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RESEARCH ARTICLE
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Substitution of Fish Meal with Mealworm (*Tenebrio molitor*) Meal for Nutritional Quality, Feed Utilization Efficiency and Growth Performance of Nile Tilapia (*Oreochromis niloticus*)

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ABSTRACT

Nile tilapia (*Oreochromis niloticus*), a globally significant aquaculture species, is crucial for food security.[1] However, the industry's reliance on fishmeal necessitates sustainable protein alternatives. Mealworm (*Tenebrio molitor*) meal has emerged as a promising candidate due to its high nutritional value. This study, therefore, evaluated the effects of substituting fishmeal with mealworm meal on the growth performance and nutritional quality of Nile tilapia. Over a 49-day feeding trial, juvenile tilapia (initial weight: 1.83±0.06g) were assigned to six treatments in a completely randomized design. The diets included a commercial control and five formulations where fishmeal was replaced by mealworm meal at 0%, 25%, 50%, 75%, and 100%. Results indicated that dietary fishmeal replacement significantly impacted Feed Utilization Efficiency (EPP), Protein Efficiency Ratio (PER), and Specific Growth Rate (SGR) ($P\geq0.05$), while Total Feed Intake and Survival Rate were unaffected ($P\geq0.05$). Optimal growth performance was achieved at the 25% substitution level, yielding the highest SGR (3.12±0.14%/day), EPP (72.04±5.29%), and PER (2.35±0.10). Notably, the nutritional composition of the fish flesh was maximized at a 50% substitution level, which resulted in the highest concentrations of total essential amino acids (22.3g/100g protein) and LC-PUFAs (EPA+DHA; 9.32g/100g fat). These findings demonstrate that while a 25% substitution is optimal for growth, a 50% inclusion can enhance the product's nutritional value, highlighting mealworm meal's potential as a versatile and sustainable ingredient in tilapia aquafeeds.

Keywords: Tilapia, Feed, Nutrition, Mealworm, Growth.

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INTRODUCTION

Food security has become a primary global concern as the world population continues to rise and is projected to reach 9.3 billion by 2050 (FAO, 2017). As a result, food resources, exceptionally high-quality protein sources, are becoming increasingly important. Among animal-based foods, fish is a vital source of protein, providing essential macronutrients (proteins and fats) as well as micronutrients (vitamins and minerals), making it a highly valuable food for human consumption (Ahmed et al., 2021). Aquaculture plays a crucial role in meeting the growing demand for fish, especially as wild fish stocks continue to decline. Over the past two decades, aquaculture has expanded rapidly, supported by technological advancements that have enabled more efficient food production. From 2000 to 2022, global aquaculture production increased significantly from 43 million metric tons (Mt) to 120.1 million Mt (Verdegem et al., 2023).

Nile tilapia (*Oreochromis niloticus*) is a widely cultivated freshwater species with a long history in aquaculture. This species has a high growth rate and

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strong adaptability to diverse environmental conditions, making it suitable for intensive culture systems (Sayed & Fitzsimmons, 2022; Mengistu et al., 2022). Nile tilapia has been introduced globally and is one of the most farmed freshwater fish species, second only to carp (Munguti et al., 2022). The growth performance of Nile tilapia is heavily influenced by its diet. A nutritionally balanced feed is essential to support optimal growth. However, fishmeal—the primary protein source in aquafeeds—faces increasing limitations in availability, rising costs, and competition from other industries (Munguti et al., 2022). These constraints have spurred the search for alternative protein sources, including insect-based meals, which have shown great promise due to their rich nutritional profiles. Insects are known to contain high levels of both macro- and micronutrients (Anggraeni et al., 2024), with protein contents ranging from 50 to 82% on a dry matter basis—comparable to that of fishmeal, which typically contains 60 to 72% protein (Hameed et al., 2022).

