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MEALWORM (TENEBRIO MOLITOR) AS AN ECO-FRIENDLY FEED SOURCE TO IMPROVE BONE HEALTH AND AMINO ACID COMPOSITION IN LABEO ROHITA FINGERLINGS

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Abstract
Aquaculture industry is still searching a way to find an alternative to conventional fishmeal as an eco-friendly and sustainable alternative. This study offers an inquiry into the dietary supplement of mealworm (Tenebrio molitor) as an alternative sustainable source of protein in the diet of Labeo rohita fingerlings considering their mineralization of bones and amino acids. The five experimental diets were prepared by adding mealworm meal at 0%, 25%, 50%, 75%, and 100 percent inclusion levels and were tested for eight weeks. After the end of experiment, analysis of fish bone samples was done for calcium, iron and phosphorus content. Moreover, amino acids analysis were conducted using high performance liquid chromatography for all dietary groups. Results of this study indicated significant benefits in bone mineralization of rohu by using mealworms in feed. It was observed that calcium was highest at the inclusion level of 25% with the phosphorus being significantly elevated at the inclusion rates of 50% and 75% which implied a synergistic effect that was beneficial in terms of bone formation in rohu. Iron (Fe) intake was more evident at 75% and 100% inclusion levels, and this meant a better bioavailability. Amino acids analysis showed that the higher the mealworm in the diets showed the more positive outcome on both essential and non-essential amino acids. The 75% inclusion produced ideal levels of essential amino acids such as lysine and methionine that are important in fish. However, 100% replacement resulted in a small decreasing impact on some of the amino acids suggesting that there is an optimum limit for mealworm dietary inclusion and fish meal replacement in rohu. Therefore, a sustainable alternative to fishmeal in L. rohita diet is mealworm that is a eco-friendly as well improves the fish composition. It provides health benefits by strengthening the bones, amino acids composition, and overall fish quality of this commercial and indigenous fish, besides environmental and economic solutions in aquaculture industry.

INTRODUCTION

It is a rapidly growing industry, where the population of the world is getting greatly reliant on aquaculture as the source of protein in the diet. World and aquaculture increasingly is becoming popular as the significant answer to the challenge of meeting the demands of the human population which is increasing in size. Nonetheless, the increased pressure on the growth of the industry has deepened the need of sustainable and economically viable feed ingredients (Makkar 2018). Conventionally, fishmeal has been used as the main (protein) ingredient in fish feeds, owing to the nutritive properties encompassing essential protein and fatty acids, vitamins, and many minerals (Kolawole and Mustapha 2023). However, the rising price, unpredictable supply of the fishmeal, and its impact on the environment caused by overfishing have contributed to the fact that the fishmeal cannot be considered a sustainable solution over the long term(Hall 2010). Similarly, although similar alternative sources of proteins such as soybean meal are less expensive and easy to get, their abuse in the aquafeeds is frequently limited by anti-nutritional aspects and unbalanced amino acid initiations (Gutasi 2021, Serra, Pastorelli et al. 2024). This is not only an eye-opener to the necessity of new and environmentally friendly sources of protein, but it also shows how we are leading ourselves to a path of over dependence and dependency on traditional protein sources.

To meet growing challenges in the aquafeed industry, insects have also been acting as a very potential and sustainable alternative source of protein (Hameed, Majeed et al. 2022). The mealworm (*Tenebrio molitor*) is one of the most promising species among others because of its high protein concentration (47-60 % dry weight), content of a high amount of lipids (31-43 %), as well as its goods profile of amino acids, including large proportions of essential amino acids, like lysine, methionine and threonine(Hong, Han et al. 2020, Kröncke and Benning 2023, Syahrulawal, Torske et al. 2023). Studies have shown that mealworm meal may replace fishmeal by up to 100 per cent without negatively affecting the growth outcome of several fish species, and (Hua 2021, Shafique, Abdel-Latif et al. 2021). Also, more mealworm can be effectively grown on organic waste substrates, which is aligned with circular economy concepts and limits the negative impact on the environment of aquaculture(Moruzzo, Riccioli et al. 2021, Debbarma, Deb et al. 2025) .

The literature already suggested the nutritional value of mealworms as an aquafeed ingredient with some implications of its impact on growth parameters and overall nutrient utilization (Syahrulawal, Torske et al. 2023). In the case of *L. rohita*, one of the main carp species of high economic and nutritional value in South Asian aquaculture, the growth, and to a certain level amino acid composition have been studied as an impact of the use of different protein sources. A crucial role of the necessary amino acids, as methionine and lysine, in the growth and muscle processes of *L. rohita* is already reported (Syahrulawal, Torske et al. 2023). Similarly, the presence of minerals such as calcium, phosphorus, and iron in bone mineralization and skeletal health in fish has been extensively proved where the absorption of minerals by the fish in the diet and water has been highly reported (Khalili Tilami and Sampels 2018, Mmanda 2025). However, no study is reported on how the two factors i.e. bone mineralization (calcium, iron, and phosphorus content) and the whole amino acid profile of the *L. rohita* fingerlings are affected by mealworm (Abd Rahman Jabir 2012). The implication of these synergistic effects is important in designing the best and well-balanced diets, which not only achieve a strong growth but also lead to improved skeletal and general physiological fitness, thus future prospects on sustainable aqua farming.

