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INCLUSION OF GIANT MEALWORM (ZOPHOBAS MORIO) AS AN ALTERNATIVE PROTEIN SOURCE IN DIETS FOR P.VANNAMEI SHRIMP (BOONE, 1931)

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ABSTRACT
This work investigates the inclusion of giant mealworm (Zophobas morio) as an alternative protein source in diets for the shrimp Penaeus vannamei (Boone, 1931). The main objective is to evaluate the effects of this inclusion on the growth performance of shrimp. The methodology used involved the formulation of experimental diets with different levels of inclusion of Zophobas morio and the performance of feeding trials under controlled conditions. The results indicated that the inclusion of giant mealworm can fully replace fishmeal without compromising shrimp performance, as well as presenting additional benefits in terms of health and environmental sustainability. It is concluded that Zophobas morio is a viable and promising alternative for the formulation of shrimp diets, contributing to the diversification of protein sources in aquaculture.

Keywords: Zophobas Morio. Alternative Protein. Penaeus Vannamei.

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INTRODUCTION

Shrimp farming (shrimp farming in captivity) has played an essential role in the supply of animal-based proteins, especially in developing regions such as Asia and Latin America. Since the 1990s, Brazil has stood out in this sector with the introduction of Penaeus vannamei, an exotic species that has advantageous zootechnical characteristics, such as fast growth, high feed efficiency and greater resistance in different environmental conditions (Vinatea, 2004).

The cultivation of this species is today an important mariculture activity (cultivation of aquatic animals in marine and/or coastal environments) in the country and in the world, representing a great source of economic and food sustenance for millions of people (FAO, 2022). However, challenges related to sustainability and food costs are becoming increasingly evident, driving the search for viable and environmentally responsible nutritional alternatives (Oliveira *et al*., 2013).

Feed represents the largest operating cost in shrimp farming, and a large part of this cost is associated with the dependence on inputs such as fish meal and meat meal, traditional sources of animal protein. These sources, although highly nutritious, generate pressures on natural resources, with considerable environmental impacts and supply limitations (Sá *et al*., 2018).

Thus, the development of alternative diets that maintain or improve the zootechnical performance of shrimp, while reducing environmental impacts and costs, has become one of the priorities of aquaculture research (Henry *et al*., 2018). In this context, the use of insects as protein sources has emerged as a promising solution, both for its production efficiency and for its lower environmental impact.

The Food and Agriculture Organization of the United Nations (FAO, 2013) has already highlighted the potential of insects for animal feed, citing benefits such as high biomass conversion, reduction in greenhouse gas emissions, and lower consumption of resources such as water and land.

Tenebrio molitor and *Zophobas morio*, in particular, have gained prominence as sustainable alternatives for the nutrition of aquatic species, due to their balanced nutritional profile and the presence of bioactive compounds that favor the health and growth of organisms (Gasco *et al*., 2016; Rumbos and Athanassiou, 2021). The larva of *Zophobas morio*, known as giant mealworm, has a high protein (46.8%) and lipid (33.6%) content, in addition to containing chitin, a polysaccharide that can benefit the immune

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system of shrimp and improve intestinal health (Araújo *et al*., 2019; Benzertiha *et al*., 2020).

Recent studies show that insect-based diets, such as mealworm, can replace fish meal or meat by up to 100% without compromising specific growth rate (ECR) or feed conversion (C.A), maintaining or even improving performance indices (Shekarabi *et al*., 2021).

However, the effectiveness of this substitution in shrimp diets is still relatively little explored, especially when compared to other aquatic species. Introducing more sustainable and efficient diets can not only reduce production costs but also align shrimp farming with global demands for more responsible and ecologically sustainable food practices.

