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# *Tenebrio molitor* larvae meal as fish meal alternative: impacts in juvenile narrow-clawed crayfish (*Pontastacus leptodactylus*)

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**Abstract:** A 60-day trial examined replacing fish meal (FM) protein with defatted *Tenebrio molitor* (TM) larvae meal in diets for juvenile narrow-clawed crayfish (*Pontastacus leptodactylus*). Six isonitrogenous and isoenergetic diets were formulated to replace 0%, 20%, 40%, 60%, 80%, or 100% of FM protein with TM meal. The treatments were designated as FM100/TM0, FM80/TM20, FM60/TM40, FM40/TM60, FM20/TM80, and FM0/TM100, respectively, and corresponded to actual dietary TM meal inclusion levels of 0%, 4.45%, 8.90%, 13.35%, 17.80%, and 22.25%, respectively. A total of 600 stage II juvenile crayfish were randomly allocated to six dietary treatments. Each treatment comprised four replicate boxes, with 25 crayfish stocked in each box. Growth performance did not differ significantly among the FM100/TM0, FM80/TM20, and FM60/TM40 treatments ($P > 0.05$). Replacement levels above 40% significantly reduced growth, and the FM0/TM100 treatment resulted in the lowest final weight. Survival did not differ significantly among treatments ($P > 0.05$), although the numerically lowest survival rate was observed in FM0/TM100 (48%). Molting frequency and cheliped injury also did not differ among treatments. Normal hepatopancreatic histology was observed in the FM100/TM0 and FM80/TM20 treatments, while pigment deposition, increased B-cell numbers, degeneration, and necrosis were detected at replacement levels of 40% and above. Intestinal histology remained normal up to 40% replacement, whereas luminal content stasis and significant reductions in villus length were observed at replacement levels of 60% and above. Under the conditions of this 60-day trial, replacement of up to 20% of FM protein with defatted TM meal did not significantly impair growth, survival, or the evaluated intestinal and hepatopancreatic histology.

**Keywords:** Crayfish, fish meal replacement, growth performance, histopathology, *Tenebrio molitor*.

# Balık ununa alternatif olarak Tenebrio molitor larva unu: Jüvenil dar kıstkaçlı kerevitlerdeki (*Pontastacus leptodactylus*) etkileri

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**Öz:** Bu 60 günlük çalışmada, balık ununun (FM) proteininin yağı alınmış *Tenebrio molitor* (TM) larva unu ile ikame edilmesinin, juvenil kerevit (*Pontastacus leptodactylus*) diyetlerindeki etkileri incelenmiştir. Altı izonitrojenik ve izoenerjetik yem, FM proteininin %0, %20, %40, %60, %80 veya %100'ünün TM unu ile ikame edilmesine göre formüle edilmiştir. Deneme grupları sırasıyla FM100/TM0, FM80/TM20, FM60/TM40, FM40/TM60, FM20/TM80 ve FM0/TM100 olarak adlandırılmıştır. Bu ikame düzeyleri, yemlerde sırasıyla %0, %4,45, %8,90, %13,35, %17,80 ve %22,25 gerçek TM unu kullanım oranlarına karşılık gelmektedir. II. evredeki toplam 600 juvenil kerevit, altı deneme grubuna rastgele dağıtılmıştır. Her deneme grubu, her birinde 25 kerevit bulunan dört tekerrür kutusundan oluşmuştur. Büyüme performansı FM100/TM0, FM80/TM20 ve FM60/TM40 grupları arasında istatistiksel olarak farklılık göstermemiştir ($P > 0,05$). %40'ın üzerindeki ikame düzeyleri büyümeyi önemli ölçüde azaltmış ve en düşük son ağırlık FM0/TM100 grubunda belirlenmiştir. Yaşama oranı gruplar arasında istatistiksel olarak farklılık göstermemiştir ($P > 0,05$); bununla birlikte, sayısal olarak en düşük yaşama oranı FM0/TM100 grubunda belirlenmiştir (%48). Kabuk değiştirme sıklığı ve kıskaç yaralanmaları da gruplar arasında farklılık göstermemiştir. FM100/TM0 ve FM80/TM20 gruplarında hepatopankreas histolojisi normal bulunurken, %40 ve üzerindeki ikame düzeylerinde pigment birikimi, B hücresi sayısında artış, dejenerasyon ve nekroz belirlenmiştir. Bağırsak histolojisi %40 ikame düzeyine kadar normal bulunurken, %60 ve üzerindeki ikame düzeylerinde bağırsak lümeninde içerik stazı ve villus uzunluğunda önemli azalmalar belirlenmiştir. Bu 60 günlük denemenin koşulları altında, BU proteininin yağı alınmış TM unu ile %20'ye kadar ikame edilmesi büyümeyi, yaşama oranını veya değerlendirilen bağırsak ve hepatopankreas histolojisini önemli ölçüde olumsuz etkilememiştir.