Hence, this study attempted to explore (in detail) the effects that the various levels of mealworm meal (*Tenebrio molitor*) inclusion in the diet on the bone mineralization (in terms of calcium, iron, and phosphorous content) and on the amino acid composition of *L. rohita* fingerlings. These results will provide important empirical contributions to the scientific community, that the inclusion of mealworms as a sustainable protein, with high density of nutrients, in aqua-feeds. Moreover this will ensure healthier aquaculture species farming and the application of environmental sustainability in the fish production.

Materials and Methods

The work included two experimentally related periods, as each of them was devoted to a specific nutrition result of *L. rohita* fingerlings, but on mealworm (*Tenebrio molitor*) -based diets flavor: bone mineralization, amino acid composition. The experimental work was carried out in the controlled conditions of the Fisheries laboratory, Department of Zoology, University of Okara to ensure consistency, and there could be reduced influence of external factors.

Experimental Design and Fish Husbandry

L. rohita fingerlings were sourced from the Fish Seed Hatchery, Head Balloki, Punjab. Before the experimental trials, fish were immersed into a 5 g/L NaCl solution to remove ectoparasites and then they were acclimatized to the indoor laboratory conditions (two weeks). At this stage of acclimatization, fish were kept in glass aquariums (30-liter capacity of the study concerning amino acids, and 200-liter capacity fiberglass tanks of the study concerning bone mineralization), and the baseline diet was fed on the six days of the week, subjecting them to a sort of acclimatization to apparent satiety. During both experiment phases, fish of the same initial weights were subjected by random transfer to treatment groups.

To explore the assessments of bone mineralization five test diets were prepared using different levels of mealworm (MW) to replace regular fishmeal: a control, a 25% MW, 50% MW, 75% MW, and 100% MW, as source of protein. A total of eight weeks of the experiment was carried out, 15 *L. rohita* fingerlings were stocked in each tank, and three replicates were used in each of the treatment groups in a completely randomized fashion.

In the analysis of amino acid composition 60 days nutrition trial was performed. This set up included five treatment groups and the feed had different levels of mealworms inclusion (0 control, 25, 50, 75, and 100 percent). The three replicates in each treatment group were conducted and the fifteen fish were kept in a rectangular glass (30-liter capacity) aquarium with an average weight of 3-5 grams and a body length of 5.2-5.5 cm. Diets were isonitrogenous and low in calories except the change in protein source, to allow the consistency in nutritional delivery.

During the course of both experiments, fish were fed on each of their diets, twice a day (in the morning and in the mid-afternoon) at a rate of 5 percent body weight per day in an attempt to determine their effect on bone mineralization. To limit the waste of the feed and to preserve the quality of the water, water changes were done after every 30 min during the time of feeding. The bone mineralization trial was done by monitoring the fish weight and the feed intake bi-weekly.

Feed Preparation

Ingredients used in the both studies where grounded into fine particles then were thoroughly mixed and pelleted in preparation of test feeds. Pelleted feeds were dried using dry oven at 60 C to reduce moisture content and likely to improve the storage stability of pelleted feeds. All feed samples were kept in airtight containers in cool dry conditions to avoid contamination and exposure to the air. In the prepared diets, the proximate composition which included protein, fat, ash, total minerals, calcium, iron and phosphorus was measured.

Environmental Conditions

Environmental conditions were closely followed and controlled during the two experiment trials. Temperature of water was maintained at 31 C (bone mineralization) and the range of temperatures under *L. rohita* (amino acid study) using a BOMATA weather proof IPX7 thermometer. The relative humidity was always kept at 64 percent (bone mineralization). Dissolved oxygen (DO) level was kept constant throughout (amino acid study) and measured every day (both studies) with the aid of an electronic dissolved oxygen meter. PH of the water was rechecked daily with PH paper (amino acid study) and adjusted. Periodical cleaning of tanks occurred and water changed regularly so that the sanitary conditions of the rearing were the best.

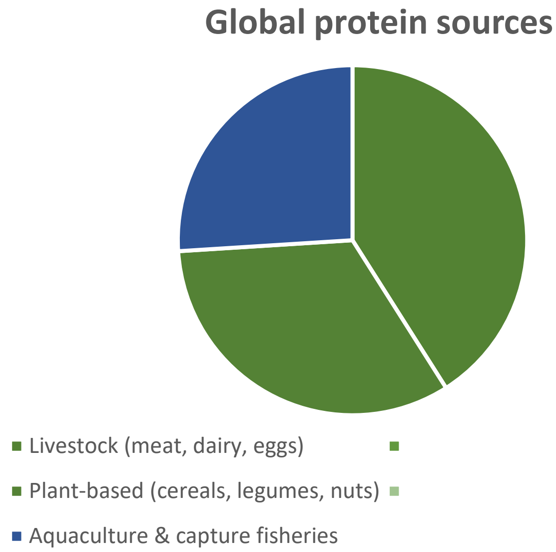

Figure S1: World aquaculture protein reliance infographic.