Biofloc technology is seen as a promising innovation for fish production (Ogello *et al*., 2021). The BFT (*Biofloc Technology*) system improves the efficiency in the use of nutrients, which are constantly recycled and reused by microorganisms in an environment with minimal water exchange (Avnimelech, 2012; Ebeling *et al*., 2006; Nisar *et al.*, 2022). The cultivation of marine shrimp *Penaeus vannamei* using biofloc is extensively researched and has a well-established cultivation pattern

Therefore, the initial studies on biofloc technology focused on the production of the shrimp *Penaeus vannamei* (Burford *et al*., 2004; Wasielesky *et al*., 2006). Since then, a lot of knowledge has been generated about this species. Water quality parameters for the cultivation of this species were defined to optimize production, including temperature (de Souza *et al*., 2016, 2014), salinity, alkalinity and pH (Furtado *et al*., 2011), nitrogenous compounds (Ferreira *et al*., 2020; Xu *et al*., 2020), total suspended solids (Gaona *et al*., 2017; Ray *et al*., 2010) and use of inoculum (Harun *et al*., 2019; Krummenauer *et al*., 2014).

Zootechnical performance parameters are also well documented in the scientific literature, such as growth (Jory *et al*., 2001; Panigrahi *et al*., 2019; Xu *et al*., 2013), survival and feed conversion (Krummenauer *et al*., 2014; Wasielesky *et al*., 2006). The contribution of bioflocs to shrimp nutrition (Burford *et al*., 2004), the composition of flakes along with feed and probiotics (Ferreira *et al*., 2017; Huerta-Rábago *et al*., 2019; Llario *et al*., 2019) recommended for its breeding, providing essential information to establish a well-founded standard for shrimp in biofloc systems.

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Therefore, this study aims to investigate the replacement of meat meal by giant mealworm meal (*Zophobas morio*) in diets for shrimp *Penaeus vannamei*. The main objective of this research is to evaluate the impacts of this substitution on the zootechnical performance of shrimp and on water quality in biofloc systems.

MATERIAL AND METHODS

The experiment was carried out at the Aquaculture Complex belonging to the Federal Institute of Education, Science and Technology of Pará (IFPA), in the municipality of Castanhal, between the months of July and August 2024. A total of 64 shrimp of the species *P. vannamei*, acquired at the Lorenville farm, located in the municipality of Curuçá-Pará, were used, the animals were taken to the aquaculture complex (IFPA) for the acclimatization period for 5 days. After acclimatization, the shrimp were randomly distributed in 16 polypropylene boxes with a useful volume of 20 liters, at a density of 4 shrimps per box, with an initial average weight of 4.76 ± 0.57 g. Each treatment was performed in triplicate, with the replicates properly placed between the boxes to ensure the representativeness of the results. For 30 days, the experiment was carried out in a biofloc system (BFT), a system that allows a high production density and non-renewal of water and constant aeration (Barbosa *et al*. 2017).

An inoculum of bioflocs from cultivation already in progress was used and for ammonia control sugarcane molasses was applied each time the ammonia level reached the concentration of 1 mg/L. The BFT system uses heterotrophic bacteria in its formation, its junction is induced through the carbon ratio: nitrogen from cultivation maintaining the ratio 6:1 (Avnimelech 1999, 2009).

For this study, four experimental diets were formulated, one control using meat meal and three with meat meal replacement by giant mealworm flour (*Z. morio*) at the levels of 25, 50 and 100%. The rations were formulated and produced in the aquaculture complex. The ingredients were weighed, mixed and pelleted. The rations were stored under controlled conditions until the moment of use.

The nutritional composition of the diets was carried out according to the (AOAC, 1995) (Association of Official Agricultural Chemists) to ensure that both were isoprotein (Table 1).

Table 1: Composition of ingredients in the formulation of diets for fattening in a system of bioflocs.

	Treatments			
Composition (%)	**0%**	**25%**	**50 %**	**100 %**
Meat Meal (%)	66	49,5	33	0
Rice F. (%)	4	3	5	2
Corn F. (%)	5	5	3	2
F. of wheat (%)	22	23	23	27
F. of Tenebrio (%)	0	16,5	33	66
Soybean Oil (%)	2	2	2	2
Premix vit min	1	1	1	1
PB feed*	35,43	35,51	35,63	35,97
EB feed (Kcal)*	376,8	413,48	452,11	525,32
EB:PB *	10,64	11,65	12,69	14,61
ED feed (Kcal)*	3542,81	3550,68	3562,66	3596,83

CP-crude protein, EB- crude energy, ED-digestible energy.

