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Revisiones / Reviews

Replacement of fishmeal with *Tenebrio molitor* meal in diets for crustaceans: Effects on growth and immune response

Reemplazo de la harina de pescado por harina de *Tenebrio molitor* en dietas para crustáceos: efectos en el crecimiento y la respuesta inmune

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ABSTRACT
The use of fishmeal (FM) as a protein source in aquaculture is one of the main criticisms of this industry. Increasing production more sustainably is a current imperative for this sector. On the other hand, insect farming has become a promising alternative for aquafeeds, since several species can be produced using organic waste. Insects are organisms rich in nutrients, they contain high amounts of energy, and they have a balanced amino acid profile, unlike protein sources of plant origin that are generally deficient in certain essential amino acids. The aim of the manuscript is to present a review of recent research on the replacement of FM by *Tenebrio molitor* larvae meal (TMM), a beetle that has become popular as an alternative in aquaculture diets, and its effects on crustaceans' growth and immune response. Studies indicate that TMM can completely substitute FM in *Litopenaeus vannamei*, while the substitution levels that worked best for *Macrobrachium rosenbergii* and crayfish range from 12 to 50%. No studies were found in Amazonian crustaceans or even in South American freshwater prawns, which opens a window of opportunities for the development of new lines of research.

Keywords: aquaculture; feeding; insects; crustacean; health

RESUMEN
El uso de harina de pescado (FM) como fuente proteica en la acuicultura es una de las principales críticas sobre esta industria. Aumentar la producción de un modo más sostenible es un imperativo actual para este sector. Por otra parte, la cría de insectos se ha tornado en una alternativa prometedora para la alimentación acuícola, ya que varias especies pueden producirse usando residuos orgánicos. Los insectos son organismos ricos en nutrientes, alta energía, y un perfil equilibrado de aminoácidos, a diferencia de las fuentes proteicas vegetales que son deficientes en ciertos aminoácidos esenciales. El objetivo del manuscrito es presentar una revisión de las investigaciones recientes sobre la sustitución de la FM por la harina de larvas de *Tenebrio molitor* (TMM), un coleóptero que se ha vuelto popular como alternativa en dietas acuícolas y sus efectos en el crecimiento y la respuesta inmune en crustáceos. Los estudios indican que la TMM puede sustituir completamente a la FM en *Litopenaeus vannamei*, mientras que los niveles de sustitución que funcionaron mejor para *Macrobrachium rosenbergii* y el cangrejo de río van desde 12 a 50%. No se encontraron estudios en crustáceos amazónicos ni sudamericanos, lo que abre oportunidades para nuevas líneas de investigación.

Palabras clave: acuicultura; alimentación; insectos; crustáceos; salud

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1. INTRODUCTION

According to statistics reported by FAO (2021), the global harvest of crustaceans reached 10.5 million tons in 2019, totaling a sales value of approximately 276.6 billion dollars. In productive terms, the growth rate of crustacean farming has been on average 10% annually in the last two decades, and in 2019 it represented 9% of the global aquaculture harvest (Tacon, 2020). Recent data taken from FAO (2021) and Tacon (2020), reveals that nearly 92% of global crustacean production depends on just six marine species, which are the following: *Litopenaeus vannamei* (5.24 million tons), *Procambarus clarkii* (2.16 million tons), *Eriocheir sinensis* (0.78 million tons), *Penaeus monodon* (0.77 million tons), *Macrobrachium rosenbergii* (0.27 million tons), and *M. nipponense* (0.23 million tons). It is also important to indicate that almost 90% of farmed crustaceans are yielded in Asia, with the People's Republic of China being the nation responsible for producing half of the world's production (Costa-Pierce & Chopin, 2021; FAO, 2021; Naylor et al., 2021).

The increasing dependence on fishmeal for the production of aquafeeds, especially for carnivorous species such as crustaceans, has caused an excessive increase in the operating costs of aquaculture worldwide (Dawood, 2021). This worrying situation may even worsen due to overfishing of the resources from which fishmeal is processed, which is causing a constant decrease in the production of this vital protein feedstuff (Dawood, 2021).

Meals made from insects have become feedstuffs that are gaining a lot of interest in recent years due to their potential as possible protein substitutes for fishmeal (Röthig et al., 2023; Shafique et al., 2021). This is because insects have a high content of nutrients that not only include proteins, but also unsaturated fatty acids, vitamins, fibers, and minerals (Makkar et al., 2014; Smetana et al., 2019).

