and non-essential FAs. Notably, the results revealed a significant decline in the levels of EPA and DHA in shrimp muscle as the proportion of FM was replaced by MW. This finding highlights the inadequacy of MW in providing these critical PUFAs, which are essential for optimal growth, development, and overall health in aquatic organisms (Sargent *et al*., 1993; Tocher *et al*., 2008).

The predominant FAs identified in shrimp muscle, in decreasing order, were palmitic (C16:0), linoleic (C18:2c), oleic (C18:1c), stearic (C18:0), tricosylic (C23:0), and DHA. The observed shift in FA composition, particularly the emergence of oleic acid as the predominant FA in diets with higher MW inclusion, highlights the influence of dietary composition on the lipid profile of shrimp. Oleic acid, a monounsaturated FA (MUFA), is associated with various health benefits, including anti-inflammatory properties and improved cardiovascular health (Kris-Etherton *et al*., 2002). The increase in oleic acid levels in the T100 treatment suggests that MW may contribute a higher proportion of this beneficial FA compared to FM, which is critical for maintaining the nutritional quality of shrimp muscle.

The total saturated FA (SFA) content was lowest in the T100 treatment and highest in T0. The primary SFAs identified were palmitic (C16:0), stearic (C18:0), and tricosylic (C23:0), while myristic (C14:0), pentadecanoic (C15:0), and arachidic (C20:0) acids contributed minimally to the total FAs. This variation in SFA content aligns with findings from Ouraji *et al*. (2009), who reported that dietary composition significantly affects the SFA profile in shrimp. The reduction in SFA content in the T100 treatment may indicate a favorable shift towards a lipid profile that is less saturated, which is beneficial given the adverse health implications associated with high saturated fat intake in both humans and aquatic organisms (Mozaffarian and Wu, 2011).

In terms of total MUFAs, the lowest levels were recorded in the T0 treatment, while the highest were observed in T100. Oleic acid (C18:1c) was the most abundant MUFA across all groups, with myristoleic acid (C14:1) detected only in the T60 group. The increase in MUFA levels in the T100 treatment supports the notion that MW can enhance the nutritional profile of shrimp diets. This observation is consistent with Sánchez-Muros *et al*. (2016), who demonstrated that IMs can significantly alter the FA composition of shrimp, leading to higher MUFA levels, which are generally considered beneficial for health.

The total PUFAs content was lowest in T100 and highest in T0, with linoleic acid (C18:2c) and DHA (C22:6) being the predominant PUFAs across all groups. The highest relative levels of these PUFAs were observed in T0, indicating that the substitution of FM with MW compromises the availability of essential FAs in shrimp diets. This decline in PUFAs content is particularly concerning, as PUFAs, especially EPA and DHA, are crucial for various physiological functions, including growth, reproduction, and immune response in shrimp (Sargent *et al*., 1993; Tocher *et al*., 2008). The results corroborate the findings of Barroso *et al*. (2017), who emphasized that while IMs can serve as a sustainable protein source, they often lack adequate levels of long-chain PUFAs, necessitating dietary supplementation to meet the nutritional needs of aquatic species.

The observed alterations in FA composition highlight the critical need for careful dietary formulation in aquaculture, particularly when incorporating alternative protein sources like MW. While MW presents a sustainable option for reducing reliance on traditional FM sources, its FA profile does not fully meet the nutritional requirements of *L. vannamei* shrimp. Future research should focus on optimizing dietary strategies that combine MW with other ingredients rich in PUFAs or supplementing diets with marine oils to enhance the overall nutritional quality of shrimp feeds. To address this limitation, several strategies can be implemented: supplementing shrimp diets with marine oils rich in EPA and DHA, fortifying MW with these essential FAs during its rearing by feeding it enriched diets, and exploring alternative insect species that naturally contain higher levels of PUFAs. Additionally, optimizing dietary formulations that combine MW with other ingredients rich in PUFAs and conducting further research into the nutritional profiles of various feed ingredients can enhance the overall quality of shrimp diets.

Conclusions

This study provides valuable insights into the proximate composition and FA profiles of FM and MW, highlighting their respective roles as dietary components for *L. vannamei* shrimp. The analysis revealed that while FM offers a higher protein content and superior digestibility, MW presents a viable alternative protein source, particularly in regions where FM is scarce or economically unfeasible. However, the nutritional potential of MW is tempered by its lower levels of long-chain PUFAs, specifically EPA and DHA, which are critical for the growth, reproduction, and overall health of shrimp. The incorporation of MW into shrimp diets resulted in significant changes in the proximate composition of shrimp muscle, notably an increase in crude protein but a decrease in lipid and mineral content. These findings suggest that while MW can enhance protein levels, it may compromise the overall nutritional adequacy of shrimp diets, necessitating the inclusion of additional lipid sources or mineral supplements to ensure optimal growth and health. The observed shifts in FA profiles, including a decline in essential PUFAs and an increase in MUFAs, further underscore the need for careful dietary formulation when substituting FM with MW. Finally, while MW presents a sustainable alternative to traditional FM in aquaculture, its limitations in providing essential FAs necessitate strategic dietary supplementation. Future research should focus on optimizing dietary formulations that combine MW with lipid-rich ingredients or explore the fortification of MW with long-chain PUFAs to enhance the nutritional quality of shrimp feeds. By addressing these challenges, aquaculture can leverage the benefits of IMs while ensuring the health and productivity of cultured shrimp.

Conflicts of interest

The authors declare no conflict of interest

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