**Anahtar Kelimeler:** Balık unu ikamesi, büyüme performansı, histopatoloji, kerevit, *Tenebrio molitor*.

## INTRODUCTION

Crayfish aquaculture has attracted increasing attention in recent decades due to its high market value, nutritional quality, and rising demand in both local and international markets. Species such *as Procambarus clarkii, Cherax quadricarinatus*, and *Pontastacus leptodactylus* are among the most cultured crayfish globally. China dominates global crayfish production, contributing over 90% of the total output, while Europe, the United States, and Australia also make notable contributions (Chen et al., 2026). Crayfish are typically farmed in ponds, rice fields, or integrated aquaculture systems, reflecting their adaptability and omnivorous feeding habits. However, natural stocks of native *P. leptodactylus* populations have declined due to pollution, diseases, overfishing, climate change, and competition from invasive species (Harlıoğlu, 2024). *P. leptodactylus* is also an economically important species in Türkiye. While annual production reached approximately 5,000 tons before the 1980s, it has declined to around 1,000 tons in recent years (Uzun Gören, 2025). Various commercial feeds containing fishmeal are used in intensive culture to support the decreasing natural stocks of crayfish (Alvanou et al., 2023). However, such intensive aquaculture practices have raised sustainability concerns, particularly regarding feed costs, dependence on fish meal, and the environmental footprint of production (Özdoğan et al., 2026).

Fish meal (FM) is the primary protein source in aquatic feeds. However, rising demand for FM poses a threat to natural fish stocks (Lalander et al., 2015; Mousavi et al., 2020) and has driven up its marked price (Dalsgaard et al., 2009; Wang et al., 2022). Consequently, identifying alternative protein sources has become essential for sustainable industrial aquaculture development (Mastoraki et al., 2020). Commercial crayfish diets are particularly costly, often containing 20–50% fish meals (Qian et al., 2021). Therefore, alternative protein sources need to be looked for crayfish feed (Wang et al., 2022). Plant proteins, such as soybeans used as alternative for FM substitutions in aquatic feeds. However, their limitations, including deficiencies in essential nutrients and amino acids, particularly lysine and methionine, as well as the presence of anti-nutritional factors, can reduce nutrient utilization and feed intake (Gai et al., 2012; Mousavi et al., 2020; Wang et al., 2022). Recently, insects have emerged as a promising alternative protein source for fish meals in aqua culture feeds. Insects reared on organic waste can provide high-quality animal protein while contributing to nutrient recycling and environmentally sustainable production (Sánchez-Muros et al., 2016). Insect larvae meal is recognized as a valuable ingredient because they supply protein, essential amino acids, lipids, carbohydrates, vitamins, and trace elements (Chen et al., 2009). In addition to their nutritional value, insect meals contain substantial levels of bioactive compounds and chitin which may exert antioxidant, antibacterial, and immunostimulatory effects, thereby enhancing the health and immunity of fish and shrimp (Motte et al., 2019; Mousavi et al., 2020). Mealworm TM meal provides a balanced amino acid profile and contains proximately 47- 69% crude protein and 30- 47% crude lipid (Gasco et al., 2016; Katya et al., 2017; Gasco et al., 2023). Numerous studies have investigated the use of TM in fish diets and its effects on growth and health (e.g. Sánchez-Muros et al., 2016; Piccolo et al., 2017; Sankian et al., 2018). Nevertheless, potential drawbacks of insect meals in animal feeds must be considered. High dietary levels of insect meals have been associated with adverse effects, such as reduced villus length and growth suppression in fish (e.g., Dumas et al., 2018; Cardinaletti et al., 2019), as well as increased B-cells prevalence in the hepatopancreas (Zarantoniello et al., 2023) and growth inhibition in decapod crustaceans (He et al., 2022).