Mineral Content Analysis

The samples of feed formulations at the end of the eight-week feeding trial on bone mineralization were examined regarding the amount of calcium, iron, and phosphorus. Concentration of calcium and iron was measured using the method APHA 3120 B (2022) that includes the digestion of feed samples with nitric acid and their analysis using an atomic absorption spectrophotometer. The measurement of the phosphorus was done by the AOAC 995.11 method (2023) by using a colour-developing reagent to produce a colored complex and the absorbance was read at 410 nm. All the analyses were carried out in three replicates, and the results were calculated as means of concentration in milligrams per kilogram of feed (mg/kg).

Bone Mineralization Assessment

In order to measure the level of bone mineralization towards the end of the eight-week feeding trial, five specimens were randomly chosen from each replicate tank, sacrificed and their vertebrae dissected. Bones were dried at 60 °C in 24 hours to find dry weight and it was then carefully cleaned of the soft tissue. The weight of ash was then ascertained by incinerating the dried bone sample in a muffle furnace at 550°C after a period of one day and the result was calculated as a proportion of ash weight of the dry bone. Also, the ash portion of bone consist of calcium and phosphorus and its percentage was measured with the help of an atomic absorption spectrophotometer. Colorimeter technique was used for calcium and phosphorus content, respectively.

Amino Acid Analysis

Fish samples of both groups were taken after eight weeks of the nutrition trial to determine the differences in the amino acid contents of each group of fish. Kjeldahl method was used to determine the crude protein value. The amino acids and amino acids including the essential and non-essential amino acids were measured using a standard hydrolysis method: 24-hour hydrolysis of muscle protein was done using 6N hydrochloric acid at 110C in an anaerobic condition. The hydrolysed extracts were next neutralized with 6N NaOH and were derivatized by an AccQ-Fluor Reagent kit (Technique 052880, Waters). The derivatized samples were injected in the high-performance liquid chromatography (HPLC) system (1525, Waters) with the C18 RP column and fluorescence detector (2475, Waters). Identification and quantification of amino acids was made based on measurement of their retention times and peak areas as compared to commercial standards (WAT088122, Waters). In case of tryptophan analysis, minced muscle was digested with NaOH (5% w/v) for 24h after which it was neutralized at pH 7.0 by using 6N HCl followed by determination of tryptophan content using spectrophotometer (530 nm). The data of all the amino acids was expressed as mean with standard deviation.

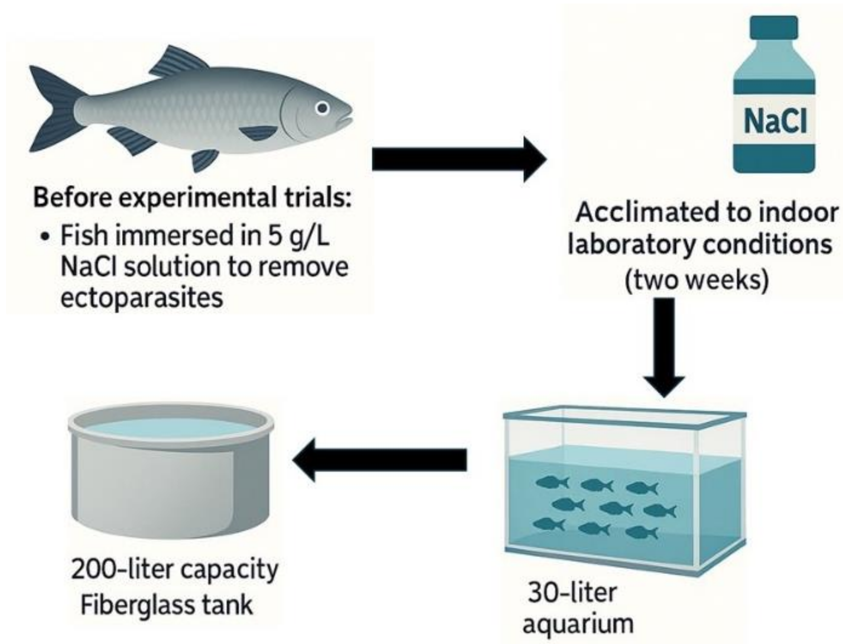

Figure S2: Experimental design flowchart (acclimatization, tanks, feeding).

Statistical Analysis
In the case of bone mineralization data, the T-tests, standard errors of the data (SE), standard deviation of the data (STD) and means of the Calcium, iron and phosphorus data of each diet group were carried out. One-way ANOVA analysis was used to do the comparison of mineral content of feeds at different concentrations of the MW-based feeds. Post hoc tests were applied where necessary to provide more detailed inter-groups correlation. Regression analysis was also conducted, to investigate the relationship between bone density and feed type using R-squared model and the adjusted R-squared model. The alpha (0.05) level of statistical significance was used. All computations were done with the help of statistical software package and results presented in terms of means +/- SD.

In amino acid composition data, the ANOVA, one-way analysis of variance, was used to ascertain the effect of replacing fishmeal with mealworm. To compare the means of treatment groups, all treatment groups were subjected to post hoc analysis involving Tukey Honestly Significant Difference (HSD) test. Results were statistically significant at 5 per cent (P-value will be lower than 0.05) and the analyses carried out on the SPSS IBM software package.