Mealworm *(Tenebrio molitor)* is one such insect with great potential as an alternative protein source in aquafeeds. Mealworm meal contains approximately 53% protein, 28% fat, 6% fiber, and 5% moisture on a dry weight basis (Mariod, 2022). It has been extensively tested in various aquatic species and has demonstrated promising results. For instance, in Pacific white shrimp (Litopenaeus vannamei), a 50% replacement of fishmeal with mealworm meal resulted in optimal performance with a daily growth rate of 2.6% and an FCR of 1.2 (Zhang et al., 2022). In African catfish (Clarias gariepinus), partial replacement (20%) led to improved growth performance, with an SGR of 3.8%/day and a PER of 3.3 (Ng et al., 2022). Differences in the optimal replacement levels among studies may be attributed to species-specific physiological requirements and environmental conditions.

Although fishmeal replacement may positively influence growth, excessive substitution could lead to nutritional imbalances or adverse effects due to unknown anti-nutritional factors (Li et al., 2021). Thus, further research is necessary to evaluate the potential of mealworm meal in various species and to understand its nutritional characteristics better. This study aims to evaluate the effects of replacing fishmeal with mealworm (T. Molitor) meal on total feed intake, feed utilization, and growth performance of Nile tilapia. Experimental diets were formulated with incremental levels of fishmeal replacement to comprehensively assess the impact of mealworm inclusion on the performance of this important aquaculture species.

MATERIALS & METHODS

The feeding trial was conducted for 49 days at the Fish Hatchery Center (Balai Benih Ikan) in Mijen, Semarang, Indonesia. The experiment followed a completely randomized design (CRD) consisting of six dietary treatments with three replicates each. A commercial diet containing 30% crude protein was used as the control (Treatment K), while five experimental diets were formulated to contain incremental levels of fishmeal (FM) replacement with mealworm meal (Tenebrio molitor; BM). All experimental diets were formulated to be isonitrogenous (30% crude protein), isolipidic (12% crude fat) and isoenergetic, ensuring that differences in fish performance could be attributed to the protein source rather than variations in overall nutrient composition.

The levels of fishmeal replacement by mealworm meal ranged from 0% to 100%, resulting in the following dietary treatments: Treatment A: 0% BM (100% FM); Treatment B: 25% BM + 75% FM; Treatment C: 50% BM + 50% FM; Treatment D: 75% BM + 25% FM; Treatment E: 100% BM (0% FM). All diets were pelleted and dried prior to feeding. The ingredient composition and proximate analysis of each diet are presented in Table 1.

Table 1: Composition of ingredients and proximate analysis of the experimental diets

Material	Composition (DM) g/100g					
	Control	A (0%)	B (25%)	C (50%)	D (75%)	E (100%)
Fish meal	-	32.5	24.38	16.25	8.13	0.00
Mealworm meal	-	0.00	8.12	16.10	24.15	32.50
Soybean meal	-	25.60	25.50	25.50	25.50	27.50
Rice bran flour	-	11.60	14.00	14.00	14.00	13.20
Corn flour	-	10.00	10.00	10.00	10.00	10.00
Wheat flour	-	7.50	7.00	7.30	7.30	7.30
α – selulosa	-	0.00	0.00	1.55	3.62	4.40
Fish oil	-	7.7	5.7	4	2	0
Corn oil	-	1.1	1.3	1.3	1.3	1.1
Vitamin mix	-	1.5	1.5	1.5	1.5	1.5
Mineral mix	-	1.5	1.5	1.5	1.5	1.5
Carboxymethyl Cellulose	-	1	1	1	1	1
Total		100	100	100	100	100
Protein*	31.02	30.08	30.12	30.08	30.04	30.05
Fat*	4.12	11.96	11.86	12.13	12.12	12.17
Nitrogen Free Extract (NFE)	35.23	28.17	29.85	31.09	32.07	33.32

Note: Proximate Analysis conducted by the Laboratory of the Agricultural Instrument Standard Application Center, Central Java.