TM meal has been reported as a viable protein source for shrimp (Panini et al., 2017; Choi et al., 2018; Feng et al., 2019; Motte et al., 2019; Cai et al., 2022; He et al., 2022) and for crayfish (Mazlum et al., 2021; Wang et al., 2022). As in fish culture, the economic sustainability of crayfish aquaculture necessitates the search for alternative protein sources to replace fish meal. Therefore, the present study investigated the effects of graded replacement of FM protein with defatted TM larva meal in the diets for juveniles *P. leptodactylus*, with particular emphasis on growth performance, survival, molting, cheliped injury, hepatopancreatic histology, and intestinal morphometry.

## MATERIAL AND METHOD

***Ethical Statement:*** In this study, the principles of the Local Ethics Committee were adhered to. Since the narrow-clawed crayfish (*P. leptodactylus*) is an invertebrate species, a formal ethics committee approval certificate was not required under current regulations; however, all experimental procedures were conducted in strict compliance with animal welfare and ethical guidelines.

***Diet preparation:*** Defatting of TM larvae meal was performed using a modified solvent-extraction procedure based on Ido et al. (2019). Briefly, the ground meal was mixed with ether, the lipid-containing solvent was removed, and the defatted meal was dried before diet preparation. The formulations of experimental diets are presented in Table 1. Six diets were formulated by replacing 0%, 20%, 40%, 60%, 80%, or 100% of FM protein with defatted TM meal. The treatments were designated as FM100/TM0, FM80/TM20, FM60/TM40, FM40/TM60, FM20/TM80, and FM0/TM100, respectively. These treatment codes indicate

the relative percentages of FM protein retained and replaced by TM meals, respectively. The replacement levels corresponded to actual dietary TM meal inclusion levels of 0%, 4.45%, 8.90%, 13.35%, 17.80%, and 22.25%, respectively. The diets were formulated to be approximately isonitrogenous (38.00%–38.42% crude protein) and isoenergetic (calculated gross energy: 20.79–20.83 MJ $kg^{-1}$). The diets were subsequently pelleted by extrusion through a meat grinder fitted with a 1 mm diameter disc.

**Table 1.** Formulation and chemical composition of experimental diets (g/100 g).

| Ingredients | Defated TM meal | FM100/TM0 | FM80/TM20 | FM60/TM40 | FM40/TM60 | FM20/TM80 | FM0/TM100 |
|---|---|---|---|---|---|---|---|
| *Tenebrio molitor* | | 0 | 4.45 | 8.9 | 13.35 | 17.8 | 22.25 |
| Fish meal | | 25 | 20 | 15 | 10 | 5 | 0 |
| Soybean meal | | 23 | 23 | 23 | 23 | 23 | 23 |
| Corn starch | | 18 | 18 | 18 | 18 | 18 | 18 |
| Wheat meal | | 23 | 23.75 | 24.48 | 25.22 | 25.96 | 26.7 |
| Fish oil | | 8 | 7.8 | 7.62 | 7.43 | 7.24 | 7.05 |
| Vitamin mix[1] | | 1 | 1 | 1 | 1 | 1 | 1 |
| Mineral mix[2] | | 1 | 1 | 1 | 1 | 1 | 1 |
| Pellet binder | | 1 | 1 | 1 | 1 | 1 | 1 |
| Chemical analyses | | | | | | | |
| Crude protein (%) | 67.46 | 38.00 | 38.08 | 38.16 | 38.25 | 38.34 | 38.42 |
| Crude lipid (%) | 8.42 | 11.051 | 10.79 | 10.55 | 10.29 | 10.04 | 9.79 |
| Crude ash (%) | 6.23 | 7.57 | 7.23 | 6.89 | 6.56 | 6.22 | 5.88 |
| Gross energy Mj $kg^{-1}$ | 22.32 | 20.79 | 20.80 | 20.81 | 20.81 | 20.82 | 20.83 |

[1] Vitamin premix per kg: 4000.000 IU vitamin A; 480.000 IU vitamin D3; 40.000 mg vitamin E; 2400 mg vitamin K3; 4000 mg vitamin B1; 6000 mg vitamin B2; 40.000 mg niacin; 10.000 mg calcium D-pantothenate; 4000 mg vitamin B6; 10 mg vitamin B12; 100 mg D-biotin; 1200 mg folic acid; 40.000 mg vitamin C; 60.000 mg inositol.
[2] Mineral premix per kg: 23.750 mg Mn; 75.000 mg Zn; 5000 mg Cu; 2000 mg Co; 2750 mg I; 100 mg Se; 200.000 mg Mg, GE (MJ/kg) = (Protein x 0.236)+ (Lipid x 0.395)+( Carbohydrate x 0.172).