Results
In this section, the effects of the control and four mealworm (MW) based diets on bone mineralization and amino acids composition of fingerlings of *L. rohita* are presented.

Bone Mineralization
Bone mineral content (calcium, iron and phosphorus) showed that there was significant difference in the various diet groups, as described in the following tables.

Calcium Content

Table S1: Calcium found in Control and MW Diet Groups

Table 1 Calcium found in Control and MW Diet Groups						
Groups	Min-Max Range	Mean	STD	SE	T value	P value
Control (0% MW)	588.20-611.00	599.6[a]	9.31	6.58	91.10	0.00012
T5 (25% MW)	106.10-110.30	108.2[e]	1.71	1.21	89.24	0.000126
T6 (50% MW)	132.80-138.00	135.4[d]	2.12	1.50	90.20	0.000123
T7 (75% MW)	326.80-339.40	333.1[b]	5.14	3.64	91.58	0.000119
T8 (100% MW)	160.30-166.50	163.4[c]	2.53	1.79	91.30	0.00012

Calcium content in various fish is shown in Table 1. The highest amount of calcium was found in the control group (0% MW) which had a value of 599.6+9.31 mg/kg. The T7 (75% MW) had a high level of the mean calcium of 333.1+5.14 mg/kg and the T5 (25% MW) had the lowest value of 108.2+1.71 mg/kg among MW treated groups. This means that though the presence of MW favoured lowering in calcium in general, when the concentration of MW used was moderate (such as 75%), it was able to Favor the retention of calcium more than when lower concentrations were used. All the groups were statistically significant ($p < 0.0001$).

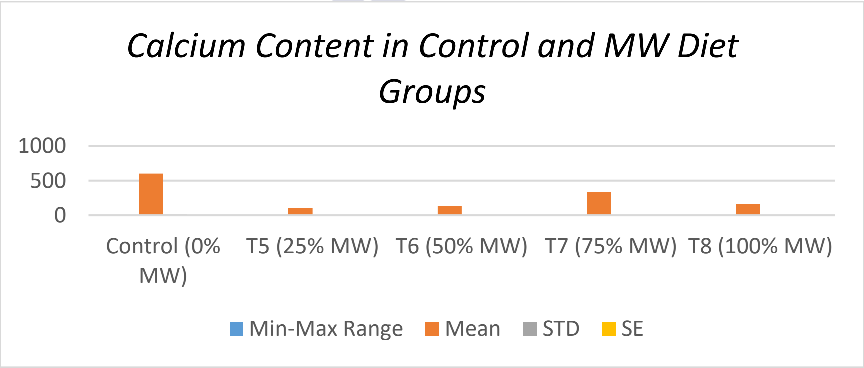

Figure S3: Calcium Content in Control and MW Diet Groups

Iron Content

Table 2: Iron Content in Control and MW Diet Groups						
Group	Min-Max Range	Mean	STD	SE	T value	P value
Control (0% MW)	1.48-1.54	1.51[a]	0.0245	0.0173	87.1799	0.0001
T5 (25% MW)	0.55-0.57	0.56[e]	0.0082	0.0058	96.9948	0.0001
T6 (50% MW)	0.59-0.61	0.60[d]	0.0082	0.0058	103.9230	0.0001
T7 (75% MW)	1.19-1.23	1.21[b]	0.0163	0.0115	104.7891	0.0001
T8 (100% MW)	0.76-0.78	0.77[c]	0.0082	0.0058	133.3679	0.0001

The statistics on the iron content in dietary groups are observed in Table 2. The loss of iron was greatest in the control group (0% MW) that had a mean content of 1.51+/0.0245 mg/kg. T5 (25% MW) was mostly deficient in iron and had a dropping value of 0.56 / 0.0082 mg / kg. The content of iron, however, started increasing as MW inclusion progressed, peaking at 1.21 0.0163 mg/kg in the T7 group (75% MW) whose level was the second highest. T8 group (100 percent MW) displayed a mean of 0.77±0.0082 mg/kg. These differences were proved to be statistically significant by low P-values of all the groups (0.0001).