The ingredients of the diets were crushed and mixed (as shown in figures A and B). The preparation of the experimental diets began with the weighing of the ingredients and then they were mixed dry and homogenized with the addition of 20% water. Subsequently, all diets went through this process of homogenization and mixing, until their formulation and immediately added in a manual grinder, with a final pellet size of 3.00 mm.

Figure A: Grinding of larvae for diet formulation; Figure B: Mixing of ingredients.

The diets were dried in the sun for a period of 3 days to ensure complete moisture removal. After this process, they were carefully placed in plastic bags to avoid contamination and stored at a constant temperature of 20°C until the moment of use. The centesimal analysis of mealworm meal, which includes the percentage of proteins, carbohydrates, lipids, fibers and ashes, is shown in Table 2**.**

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Table 2: Centesimal composition (based on dry matter (%)) of giant mealworm protein (Z.morio).

Centesimal composition of giant mealworm (*Zophobas morio*) (%).	
Crude Protein (%)	46,8
Dry matter (%)	96,32
Ether Extract (%)	33,6
Ash (%)	2,52
Quintina (%)	4,59
Limestone (%)	0,05
Phosphorus (%)	0,62
Crude Fibre (%)	5,1

Source: Araújo *et al.*, 2019

EXPERIMENTAL CONDITIONS

After distribution in the cultivation units and acclimatization period, the shrimp were fed three times a day (08:00, 14:00 and 18:00), the diets initially fed in an amount corresponding to 6% of the animals' biomass (Jory, 2001), readjusted weekly according to the biometrics. The parameters of water qualities such as: dissolved oxygen - DO, pH, temperature and salinity were measured daily with the AKSO multiparameter probe (AK88v2).

The analyses of alkalinity, ammonia (NH_3), nitrite (NO_2) and nitrate (NO_3) were measured twice a week using the rapid analysis kits (Aquality, LabconTest and CHECKER). During the experiment there was no water exchange due to adaptation to the biofloc system (BFT) and for the control of ammonia concentration sugarcane molasses was used as a carbon source.

ZOOTECHNICAL PERFORMANCE OF CAMEROON

Shrimp biometrics were performed weekly (Figures C and D) with the help of a digital scale (BEL engineering) to monitor the weight of the animals and adjust the amount of feed. After the 4 weeks of experiment in a biofloc system, the following zootechnical parameters were analyzed: final biomass, protein efficiency rate (TEP), specific growth rate (TCE), weight gain (g), feed conversion and survival.

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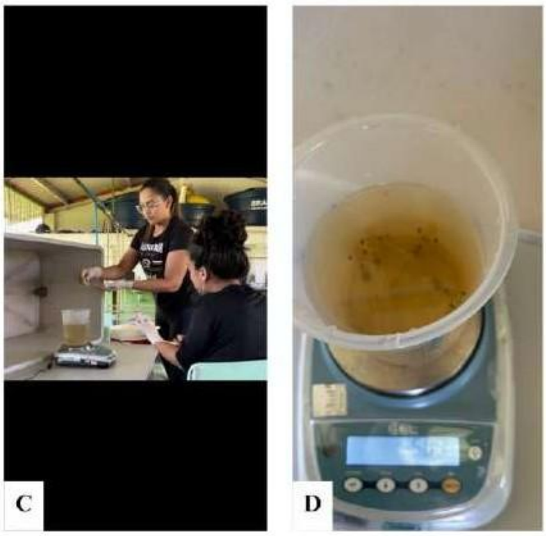
Figure C and D: Realization of shrimp biometrics.