***Experimental design:*** Crayfish juveniles were obtained through artificial incubation at the Eğirdir Fisheries Research Institute (Isparta, Türkiye). Before random allocation, all 600 stage II juvenile crayfish were individually weighed and measured. The overall initial body weight and total length of the experimental population were 0.033 ± 0.003 g and 1.051 ± 0.045 cm, respectively. These values represent the overall initial population and not separate treatment-specific means. The juveniles were randomly allocated to six dietary treatments: FM100/TM0, FM80/TM20, FM60/TM40, FM40/TM60, FM20/TM80, and FM0/TM100. Each treatment comprised four replicate rearing boxes, resulting in 24 experimental units, with 25 juveniles stocked in each box. To minimize cannibalism, three perforated bricks (18 x 10 x 8 cm) and plastic pipes (1.5 x 4 cm) were provided as shelters in each unit. The experiment was conducted for 60 days in a recirculating aquaculture system (RAS) equipped with mechanical and biological filtration, a UV reactor, heating system, blower equipment and ozone disinfection. The system, which has a tank capacity of 186$m^3$, provides a water flow rate of 14L/s and a water change of 6.5 times daily. Water temperature was maintained at 21±1 °C and dissolved oxygen rate from 8.44 to 10.4 mg/$L^{-1}$. Crayfish were hand- fed *ad libitum* twice daily (8:30 and 20:30) under a 12 h light / 12 h dark photoperiod. Because the diets were offered *ad libitum* and uneaten feed could not be quantitatively recovered, feed intake was not determined with sufficient accuracy to calculate feed conversion ratio or protein efficiency ratio. Furthermore, the experiment did not include an inert digestibility marker or quantitative fecal collection; therefore, apparent digestibility coefficients were not determined. Experimental units were cleaned every other day, and the residual feces were siphoned out.

***Growth parameters:*** At the beginning and end of the trial, all crayfish in each replicate rearing box were individually weighed using a precision balance (RADWAG AS 220.R2), and their total lengths were measured using a digital caliper (Mitutoyo CD-15APX). Individual measurements were averaged within each replicate box before the calculation and statistical analysis of growth variables. Weight gain and Specific Growth Rate (SGR) were calculated separately for each box using the box-specific mean initial and final body weights. Survival and cheliped injury rates were also calculated separately for each box, while molting events were recorded at the box level. Growth performance parameters were calculated according to the following formulas (Yu et al., 2020; Mazlum & Uzun, 2022).

Weight gain (WG) g = (final body weight (g) - initial body weight (g))

Specific growth rate (SGR, % $day^{-1}$) = (ln final body weight - ln initial body weight) / experiment days x 100

Survival rate (SR, %) = (Nf / Ni) × 100

where Nf is the number of surviving crayfish in each replicate box at the end of the trial, and Ni is the number of crayfish initially stocked in the same box.

Single cheliped injury rate at the end of the trial (%) = 100 x (Single cheliped injury crayfish numbers / The number of crayfish)

Double cheliped injury rate at the end of the trial (%) =100 x (Double cheliped injury crayfish numbers / The number of crayfish)

Total molting number during the experiment = molting numbers

***Histopathological* examination:** At the end of the trial, four crayfish were randomly sampled from each replicate rearing box (16 individuals per treatment),

euthanized, and their whole bodies were fixed in 10% neutral buffered formaldehyde solution. After two days of fixation, each crayfish was divided into 3 parts from head to tail and placed into tissue processing cassettes. Samples were routinely processed, embedded in paraffin, and sectioned at 5 µm using a rotary microtome (Leica RM 2155; Leica Microsystem, Nussloch, Germany). Sections were stained with hematoxylin-eosin (HE) and examined under a light microscope (Olympus Corporation, Tokyo, Japan). One representative longitudinal section and one representative transverse section were examined per individual. The hepatopancreas and entire gastrointestinal tract were evaluated for histopathological alterations in a blinded manner. For intestinal morphometry, at least 10 well-oriented villi from 10 microscopic fields per crayfish were measured using a ×40 objective. Individual measurements were averaged within each replicate box before statistical analysis. Morphometric measurements and microphotography were performed using the CellSens Life Science Imaging Software System (Olympus Corporation, Tokyo, Japan).