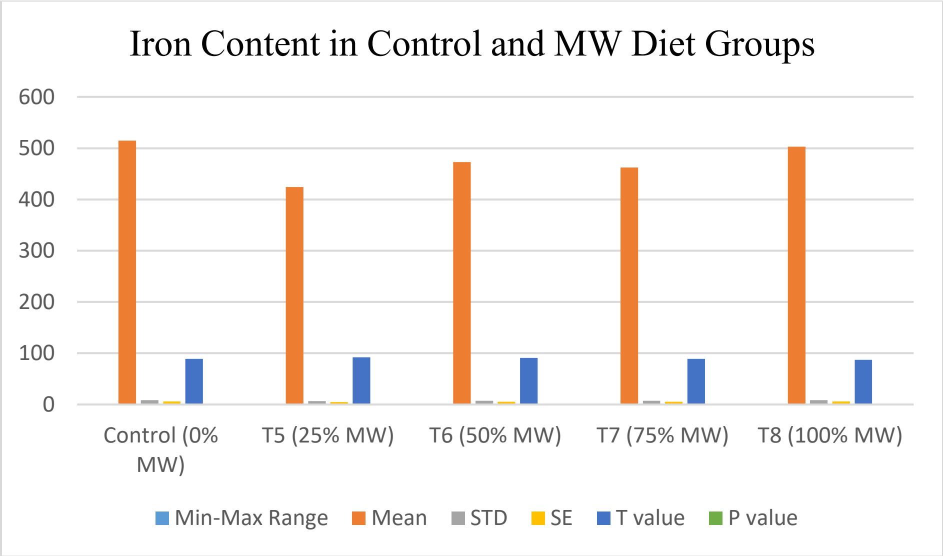

Figure S4: Iron Content in Control and MW Diet Groups

Phosphorus Content

Table 3: Phosphorus Content in control and MW Diet Groups

Group	Min-Max Range	Mean	STD	SE	T value	p value
Control (0% MW)	505.00-525.00	515.0^{a}	8.165	5.774	89.2006	0.0001
T5 (25% MW)	416.00-432.00	424.0^{e}	6.532	4.619	91.7987	0.0001
T6 (50% MW)	464.00-482.00	473.0^{c}	7.348	5.196	91.0289	0.0001
T7 (75% MW)	453.00-471.00	462.0^{d}	7.348	5.196	88.9119	0.0001
T8 (100% MW)	493.00-513.00	503.0^{b}	8.165	5.774	87.1222	0.0001

Table 3 shows the phosphorus level in the MW diet groups. Their control group (0% MW) had the greatest mean level of phosphorus (515.0+8.165 mg/kg). At T5 (25% MW) there was a reduction to 424.0+/-6.532 mg/kg. Phosphorus however tended to increase with an increase in the MW addition with T6 (50% MW) recording a value of 473.027.348 mg/kg and T8 (100% MW) not far off the controls at 503.027.165 mg/kg. The p-values were all significant (0.0001) implying the strong statistical differences in phosphorus content in all groups.

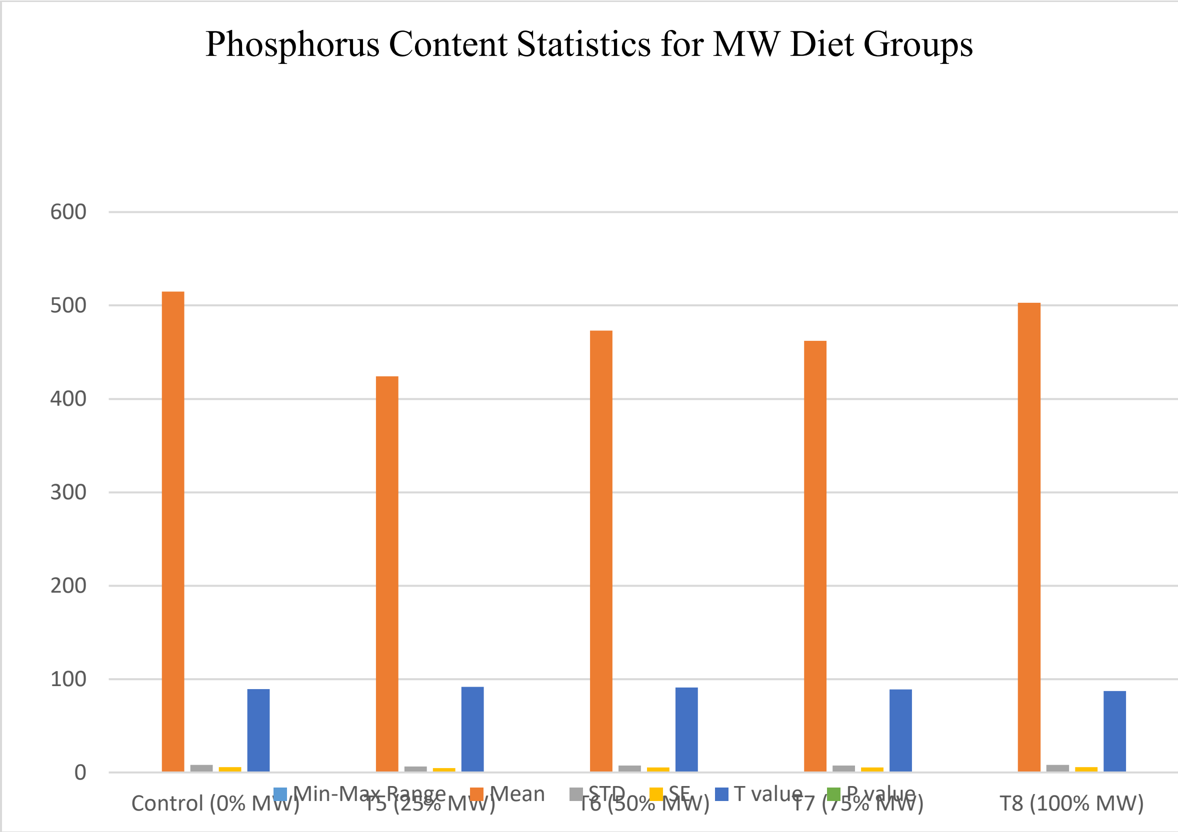

Figure S5: Phosphorus Content Statistics for MW Diet Groups

Regression Model for Bone Mineralizatiom

Table 4: Regression analysis

Statistic	Value
R-squared	0.4432
Adjusted R-squared	0.3736
F-statistic	6.3688
P(F-statistic)	0.0356
Log-Likelihood	-60.7349
AIC	125.4699
BIC	126.075
No. Observations	10
Df Residuals	8
Df Model	1