Experimental Fish and Rearing Conditions

The experimental fish used in this study were juvenile Nile tilapia (*Oreochromis niloticus*) with an initial average body weight of 1.83±0.06g and an average length of 4.75±0.14cm. The fish were randomly distributed into glass aquaria (50 × 50 × 50 cm^3) at a stocking density of 15 fish per aquarium. The rearing system used freshwater and all tanks were aerated continuously. Fish were fed twice daily to apparent satiation throughout the 49-day feeding trial. Growth performance measurements were conducted every 7 days to monitor changes in body weight and length.

Growth Performance and Feed Utilization Parameters

The following indices were used to evaluate feed utilization and growth performance:

Proximate Analysis

The proximate composition of the samples was analyzed according to the official methods of the Association of Official Analytical Chemists (AOAC, 2005). Crude protein was determined from the total nitrogen content (N × 6.25), and the carbohydrate fraction was estimated by difference

Amino Acid Profile

For amino acid analysis, samples were prepared according to AOAC (2005) and analyzed on a Waters HPLC system equipped with a fluorescence detector. A 5 µL aliquot of each sample was injected and eluted with a mobile phase consisting of 60% Acetonitrile-AccqTag Eluent at a flow rate of 1.0 $mL \cdot min^{-1}$ for subsequent quantification.

Fatty Acids Profile

Determination of the fatty acid profile was conducted using a Shimadzu GC-14B gas chromatograph (Shimadzu Corporation, Japan) equipped with a flame ionization detector and a capillary column. The analytical methodology adhered to the official AOAC (2005) procedures. The complete fatty acid composition for the diets used in the 49-day study is detailed in Table 4.

Feed Consumption Rate (TKP)

According to Weatherly (1972), total feed consumption can be calculated using the following formula:

F = C - S

Description:

F : Feed consumption (g)

C : Feed given (g)

S : Remaining feed (g)

Feed Intake (FI)

Feed Consumed = Final Feed Weight – Initial Feed Weight

Feed Utilization Efficiency (FUE)

The value of feed utilization efficiency is calculated based on the formula of Zonneveld et al. (1991), namely:

$$FUE = \frac{W_t - W_0}{F} \times 100\ \%$$

Description:

EPP : Feed Utilization Efficiency

Wt : Final fish weight of the study (g)

W0 : Initial fish weight of the study (g)

F : Total amount of feed consumed.

Protein Efficiency Ratio (PER)

The calculation of the protein efficiency ratio (PER) value uses the formula from Tacon (1987), namely:

$$PER = \frac{W_t - W_0}{Pi} \times 100\ \%$$

Description:

PER: Protein Efficiency Ratio (%)

Wt: Total weight of test fish at the end of the study (g)

W0: Total weight of test fish at the beginning of the study (g)

Pi: Weight of feed consumed x % of feed protein (g)

Specific Growth Rate (SGR)

According to Weatherley and Gill (1987), the Specific Growth Rate during the maintenance period is calculated using the formula:

$$SGR = \frac{\ln W_t - \ln W_0}{t} \times 100\ \%$$

Description:

SGR : Specific growth rate (%/day)

Wt : Total weight of fish at the end of the study (g/tail)

W0 : Total weight of fish at the beginning of the study (g/tail)

t : Time (Length of maintenance)

Survival Rate (SR)

According to Effendie (1997), survival is the percentage of cultivar survival, which can be calculated as follows:

$$SR = \frac{N_t}{N_0} \times 100\%$$

Description:

Nt: Number of fish at the end of the study

N0: Number of fish at the beginning of the study

Water Quality Monitoring

Water quality parameters observed in this study included temperature, pH, dissolved oxygen (DO), and salinity (Table 3). Measurements were conducted weekly, either in the morning or afternoon, before feeding.

Data Analysis

The effect of dietary treatments on all measured parameters was evaluated by a one-way analysis of variance (ANOVA) using SPSS version 26. Before performing the ANOVA, the data were confirmed to meet the assumptions of normal distribution and homogeneity of variance. For variables where the ANOVA indicated a significant treatment effect (P<0.05), Duncan's Multiple Range Test (DMRT) was conducted to delineate specific differences between the treatment means, including comparisons against the control group.