***Statistical analysis:*** The replicate rearing box was considered the experimental unit, with four replicate boxes per dietary treatment (n = 4). Individual measurements were averaged within each box before statistical analysis. Survival and cheliped injury rates were calculated separately for each box, and molting events were recorded at the box level. Data were tested for normality using the Shapiro–Wilk test and for homogeneity of variances using Levene's test. When these assumptions were met, differences among dietary treatments were evaluated using one-way analysis of variance (ANOVA), followed by Duncan's multiple-range test. When ANOVA assumptions were not met, the Kruskal–Wallis test was used, followed by Dunn's pairwise-comparison test with Bonferroni correction. Statistical significance was accepted at $P < 0.05$. Analyses were performed using IBM SPSS Statistics, version 20 (IBM Corp., Armonk, NY, USA), and results are presented as mean ± standard error (SE).

## RESULTS

### *Growth performance*

Growth performance results are presented in Table 2. Final body weight, final total length, weight gain and SGR differed significantly among treatments ($P < 0.05$), whereas survival did not differ significantly ($P > 0.05$). Final body weight, weight gain, and SGR did not differ significantly among the FM100/TM0, FM80/TM20, and FM60/TM40 treatments ($P > 0.05$). Replacement levels of 60% and above reduced growth, with the lowest values recorded in FM0/TM100. Although the numerically lowest survival rate was recorded in FM0/TM100 (48%), survival did not differ significantly among treatments ($P > 0.05$). Cheliped injury rates and total molting number did not differ among treatments ($P > 0.05$).

**Table 2.** Growth parameters of crayfish fed the experimental diets (mean ± SE, n = 4 replicate boxes per treatment).

| | FM100/TM0 | FM80/TM20 | FM60/TM40 | FM40/TM60 | FM20/TM80 | FM0/TM100 | df | F | *P* |
|---|---|---|---|---|---|---|---|---|---|
| Initial mean weight (g) | 0.033±0.003 | | | | | | | | |
| Initial mean length(cm) | 1.051±0.045 | | | | | | | | |
| Final mean weight (g) | 0.42±0.03$^{a}$ | 0.36±0.02$^{ab}$ | 0.34±0.02$^{ab}$ | 0.32±0.02$^{bc}$ | 0.28±0.01$^{bc}$ | 0.24±0.02$^{c}$ | 5 | 8.21 | 0.00 |
| Final mean length (cm) | 2.677±0.06 | 2.522±0.05 | 2.538±0.05 | 2.455±0.05 | 2.343±0.06 | 2.241±0.05 | 5 | 9.51 | 0.00 |
| Weight gain (g) | 0.39±0.04$^{a}$ | 0.32±0.01$^{ab}$ | 0.31±0.01$^{ab}$ | 0.30±0.02$^{bc}$ | 0.25±0.01$^{bc}$ | 0.21±0.00$^{c}$ | 5 | 8.72 | 0.00 |
| SGR (% day$^{-1}$) | 4.23±0.15$^{a}$ | 3.96±0.06$^{ab}$ | 3.90±0.06$^{ab}$ | 3.78±0.11$^{b}$ | 3.60±0.07$^{bc}$ | 3.34±0.04$^{c}$ | 5 | 12.02 | 0.00 |
| Single cheliped injury (%) | 20.06±4.80 | 17.09±4.90 | 20.76±4.21 | 23.36±6.27 | 22.58±4.06 | 17.79±9.31 | 5 | 0.18 | 0.97 |
| Double cheliped injury (%) | 1.56±1.56 | 2.86±1.65 | 3.59±2.08 | 2.08±2.08 | 1.31±1.31 | 6.25±3.99 | 5 | 0.63 | 0.68 |
| Total molting number during the experiment | 9.25±1.37 | 10.50±1.94 | 9.25±2.50 | 11.25±2.36 | 8.75±0.80 | 8.00±1.22 | 5 | 0.42 | 0.83 |
| Survival rate (%) | 65.00±5.25 | 69.00±1.91 | 63.00±4.43 | 63.00±7.72 | 65.00±3.78 | 48.00±3.27 | 5 | 2.34 | 0.08 |

* Initial body weight and total length represent the overall mean ± SD of the 600 juvenile crayfish measured before random allocation. All remaining values are presented as mean ± SE of four replicate boxes per treatment. Different superscript letters within the same row indicate significant differences among treatments ($P < 0.05$).