In this sense, the meal from the larvae of *Tenebrio molitor*, a species of beetle of the Tenebrionidae family, is one of the feeds that has been intensively evaluated as an alternative source of protein in aquaculture diets (Shafique et al., 2021). Currently, there are a good number of studies that have evaluated the potential use of *T. molitor* larvae meal as an alternative source in practical diets for fish (Gasco et al., 2016; Gu et al., 2022; Henry et al., 2018; Hoffmann et al., 2021; Iaconisi et al., 2017; Iaconisi et al., 2018; Ido et al., 2019; Jeong et al., 2020; Melenchón et al., 2021; Rema et al., 2019; Roncarati et al., 2015; Sankian et al., 2018; Su et al., 2017), however; it has not been previously considered as a protein source in crustacean diets, except in *L. vannamei*, on which there are some recent pioneering studies.

The main goal of the manuscript is to present a review of recent research on the replacement of fishmeal (FM) by *Tenebrio molitor* larvae meal (TMM), a beetle that has become popular as an alternative feedstuff for aquaculture diets, and its effects on farmed crustaceans' growth performance and immune response.

2. MATERIAL AND METHODS

The information detailed in this article was obtained from an in-depth review of the scientific literature (scientific articles, review articles, scientific notes, books, and book chapters), existing in the databases available on the Internet. We used the following key words as search terms:

<aquaculture>, <acuicultura>, <aquicultura>, <feed>, <alimentación>, <alimentação>, <insect>, <*Tenebrio molitor*>, <yellow mealworm>, <fishmeal>, <harina de pescado>, <farinha de peixe>, <replacement>, <sustitución>, <substituição>, <crustacean>, <shrimp>, <prawn>, <crab>, <crayfish>, <camarões>, <camarón>, <langostino>, and <cangrejo>.

The period analyzed was limited to the last decade (2014 to 2023). Results from dissertations, theses, or abstracts presented at scientific conferences were not included.

The results list the names of the crustacean species in which studies have been carried out to replace fish meal (FM) with *Tenebrio molitor* larvae meal (TMM), as well as the main details of the methodology used and the most outstanding results of the works found.

3. REVIEW RESULTS

After reviewing the literature, we only found a total of 12 studies published on the topic of this research. The period in which these papers were published ranges from 2017 to 2023. Eight papers (66.67%) were carried out on the White shrimp, *Litopenaeus vannamei*, which is reasonable, since it is the most important crustacean in the global aquaculture industry, with 5.45 million tons harvested in 2019, representing 52% of the crustaceans produced globally (FAO, 2021). The Giant River Prawn, *Macrobrachium rosenbergii*, is another important species for aquaculture, with 272,738 tons harvested in 2019 (FAO, 2021). The remaining three articles were studies done on other crustaceans such as the Red Claw Crayfish, *Cherax quadricarinatus*, Narrow-Clawed Crayfish, *Pontastacus leptodactylus*, and the Baltic Prawn, *Palaemon adspersus*, which do not have the same economic importance as the previous species aforementioned. Table 1 shows the results obtained from the review carried out.

Table 1.

Crustacean species, fish meal replacement level, study duration, feeding frequency (FF), initial body weight (IBW), main results, recommended fishmeal replacement level, and references of 12 studies published on the fish meal replacement with Tenebrio molitor meal in diets for crustaceans: effects on growth performance and immune response

Crustacean Species	FM Replacement Level	Study Duration	FF	IBW (g)	Main Results	Recommended FM Replacement Level	References
Litopenaeus vannamei (White Shrimp)	0, 15, 30, 60, and 100%	60 days	Four times a day	7.41	There was a significant difference in BWG, FE, FCR, and HPI among the treatments (P<0.05). TMM30 treatment showed expressively (P<0.05) higher BWG, FE, and HPI; and significantly (P<0.05) inferior FCR concerning TMM0 treatment. The levels of CHOL, TG, and GLU, showed a decreasing trend with increasing replacement of FM with TMM and showed a significant difference with the TMM0 at high levels of replacement (P<0.05). Outcomes exhibited that replacing FM with TMM had a significant effect on the activities of SOD, PO, LZM, ACP, AKP, and THC in the diets compared to the TMM0 group (P<0.05).	Up to 30% FM replacement.	Sharifinia et al. (2023)
	0, 15, 30, and 45%	66 days	Four times a day	0.42	Dietary TMM30 significantly increased the BWG, WGR, and SGR of shrimps (P<0.05) when compared with TMM0. Enzyme activity of serum AKP, CAT, SOD, proPO, and LZM differed significantly in TMM45 compared with TMM0 (P<0.05), and MDA decreased. Intestinal trypsin meaningfully increased in TMM45 compared with TMM0 (P<0.05).	Between 30 to 45% FM replacement.	Zheng et al. (2023)