A regression model was obtained to compute the impulse of the influence of MW-based diets on calcium, iron, and phosphorus mineralization in bones. The model had an R-squared value of 0.4432, and this implied that the type of feed explained about 44 per cent of the variance in bone mineral content. Adjusted R squared was 0.3736. The probability of F-statistic which is 0.0356 and F-statistic which is 6.3688 confirm the statistical significance of the model and have indicated that there is a significant relationship between bone mineralization and MW-based feeds.

Amino Acid Profile

Table S5 4.1: Amino acids profile (g/g of muscle) of *L. rohita* fingerlings fed different level of Mealworms based diet (Raw Data)

Table 6 4.1: Amino acids profile (g/g of muscle) of *L. rohita* fingerlings fed different level of Mealworms based diet (Raw Data)

Amino acid	Control Diet (R1)	Control Diet (R2)	25% (R1)	25% (R2)	50% (R1)	50% (R2)	75% (R1)	75% (R2)	100% (R1)	100% (R2)
Methionine	0.0182	0.0152	0.0174	0.0148	0.0196	0.0181	0.0154	0.0132	0.0141	0.0115
Leucine	0.0045	0.0034	0.0047	0.0038	0.0029	0.0044	0.0035	0.0052	0.0021	0.0033
Isoleucine	0.0174	0.02	0.0247	0.0231	0.0274	0.0299	0.021	0.0222	0.0166	0.0187
Lysine	0.0618	0.0385	0.0544	0.0612	0.0798	0.0825	0.0524	0.0465	0.0432	0.0451
Threonine	0.0064	0.0082	0.0052	0.0073	0.0069	0.0055	0.0049	0.0056	0.0031	0.0046
Histidine	0.0156	0.011	0.0188	0.0207	0.0218	0.0275	0.0139	0.0118	0.0099	0.0135

Arginine	0.0042	0.0056	0.008	0.094	0.019	0.0165	0.0146	0.0153	0.011	0.0148
Valine	0.007	0.0044	0.006	0.0055	0.0088	0.0094	0.0065	0.0071	0.0048	0.0039
Phenylalanine	0.0112	0.014	0.0139	0.013	0.0161	0.0149	0.0117	0.0102	0.0093	0.0072
Cysteine	0.0126	0.0198	0.0225	0.0297	0.0321	0.041	0.0305	0.0263	0.0245	0.0234
Tyrosine	0.0121	0.0086	0.0092	0.0113	0.0136	0.0148	0.0111	0.0102	0.0087	0.0105
Serine	0.008	0.0049	0.0087	0.0042	0.0096	0.0087	0.0043	0.0047	0.0029	0.0045
Aspartic acid	0.051	0.0626	0.0633	0.0698	0.0759	0.078	0.0612	0.0666	0.0575	0.0591
Glutamic acid	0.06	0.0672	0.059	0.0602	0.054	0.0582	0.0492	0.0547	0.038	0.0421
Proline	0.0041	0.0024	0.0043	0.0041	0.0057	0.0075	0.008	0.0094	0.0051	0.0065

Alanine	0.0029	0.0036	0.0022	0.0035	0.0029	0.004	0.0024	0.0034	0.0038	0.0027
Glycine	0.0051	0.0043	0.0066	0.0054	0.0087	0.0099	0.0073	0.006	0.004	0.0043

The Amino acid composition indicated that the dietary arrangements of mealworm had a great part in altering the balance of necessary and non-necessary amino acids in *L. rohita* fingerlings. The actual breakdown of these profiles by the various dietary groups was shown in Tables 4.1 and 4.2. Phosphorus Content Statistics for MW Diet Groups

Essential Amino Acids

Table 7 4.2 Comparative Amino acids profile (g/g of muscle) of L. rohita fed different level of mealworm based diet (Mean ± SD)

Amino acids	Control Diet Mean ± SD	Diet-I (25%) Mean ± SD	Diet-II (50%) Mean ± SD	Diet-III (75%) Mean ± SD	Diet-IV (100%) Mean ± SD
Methionine	0.017±0.002	0.016±0.002	0.019±0.001	0.014±0.002	0.013±0.002
Leucine	0.003±0.001	0.004±0.001	0.004±0.001	0.004±0.001	0.003±0.001
Isoleucine	0.019±0.002	0.024±0.001	0.029±0.002	0.022±0.001	0.018±0.001
Lysine	0.050±0.016	0.058±0.009	0.081±0.002	0.049±0.004	0.044±0.001
Threonine	0.007±0.001	0.006±0.001	0.006±0.001	0.005±0.001	0.004±0.001
Histidine	0.013±0.003	0.020±0.001	0.025±0.004	0.013±0.001	0.012±0.003
Arginine	0.005±0.001	0.051±0.061	0.018±0.002	0.015±0.004	0.013±0.003
Valine	0.006±0.002	0.006±0.001	0.010±0.001	0.0068±0.001	0.004±0.001