***Histopathological findings:*** Histopathological evaluation of the hepatopancreas revealed that B-cells contained a large vacuole, R-cells were characterized by small vacuoles and F-cells displayed a basophilic, non-vacuolated appearance within the epithelial tubule cells of normal tissue. Normal hepatopancreas histology was observed in the FM100/TM0 and FM80/TM20 treatments. At FM protein replacement levels of 40% and above (FM60/TM40, FM40/TM60, FM20/TM80, and FM0/TM100), pigment deposits were evident in hepatopancreatic cells, accompanied by an increase in B-cell numbers. Degeneration and necrosis of hepatopancreatic cells were also observed in these groups. While tissue architecture remained regular in FM100/TM0 and FM80/TM20, irregular histological features were apparent from FM60/TM40 onward (Figure 1).

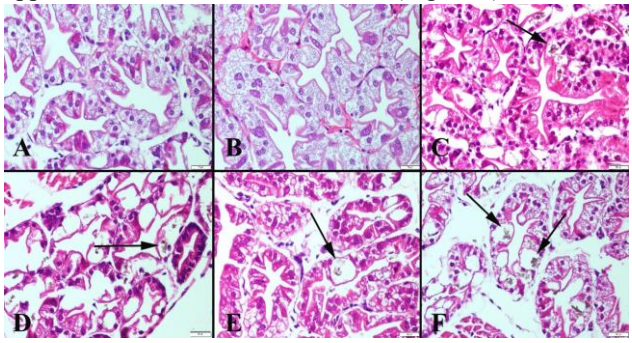


**Figure 1.** Representative histological appearance of the hepatopancreas in juvenile *P. leptodactylus*. (A) FM100/TM0: normal tissue architecture. (B) FM80/TM20: no pathological alterations. (C) FM60/TM40: mild cytoplasmic pigment deposition (arrow). (D) FM40/TM60: moderate pigment accumulation (arrow). (E) FM20/TM80: pronounced

degeneration and increased pigment deposition (arrow). (F) FM0/TM100: extensive pigment-laden cells and marked tissue disruption (arrows). H&E; scale bars = 50 µm.

Normal intestinal histology was observed in FM100/TM0, FM80/TM20, and FM60/TM40. In contrast, FM40/TM60, FM20/TM80, and FM0/TM100 exhibited pronounced luminal content stasis, particularly in the large intestine and its severity increased with increasing FM protein replacement. Additionally, a significant reduction in intestinal villus length was detected in these groups (Figure 2, Table 3).

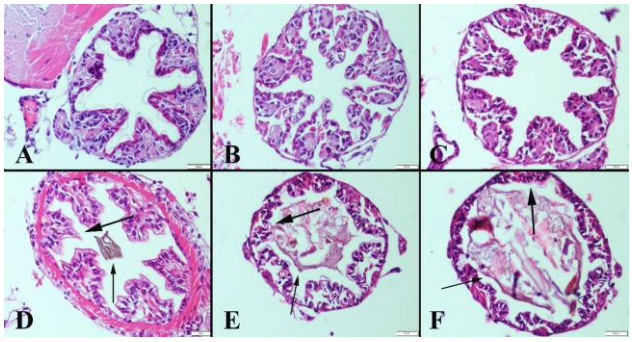


**Figure 2.** Representative intestinal histology of juvenile *P. leptodactylus*. (A) FM100/TM0: normal tissue architecture. (B) FM80/TM20: no pathological alterations. (C) FM60/TM40: normal histology. (D) FM40/TM60: luminal content accumulation (thin arrow) and reduced villus length (thick arrow). (E) FM20/TM80: increased luminal content (thin arrow) and markedly shortened villi (thick arrow). (F) FM0/TM100: extensive luminal content accumulation (thin arrow) and severely reduced villus length (thick arrow). H&E; scale bars = 50 µm.