0 and 30% dietary inclusion of TMM	28 days	Three times a day	3.04	Results showed that the growth performance of shrimp fed the TMM diet meaningfully diminished compared to that fed the control diet (P<0:05). There was no substantial difference in FE and SR among individuals fed both diets (P>0.05).		Li et al. (2022)
0, 25, 50, 75 and 100%	42 days	Four times a day	4.42	The THC, PC, and PA in the hemolymph were not meaningfully changed (P>0.05) when FM was substituted by TMM. The AGA of *L. vannamei* serum determined against dog erythrocytes was higher in the shrimp group fed TMM25. The absolute and specific activities of trypsin, chymotrypsin, lipase, α-amylase, and the patterns of proteolytic activities were not affected by the dietary treatments. The *L. vannamei* gut bacterial microbiota profile was comparable to the genera originally noticed.	Up to 100% FM replacement.	Ríos et al. (2021)
0 and 10%	65 days	Four times a day	0.17	Shrimp BWG, SGR, FCR, PER, and SR were similar to those fed with the control diet.	Up to 10% FM replacement.	Shin & Lee (2021)
0, 25, 50, 75, and 100%	56 days	To apparent satiation	1.56	Higher FBW, BWG, and best FCR in the TMM50 group. Dietary inclusion of TMM improved the SR when individuals were confronted with *Vibrio parahaemolyticus*. THC and proPO activities were found meaningfully higher when dietary TMM was included in the *L. vannamei* feeding regime, signifying that immunity and disease resistance in shrimp were significantly improved with TMM diets.	Up to 50% FM replacement	Motte et al. (2019)
0, 25, 50 and 100%	60 days	Twice daily	2.39	Higher BWG and SGR and better FCR in fish fed the TMM50 diet. Partly replacement of FM (50%) with TMM led to an up-regulated expression of immune genes, including β-1, 3-	Up to 50% FM replacement.	Choi et al. (2018)

					glucan binding proteins (BGBP), proPO, and crustin, which are the important genes in promoting disease-resistant against white spot syndrome virus (WSSV).		
	0, 25, 50, 75, and 100%	42 days	Four times a day	4.42	BWG, SGR, FI, FCR, SR, and PRR were not affected when FM was replaced by TMM (P>0.05). The BPC of the shrimp body showed no significant differences (P>0.05) between the treatments.	Up to 100% FM replacement	Panini et al. (2017)
Cherax quadricarinatus (Red Claw Crayfish)	0, 9, 18, 27, and 36%	60 days	N.A.	0.20	The highest BWG and lowest FCR were observed in crayfish fed the TMM diet at 18% FM replacement. The maximum SOD activity and the lowermost MDA content were noticed in the hepatopancreas of the individuals fed TMM18	Up to 21.9% FM replacement	Wang et al. (2022)
Pontastacus leptodactylus (Narrow-Clawed Crayfish)	0, 50, and 100%	80 days	Twice daily	0.011	Dietary replacement of FM with up to 50% TMM had positive effects on BWG, SGR, PER, and ANPU, in *P. leptodactylus* (P<0.05).	Up to 50% FM replacement	Mazlum et al. (2021)
Palaemon adspersus (Baltic Prawn)	0 and 30%	60 days	Twice daily	0.49	TMM30 diets yield higher protein and energy content in the prawn muscles. No significant differences were detected in the activities of hepatopancreas' amino acid-catabolizing enzymes.	N.A.	Mastoraki et al. (2020)
Macrobrachium rosenbergii (Giant River Prawn)	0, 4, 8, 12, and 16% dietary inclusion of TMM	70 days	Three times a day	3.26	Dietary inclusion of 12% TMM protein had the greatest effects on growth performance, immunological parameters, and resistance against *L. garvieae* and *A. hydrophila*.	Up to 12% FM replacement	Feng et al. (2019)

Legends: FM (fish meal), TMM (*Tenebrio molitor* meal), FF (feeding frequency), IBW (initial body weight), BWG (body weight gain), WGR (weight gain rate), SGR (specific growth rate), FCR (feed conversion rate), FE (feed efficiency), FI (feed intake), PER (protein efficiency ratio), ANPU (apparent net protein utilization), PRR (protein retention rate), SR (survival rate), HPI (hepatopancreas index), CHOL (cholesterol), TG (triglycerides), GLU (glucose), AKP (alkaline phosphatase), CAT (catalase), SOD (superoxide dismutase), PO (phenoloxidase), LZM (lysozyme), MDA (malondialdehyde), THC (total hemocyte count), PC (protein concentration), proPO (prophenoloxidase activity), AGA (agglutinating activity), THC (total count of hemocytes), ACP (acid phosphatase), BPC (body protein content), N.A. (no data available).