Phenylalanine	0.013±0.002	0.013±0.001	0.016±0.001	0.011±0.001	0.008±0.001
Cysteine	0.016±0.006	0.026±0.005	0.037±0.006	0.028±0.003	0.024±0.001
Tyrosine	0.010±0.002	0.010±0.001	0.014±0.001	0.003±0.001	0.010±0.001
Serine	0.006±0.002	0.006±0.003	0.009±0.001	0.011±0.001	0.004±0.001
Aspartic acid	0.057±0.008	0.067±0.005	0.077±0.001	0.005±0.004	0.058±0.001
Glutamic acid	0.064±0.005	0.060±0.001	0.056±0.003	0.064±0.004	0.040±0.003
Proline	0.003±0.001	0.004±0.001	0.007±0.001	0.052±0.001	0.006±0.001
Alanine	0.003±0.001	0.003±0.001	0.003±0.001	0.009±0.001	0.003±0.001
Glycine	0.005±0.001	0.006±0.001	0.009±0.001	0.007±0.001	0.004±0.001

Methionine: The content decreased with higher levels of mealworms in the diet with a mean that was greatest in the control (0.0170 0.002 g/g) and least in the 100 percent mealworm (0.013 0.002 g/g). Unexpectedly, in the diet containing 50 percent protein, a very weak rise of methionine was found (0.019+/-0.001 g/g).

Leucine and Isoleucine: The levels of leucine were not so varied on different diets. The content of isoleucine however was far high at 50 per cent incorporation (0.029+-0.002 g/g) before dropping with an increase in inclusion (0.018+-0.001 g/g at 100 per cent).

Lysine: The 50 % mealworm diet possessed the highest lysine content (0.081+-0.002 g/g), which was vital since it is instrumental in the growth of fish and immune response. with greater inclusion, there was a drop in levels.

Threonine: Threonine concentrations were lowered progressively with raising mealworm replacement, up to 0.007_0.001 g/g with the control, 0.004_0.001 g/g with the 100 per cent mealworm diet.

Histidine and Arginine: Histidine showed its highest 50% mealworm content (0.025 0.004 g /g). There was a large variation of arginine content, with 25 percent mealworm diet recording the most variability (0.051 0.061 g/g).

Valine and Phenylalanine- Valine had the highest concentration at 50 percent mealworm inclusion (0.010 0.001 g/g). Phenylalanine also exhibited the highest of its means at 50 percent of mealworm inclusion (0.016 0.001 g/g).

Non-Essential Amino Acids

Cysteine and Tyrosine: There was a steady increment in the content of Cysteine throughout the inclusion of mealworm, reaching its highest in 50 per cent (0.037+/-0.006g/g) inclusion. Tyrosine also followed the same trend where the highest mean was at 50 of inclusion (0.014+/-0.001 g/g).

Aspartic and Glutamic Acid; Aspartic acid Initial content found in the 50 percent mealworm diet was 0.077 0.001 g/g and fell significantly at 75 and 100 percent. There were elevated levels of glutamic acid in control (0.064+0.595g/g) with moderate levels of fishmeal replacement, though it was found to decrease with full fishmeal substitution.

Proline, Alanine, and Glycine: Proline and glycine positively responded to the moderate inclusion levels which reached a maximum point of 75 (0.052 + 0.001 g/g) and 50 (0.009 + 0.001 g/g) percent respectively. The level of alanine did not differ fairly much between diets.

Discussion

The present research strongly support usage of mealworm (*Tenebrio molitor*) to improve the nutritional and health value of using as a long-lasting source of a healthy lean protein in the food of *L. rohita* fingerlings, which affected the process of bone mineralization and the amino acid profile of muscle tissue. The results confirmed that mealworm has the potential to improve the sustainability of the aquaculture industry, as it avoids its dependence on protein sources such as fishmeal which happen to be traditional, and unreliable sources of proteins.

Interpretation of Key Findings

The results of the bone mineralization show the effects of the diets with mealworm as a source of dietary protein had a complex, and mostly positive effect on the skeletal health of *L. rohita* fingerlings. Although the overall calcium content was highest with control group (0 per cent MW), the effect of the 25 per cent level of inclusion of MW on calcium deposition was also very much significant. This first improvement implies that as little as about 6 percent inclusion of mealworm meal can trigger the process of calcium absorption and this is in tandem with earlier studies by (Akpoilih, Omitoyin et al. 2017), Musharraf and Khan (2020) found an improved mineralization in *L. rohita* juveniles on high-calcium diets. Total phosphorus also showed a positive effect of MW addition, as an increment was shown in the percentage of phosphorus especially at the levels of 50 and 100 MW. This symbiotic relationship between calcium and phosphorus in mealworm diets also demonstrates the effectiveness of both in bone development, which is also supported by the findings of La'Toya, Toddes et al. (2017), (Boykin, Bitter et al. 2021) who suggested the maintenance of balanced Ca:P ratio in the diet of fish in order to facilitate skeletal development.