**Table 3.** Intestinal villus length and width in juvenile crayfish fed the experimental diets (mean ± SE, n = 4 replicate boxes per treatment).

| | Length (µm) | Width (µm) |
|---|---|---|
| FM100/TM0 | 114.33±5.13$^{a}$ | 126.00±7.93 |
| FM80/TM20 | 120.00±1.00$^{a}$ | 126.33±7.09 |
| FM60/TM40 | 111.00±9.53$^{a}$ | 130.33±6.11 |
| FM40/TM60 | 77.66±5.50$^{b}$ | 125.33±5.50 |
| FM20/TM80 | 60.66±5.50$^{c}$ | 129.00±2.64 |
| FM0/TM100 | 25.66±4.04$^{d}$ | 128.66±5.68 |
| p value | <0.001 | >0.05 |

* Values are presented as mean ± SE of four replicate boxes per treatment. Values within the same column sharing a common superscript letter are not significantly different, whereas values with different superscript letters differ significantly ($P < 0.05$).

## DISCUSSION

The use of insect meals in aquatic feeds remains under-researched, particularly regarding crayfish growth and health. In the present study, final body weight and SGR in FM80/TM20 and FM60/TM40 did not differ significantly from those in FM100/TM0. Nevertheless, hepatopancreatic alterations were detected from FM60/TM40 onward, although intestinal histology remained normal at 40% FM protein replacement. Considering growth, survival, and the evaluated histological findings together, 20% FM protein replacement was the highest level at which no adverse response was detected under the conditions of this 60-day trial. Chitin has been reported to reduce protein digestibility in aquatic animals (Kroeckel et al., 2012). However, dietary chitin content and nutrient digestibility were not measured in the present study. Therefore, the contribution of chitin to the reduced growth at higher replacement levels could not be determined and should be considered only one of several possible explanations, together with feed intake, nutrient balance, nutrient utilization, and other diet-related factors. Conversely, Mazlum et al. (2021) reported that replacing 50% of fish meal within *P. leptodactylus* diets enhanced growth compared to a control diet containing 27% fish meal, highlighting potential differences related to formulation and inclusion levels. Similarly, Wang et al. (2022) reported that the optimal dietary replacement of FM with TM and BSFL (black soldier fly larvae) meal was 21.9% and 17.1%, respectively, for juvenile *Cherax quadricarinatus*. Choi et al. (2018) found that the replacing 50% of fish meal with TM optimized growth performance in shrimp without any adverse effects. Panini et al. (2017) observed that complete replacement of fish meal with TM in *Litopenaeus vannamei* diets resulted in growth comparable to a control diet containing 20% fish meal (Panini et al., 2017). Conversely, Cai et al. (2022) reported that replacing fish meal with 60 g/kg TM reduced growth performance and nutrient utilization in Pacific white shrimp. Motte et al. (2019) found the highest weight gain and most efficient feed conversion ratio (FCR) in *L. vannamei* when 50% of FM was replaced by TM, compared to a control diet containing 25% fish meal. He et al. (2022) reported optimal growth in *L. vannamei* fed a diet comprising 75% commercial feed and 25% fresh *Hermetia illucens* (BSFL), while *Feng* et al. (2019) demonstrated that 12% TM protein inclusion maximized growth in *Macrobrachium rosenbergii*. These variable outcomes likely reflect differences in TM processing methods, nutritional profiles of experimental diets, feeding duration, and the fish meal content of the control diets. Similarly, Özdoğan (2026) reported that replacement of up to 60% of FM protein with defatted TM meal-maintained growth during the first 88 days in *Chindongo socolofi*, whereas after 170 days only the 20% replacement group remained comparable to the control and higher replacement levels were associated with hepatic and intestinal alterations.

A limitation of the present study is that feed intake, feed conversion ratio, protein efficiency ratio, and apparent digestibility were not determined. Consequently, it was not possible to establish whether the reduced growth observed at higher FM protein replacement levels resulted from reduced feed intake, lower nutrient digestibility or utilization, or other diet-related factors.

***Histomorphology of hepatopancreas and gut:*** In the present study, several pathological changes were observed with increasing replacement of FM protein by TM meal. Pigment deposits were first detected in FM60/TM40 and became more pronounced at higher replacement levels. Degenerated and necrotic

hepatopancreatic cells were observed from FM60/TM40 onward. B-cell numbers were also increased in treatments with higher FM protein replacement levels. Zarantoniello et al. (2023) reported an increase in B cells in the hepatopancreas of prawn fed diets supplemented with BSF larvae, compared to the control diet. Rahimnejad et al. 2019 also observed a higher number of B cells in the hepatopancreas of *L. vannamei* fed diets containing high levels of silkworm (*Bombyx mori* L.) pupae which are rich in chitin.