Effects of dietary TMM on crustacean´s growth performance

Through the work carried out, we were able to verify that the inclusion of TMM in practical diets to replace FM produced quite encouraging results in the five species of crustaceans reported in the literature. Thus, Panini et al. (2017), pointed out that the performance of White shrimp was not affected by replacing FM with TMM in the diets that they used. However, they suggested that the use of TMM should be complemented by methionine to meet the White shrimp amino acids needs. Indeed, Sharifinia et al. (2023) concluded that there is strong evidence that TMM can effectively replace FM in the diet of *L. vannamei*, resulting in enhanced growth of this crustacean. Furthermore, Zheng et al. (2023) found no decrease in growth performance or feed utilization due to the dietary treatment with TMM proteins. Consequently, these authors presumed that the entire dietary treatment provided suitable quantities of critical nutrients.

Another preliminary conclusion from this review is that even at low supplementation rates, TMM may be considered as a form of functional feed. For instance, a compound feed comprising ~12 to 25% TMM improved the growth parameters of *L. vannamei*, *C. quadricarinatus*, *P. leptodactylus*, and *M. rosenbergii* (Choi et al., 2018; Motte et al., 2019; Wang et al., 2019 al., 2022; Mazlum et al., 2021; Feng et al., 2019).

As can be seen, most data have been generated for *L. vannamei*. In this species, FM can be fully replaced with TMM without detrimental effects (Panini et al., 2017; Ríos et al., 2021). Also, TMM was able to replace between 12 and 50% of the FM without negative effects in *C. quadricarinatus*, *P. leptodactylus*, and *M. rosenbergii* (Wang et al., 2022; Mazlum et al., 2021; Feng et al., 2019). Methionine seems to be a limiting factor in *L. vannamei* because FM could only be fully replaced with TMM if lysine and methionine supplements were also provided (Motte et al., 2019; Panini et al., 2017; Ríos et al., 2021). Prominently, the total replacement of FM with TMM, at least in White shrimp feeds is probable, if these species-dependent limitations are considered.

Effects of dietary TMM on crustacean´s immune response

Another interesting aspect that has been confirmed in this review is that there is sufficient scientific evidence to assume that the inclusion of TMM to replace FM does not reduce the immune response of crustaceans, but, on the contrary, strengthens it. For instance, the study published by Sharifinia et al. (2023) found that replacing FM with TMM up to 30% in the diet of *L. vannamei* increased the counts of THCs, although this increase was not significant. These findings were those described by Feng et al. (2019) who observed a significant increase in *Macrobrachium rosenbergii* THC-fed diets containing TMM protein in comparison with the control group. According to these authors' findings, the level of LZM enzyme activity exhibited a growing trend with increasing the quantity of replacing FM with TMM up to the level of 60%, which displayed that dietary TMM might expand the response of the inborn immune system of White shrimp. Furthermore, Zheng et al. (2023) found that replacing 30% of FM with TMM increases not only growth performance but also intestinal bacterial diversity. Indeed, these authors pointed out that TMM replacement of <45% FM expands antioxidant capacity and immunity in the serum, as well as intestinal trypsin. In their study, the immune performance of *L. vannamei* still increased in TMM45.

Dietary supplementation of TMM improves the immune responses in White shrimp. For instance, the study of Motte et al. (2019) showed that the immune performance of *L. vannamei* increased as the percentage of TMM replacing FM increased. Also, Ríos et al. (2021), suggest that TMM can act as a feasible substitute for FM, without causing any harmful effects on gut microbiota or the immune system of *L. vannamei*. Besides, Shin & Lee (2021) also revealed that dietary supplementation of the TMM can improve the innate immune responses and antioxidant enzyme activities of *L. vannamei*. Finally, Choi et al (2018) concluded that dietary inclusion of 12% TMM protein had the best effects on growth performance, immunological parameters, and resistance against *Lactococcus garvieae* and *Aeromonas hydrophila*. These authors support the inclusion of TMM protein in functional aquafeeds.

Overall, the findings of this paper suggest that TMM is a promising alternative protein source for this shrimp species, as it enhances both growth performance and the immune system, which only contrasts with the results obtained by Li et al. (2022), which indicates that TMM was less effective for *L. vannamei* compared to the control diet (0% of TMM). Sharifinia et al. (2023) recommend the use of TMM in the diet of farmed shrimps, given its lack of adverse impacts on growth performance and its potential to reduce environmental consequences resulting from its production.

Finally, we can conclude the article by stating that there is ample evidence that dietary TMM confers growth and health advantages to most of the crustaceans found in the analysis. Therefore, TTM can be considered a promising alternative protein source for aquafeeds. On the other side, no studies were found in Amazonian crustaceans or even in South American freshwater prawns, which opens a window of opportunities for the development of new lines of research.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORSHIP CONTRIBUTION

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