STATISTICAL ANALYSIS

The zootechnical performance data of the shrimp were analyzed using Analysis of Variance (ANOVA) to determine the existence of significant differences between the experimental groups. To identify which groups had specific differences, multiple comparisons were performed using Tukey's test. The level of significance adopted for all analyses was 5%.

RESULTS AND DISCUSSION

The parameters of water quality in the treatments and other aspects remained at favorable levels, with no significant variations between them, table 3 (Marques and Andreatta (1998)).

Table 3: Water quality parameters measured in the experimental units in a biofloc system.

Analysis of water quality parameters in the experimental units (± standard deviation).				
Parameters	0%	25%	50%	100%
Temperature (°C)	27.41±0.51	27.16±1.27	27.34±0.53	27.27±0.49
O.D(mg/L)	5.91±0.22	5.94±0.25	5.88±0.22	5.88±0.22
ph	8.26±0.99	8.30±0.68	8.30±0.87	8.24±0.17
Salt (ppm)	17.75±1.28	18.90±1.21	18.46±1.18	18.45±1.20
Total alkalinity (mg CaCO3/L)	116.70±43.3	120.8±54.3	128.3±63.62	125± 44.51
N-NH3 (mg/L)	0.81±0.72	0.70±0.62	0.80±0.73	0.83±0.85
N-NO2 (mg/L)	2.14±0.65	1.70±0.96	1.83±0.70	1.78±0.76
N-NO3 (mg/L)	50	50	50	50

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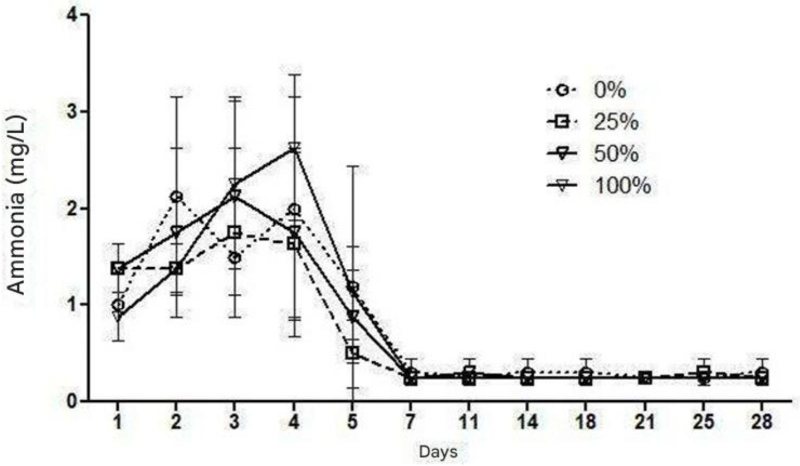
The concentration of Dissolved Oxygen remained between 5.2 – 6.5 mg/l in all treatments. The temperature varied between 26.5 – 28.5°c, the pH (8.0 – 8.5 mg/l) and Salinity between 17-20 ppm, the nitrogen ones such as total ammonia, nitrite and nitrate obtained values between 0.70 – 0.83 mg/L; 1.70-2.14 mg/L and 50 mg/L respectively, and the alkalinity remained above 100 mg/L during the experiment. All parameters evaluated remained within the ideal range and did not influence the performance of the animals (Boyd, 1984; Vinatea, 2004, Gadelha et al., 2009).

Ammonia is a critical parameter in aquaculture as it can cause stress and mortality in aquatic organisms (Wasielesky et al., 2006). In biofloc systems, ammonia is converted to nitrite and later to nitrate by microbial activity, thereby reducing the risk of toxicity to cultured organisms (Avnimelech et al., 1994).

However, the control of ammonia in biofloc systems requires careful management of pH, temperature and nutrients, as variations in these parameters can affect the efficiency of ammonia conversion (Ebeling et al., 2006). Therefore, the ammonia levels do not present significant differences between the treatments adopted (Graph 1).

Graph 1: Ammonia concentration throughout the experiment.