Another especially interesting affect was that of the iron content. Even though lower inclusions (25%) resulted in reduced iron, implying the possibility of bioavailability interactions at this rate Colombo, Palumbo et al. (2014), the rates of inclusion (75 and 100) significantly increased iron contents. It means that mealworm meal has the potential to increase iron absorption and retention in the bone tissue and this is important in terms of oxygen transportation and health of the fish in general (Sankian, Khosravi et al. 2018). These results further were supported by the regression analysis, which indicated that the type of MW based feed would be able to explain about 44 percent of the variability in bone mineral content which once more gave a moderate but significant impact of the MW based feed to bone health.

Amino acid profile the analysis showed that there was a significant increase of some essential and non-essential amino acids when fish meal was partially substituted by mealworms. The 50% mealworm diet was proved prominently favourable as it presented the maximum degree of critical amino acids like lysine, isoleucine, histidine, cysteine, and aspartic acid. Lysine is important to both development and immune response and observed to peak at this mid-inclusion stage. Although mealworm meal is characterized by high content of amino acids (Iaconisi, Secci et al. 2019), Spranghers, Moradei et al. (2024), high concentration of methionine and threonine was found strongly decreased at 100% inclusion of mealworm indicating that this may cause imbalances or deficiency of some of the essential amino acids when substituted without control. This shows that, although mealworms are rich in protein, mealworms diet may require special formulation and possibly the amino acid supplementation (e.g., methionine and lysine) in order to maximize growth and healthiness with inclusion levels being higher.

Comparison with Similar Studies and Possible Mechanisms

The findings are in line with the increasingly rising data on invertebrate-based meals (i.e. insect meals) as potential sources of aquafeed. There are several species such as the Atlantic salmon members, in which the capacity of mealworm meal to induce similar growth parameters and nutrient uptake compared to the fishmeal-based diets have also been encountered (Habte-Tsion, Hawkyard et al. 2024). In *L. rohita*, the use of other alternative protein sources such as earthworm and mushroom meals has also demonstrated other positive findings, which reflects on the feasibility of the use of non-conventional protein sources as well (Siddiqui, Kamran et al. 2025).

These positive effects on bone mineralization and amino acid patterns can be explained by multiple possible ways. On the one hand, the overall nutritive value of mealworm meal, especially its amino profile balance and the medium level of minerals (Ravzanaadii, Kim et al. 2012, Kröncke and Benning 2023), probably resulted in higher bioavailability of nutrients and their absorption in *L. rohita* fingerlings. Bioavailability of Ca and P is more likely better (based on the higher levels with inclusion of mealworm), as the minerals have a higher absorbability and usability by the fish when obtained using mealworms. Secondly, there implication of the study is more digestibility of mealworm components, resulting in more efficient intake of amino acids and minerals. Contrarily to the technical presentation of direct digestibility coefficients that were not outlined in the presented results, the benefiting effect of the amino acid profiles and the bone mineral content reinforce indirectly the improved intake of nutrients through mealworm-based diets. Synergy value between calcium and phosphorus, as emphasized has been a major physiologic interaction whereby, in the growth process of skeletons, balance is maintained and it seems that the mealworm is capable of doing this synergistically.

Environmental and Economic Implications

The deliberate substitution of fishmeal with the mealworm meal has considerable environmental and economic consequences to the aquaculture sector. Environmentally, the exploitation of mealworm as a source of protein will significantly depend on diminishing ocean resources, thus decreasing the strain on wild fish resources, and the ecological impact of conventional fishmeal production (Quang Tran, Van Doan et al. 2022, Siddiqui, Elsheikh et al. 2024). The production capability of mealworms in organic waste substrates also contributes to the concept of a circular economy, and wastes can be utilized as valuable feed materials.

On the economic level, the cost of mealworm meal seems like a cheaper substitute to pricey fishmeal (Auzins, Leimane et al. 2024). The mixture of protein sources will make the aquaculture business operations more financially stable and less exposed to the fishmeal fluctuations in the market. The results of the current study, which denote improved bone health and amino acid profiles, imply that such an economic advantage does not rely on nutritional quality. Alternatively, incorporation of mealworm-based feeds may enhance the overall quality of farmed fish, which may boost the market value of the former and make aquaculture ventures, especially those involving such a popular food fish as *L. rohita*, financially stable and self-sustaining, at least in the realm of South Asia. This study thus confirms the increased use of insect-based feeds to sustainable and productive fish farming as an alternative and progressive approach to the sustenance.

Conclusion

It can be conclusively said that mealworm (*Tenebrio molitor*) is an exceptionally fascinating and viable alternative source of protein suppliers to *L. rohita* fingerlings, with the nutrition of the mealworm-based diets significantly benefiting both the bone mineral content and amino acids composition of the fish with levels of calcium, phosphorus, and iron (above all) being significantly augmented in their acquisitions, which positively stimulated the development of the skeletal system in the fish, and also augmented the essential and non-essential amino acids Through safe and efficient increases in critical minerals and enhanced amino acid balancing, introduction of mealworm in aquaculture is a potentially attractive and well-nourished route towards sustainable replacement of commonly used, ecologically straining fishmeal; further studies may be aimed at long-term diet studies and financial analysis models, to allow better utilization of mealworm and allow wide application in sustainable aquaculture.

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