The hepatopancreas is composed of several cell types, including B cells (vesicular), E cells (undifferentiated), R cells (reabsorption), F cells (fibrillar), and M cells (basal). B-cells are characterized by a single large vesicle (Silva et al., 2018). and are generally considered responsible for intracellular digestion, with ingested material stored in extensive cytoplasmic vacuoles (Ribeiro et al., 2014). They are also implicated in nutrient absorption, digestion, and waste product accumulation, and their numbers have been reported to increase under pathological conditions (Zilli et al., 2003; Silva et al., 2018). In contrast, Vogt (2019) argued that B cells do not play a role in nutrient absorption, storage or metabolization but rather function to clear the tubules of residual material following nutrient reuptake by R-cells. In the present study, an increase in B cells and necrotic areas within the tubules was observed at FM protein replacement levels of 40% and above (FM60/TM40, FM40/TM60, FM20/TM80, and FM0/TM100). The increased B-cell numbers may reflect changes in intracellular digestion or the clearance of residual material at higher replacement levels. Dietary chitin may be one contributing factor, as previously proposed by Özdoğan et al. (2025), but chitin was not measured in the present study and a direct association cannot be established. Alternatively, Zarantoniello et al. (2023) suggested that similar B-cell responses could be associated with differences in dietary lipid composition.

Villus morphology is one of the most frequently analyzed parameters for assessing intestinal health and growth performance (Melenchón et al., 2022). In crayfish, longer intestinal villi enhance nutrient absorption capacity (Sang et al., 2010; Saputra et al., 2019). In the present study, normal intestinal tissue histology was observed in FM100/TM0, FM80/TM20, and FM60/TM40. However, luminal content stasis and a significant reduction in villus length were observed in FM40/TM60, FM20/TM80, and FM0/TM100, indicating a reduced intestinal absorptive surface and potentially lower nutrient absorption (Xu et al., 2003). Previous studies have shown that chitin and its derivatives can impair dietary nutrient absorption in the intestine (Alegbeleye et al., 2012; Li et al., 2016). Nevertheless, the cause of the villus shortening observed in the present study cannot be attributed specifically to chitin because dietary chitin, nutrient digestibility, and intestinal microbiota were not measured. Differences in feed intake, nutrient availability, and the overall composition of the experimental diets may also have contributed to the observed intestinal changes. Similarly, He et al. (2022) reported a significant reduction in intestinal fold height and decreased intestinal muscular thickness in *Litopenaeus vannamei* fed a 100% fresh BSFL diet. Several other studies have also documented reduced villus height in fish fed insect-based diets (Dumas et al., 2018; Cardinaletti et al., 2019; Józefiak et al., 2019). In contrast, Choi et al. (2018) observed no pathological changes in shrimps across different diet groups, and Cai et al. (2022) reported that the replacing 60 g/kg fish meal with TM did not affect intestinal and hepatopancreas histology. These discrepancies may be attributable to differences in feeding duration, crustacean or fish species, or inclusion levels of insect meals. Overall, growth performance and intestinal histology remained comparable to FM100/TM0 at 40% FM protein replacement; however, hepatopancreatic alterations were already evident at this level. Therefore, considering growth, survival, and both evaluated tissues together, replacement of up to 20% of FM protein with defatted TM meal did not produce an adverse response under the conditions of this 60-day trial.

## AND CONCLUSION

Under the conditions of this 60-day trial, replacement of up to 20% of FM protein with defatted TM meal did not significantly impair growth, survival, or the evaluated intestinal and hepatopancreatic histology of juvenile *P. leptodactylus*. Although growth remained comparable to the control at 40% replacement, hepatopancreatic alterations were detected from this level onward. Intestinal content stasis and significant villus shortening were observed at replacement levels of 60% and above, while complete FM protein replacement resulted in the lowest growth performance; survival did not differ significantly among treatments. Therefore, the present findings support the short-term tolerability of 20% FM protein replacement under the tested conditions but do not establish broader dietary safety. Further studies should quantify feed intake, nutrient digestibility, and dietary chitin and evaluate responses over longer feeding periods.

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