Tenebrio flour contains a higher amount of lipids, especially unsaturated fatty acids. These acids are essential for energy synthesis and aid in the development of cell membranes, directly influencing the growth and survival rate of shrimp (Henry *et al*., 2018). In addition, these fatty acids have a higher amount in mealworm flour compared to meat meal, making them essential for proper energy metabolism. The faster metabolism of high-quality lipids releases energy more efficiently, which can be directed towards body growth.

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Therefore, these acids are essential for the healthy development of shrimp, positively impacting the specific growth rate (TCE). Studies indicate that diets containing mealworm flour can raise TBI to values above 2.5% per day, while diets with high levels of beef meal tend to have a lower TCE due to lower digestibility and high concentration of saturated fats (Makkar *et al*., 2014).

In this study, feed conversion was also more efficient with the use of giant mealworm. Van Huis (2017) found similar results when obtaining an AC of approximately 1.2-1.4 in diets based on giant mealworm, compared to values higher than 1.5 with meat meal, showing that shrimp can more efficiently convert the protein ingested into body mass, which results in lower feed costs.

In a similar study, Sá *et al*. (2018), when working with the replacement of meat meal by fish meal, observed that the inclusion of up to 20-30% of meat meal in the diet maintained an efficient feed conversion (FC), with values between 1.3-1.5, in addition to an average weight gain of 30-35 g in 60-day cycles.

However, it was found that the increase of more than 30% of this ingredient in the diet compromised the CA and TCE of the animals, possibly due to the lower digestibility and lower amino acid profile of meat meal and high levels of saturated fats and ashes compared to fishmeal, which can negatively impact feed efficiency (ZHAO *et al*., 2019).

In this work, the inclusion of giant mealworm (*Z. morio*) in the diets of *P. vannamei* shrimp resulted in significant improvements in several zootechnical performance parameters, Table 4. Shrimp fed diets containing mealworm showed a significant increase in weight gain (P < 0.05) compared to animals fed the control diet. In addition, the feed conversion ratio (C.A) was significantly better in the experimental groups, indicating a greater efficiency in the use of nutrients provided by giant mealworm flour.

Table 4: Zootechnical parameters of gray shrimp *Penaeus vannamei* fed diets containing different levels of substitution of protein meal of animal origin by protein meal of giant mealworm (*Z. morio*).

Zootechnical parameters of the replacement of meat meal by giant mealworm protein meal (*Z.morio*).				
	0%	**25%**	**50%**	**100%**
Starting Weight	4,76	4,76	4,76	4,76
Final Weight (g)	7.30±0.94	7.41±0.73	8.51±1.15	10.80±0.81
Final biomass (g)	8,12	9,03	12,3	23,2
Specific growth rate (%/day)	1,09%	1,21%	1,49%	2,56%
Protein Efficiency Rate	0,52	0,57	0,78	1,49
Feed Conversion	5,29	4,78	3,51	1,86
Survival (%)	100%	100%	100%	100%

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Compared to other studies, the research by Sánchez-Muros *et al*. (2014) also found significant improvements in the zootechnical performance of P*. vannamei* shrimp when fed diets containing insect meal. In addition, a recent study by Henry *et al*. (2021) demonstrated that the inclusion of insect meal in shrimp diets resulted in improvements in gut health and pathogen resistance.

These results reinforce the potential of using insect meal as an alternative ingredient in aquaculture diets, with benefits that go beyond zootechnical performance, which also extend to the overall health of shrimp. Gasco *et al*. (2016) also observed results similar to those obtained in this study, with the protein efficiency ratio (TEP) being significantly higher in diets containing mealworm flour, reaching values between 2.0 and 2.3.

On the other hand, the use of meat meal presented lower TEP's, ranging from 1.5 to 1.7. These results indicate a greater capacity for shrimp to use the protein contained in meal, which reinforces its potential as an alternative ingredient in aquaculture diets.

These studies demonstrate that mealworm meal offers a more balanced and suitable nutritional profile for shrimp growth than meat meal, providing better digestibility, protein retention, and gut health. Insects are considered to be very advantageous in terms of sustainability, due to their rapid reproduction, high growth rate, low environmental impact, and efficiency in food conversion.

They are able to develop from different materials, such as organic waste, as well as being a rich nutritional source of protein, fatty acids, and minerals. They can also be consumed in different ways (Nowak *et al*., 2016; Van Huis *et al.,* 2013; Araújo *et al*., 2019).

Rumbos and Athanassiou (2021) highlight that giant mealworm (*Z.morio)* is a promising source of nutrients and anti-microbials, with potential for use in aquaculture and poultry feeds. The production of this insect has a reduced environmental impact, contributing to sustainability. In addition, giant mealworm can be used to reduce waste, offering a viable and sustainable alternative to traditional animal protein production.

Therefore, the use of shrimp diets that do not depend on fishmeal to achieve good zootechnical results emerge as a promising opportunity for producers to associate themselves with new markets that add value to the shrimp produced (Amaya *et al*., 2007; Soares *et al*., 2011).

The final weights of the shrimp submitted to the four treatments were analyzed by ANOVA and multiple comparisons by Tukey's test with a significance level of 5%. ANOVA

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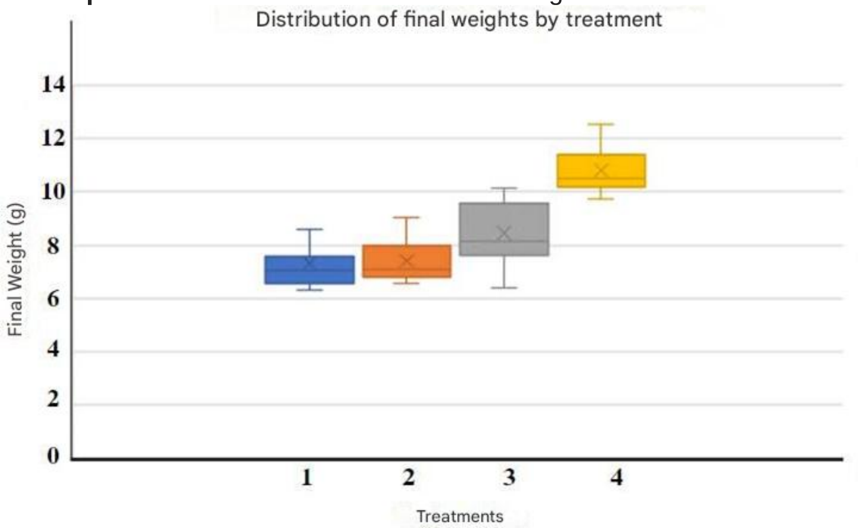
indicated a significant difference between treatments (F=45.83F = 45.83F=45.83, p<0.0001p < 0.0001p<0.0001). Tukey's test revealed that Treatment 4 was significantly superior to the others ($p < 0.05$), while no significant differences were observed between Treatments 1, 2 and 3 (Graph 2).

Graph 2: Differences between the final weights in each treatment.



1=0% treatment; 2=treatment 25%; 3=50% treatment and 4=100% treatment

Although the *post-hoc* analysis did not identify statistically significant differences between Treatments 1, 2 and 3, the data suggest a trend towards improvement in the final weights of the shrimp as the treatments progressed, especially between Treatments 2 and 3. This trend of weight gain, visually observed in the box plot, may indicate a potential optimization in the diets applied to these treatments, although the difference was not statistically significant. Treatment 4, on the other hand, showed a substantially higher weight gain, which reinforces the efficiency of the diet tested in this group and its ability to improve the zootechnical performance of shrimp.

CONCLUSION

The results obtained with the replacement of up to 100% of animal flour with giant mealworm flour were extremely promising. The significant improvements in several aspects, including the nutritional quality and sustainability of the final product. Giant mealworm has been shown to be a viable and efficient alternative, providing benefits for both the animals and the environment. These findings pave the way for future research

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and industrial applications, highlighting the innovative potential of this substitution in the food sector.

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