RESULTS

Based on these results, the experiment showed a significant effect on feed utilization efficiency (FUE), protein efficiency ratio (PER), and specific growth rate (SGR) (P<0.05). On the other hand, the substitution of fish meal with mealworm meal did not have a significant effect on the survival rate (SR) (P≥0.05). The results of the Dunnett's test are presented in Table 2. The best Total Feed Intake (TFI) result was observed in the 25% Mealworm Meal treatment group (B), where 5% of fish meal was substituted with 25% Mealworm Meal, yielding a TFI of 3.12±0.22g. The lowest TFI was recorded in the 100% Mealworm Meal treatment group (E), which used a commercial feed with 100% protein replacement, showing a TFI of 1.54±8.42g. The total feed intake values throughout the study are presented in Fig. 1.

Table 2: Dunnett's test results comparing experimental treatments with the control treatment

Variable	TKP	EPP	PER	SGR
Control vs Mealworm meal 0%	0.004*	0.658	0.994	0.787
Kontrol vs Mealworm meal 25%	0.004*	0.559*	0.102*	0.227*
Kontrol vs Mealworm meal 50%	0.001*	0.999	0.516	0.797
Kontrol vs Mealworm meal 75%	0.006*	0.765	0.441	1.000
Kontrol vs Mealworm meal 100%	0.165*	0.065*	0.001*	0.273

Note: * Most influential value; Fishmeal substitution also showed a significant effect (p<0.05) on feed intake (FI), but no significant effect (p>0.05) on feed conversion ratio (FCR). The best treatment was found in the 25% Hongkong mealworm substitution treatment.

Table 3: Water Quality During 49 Days of Experiment

Variable	Value	References
Temperature (°C)	25.5-29	25-30*
pH	7.1-8.65	6.5-8.5*
DO (mg/l)	4.2-6.1	>5*

Note: *Based on the national quality standards of BSN (2005).

Table 4: Fatty acid profile of the feed used in the maintenance of Nile tilapia during 49 days

Fatty Acids (%)	K	A (0%)	B (25%)	C (50%)	D (75%)	E (100%)
SAFA						
10:0	0.640.07	0.73±0.04	0.85±0.01	0.72±0.05	0.70±0.01	0.66±0.08
11:0	0.87±0.03	2.60±0.03	0.91±0.05	1.80±0.04	1.90±0.05	0.99±0.04
12:0	0.35±0.06	0.36±0.02	0.30±0.03	0.37±0.08	0.35±0.03	0.41±0.01
13:0	2.40±0.09	2.72±0.03	2.28±0.04	3.22±0.07	2.85±0.04	2.43±0.07
14:0	14.20±0.02	15.80±0.08	1.,51±0.02	16.10±0.02	13.55±0.02	14.75±0.03
15:0	0.24±0.09	0.91±0.03	0.40±0.02	0.56±0.04	0.45±0.02	0.30±0.09
16:0	10.42±0.04	13.45±0.05	15.,04±0.06	14.83±0.02	13.64±0.06	10.48±0.08
17:0	6.12±0.06	5.92±0.03	8.55±0.02	6.69±0.03	6.15±0.02	6.16±0.03
18:0	8.60±0.01	9.50±0.07	10.,08±0.08	8.12±0.09	7.08±0.08	8.76±0.02
20:4	2.25±0.02	2.97±0.02	4.,17±0.05	3.10±0.02	2.17±0.05	2.39±0.05
MUFA						
14:1	5.38±0.03	2.77±0.02	3.08±0.02	2.82±0.09	2.04±0.02	2.46±0.09
15:1	0.20±0.05	1.39±0.09	1.19±0.04	1.78±0.02	1.30±0.04	1.24±0.03
16:1	7.55±0.01	8.05±0.02	9.22±0.03	8.72±0.05	8.22±0.03	7.58±0.02
18:1	4.13±0.04	4.56±0.04	5.75±0.02	5.37±0.09	4.77±0.02	4.16±0.09
18:2c	5.42±0.08	5.77±0.02	6.04±0.02	5.12±0.02	5.04±0.02	5.48±0.02
PUFA						
18:3ω3	1.18±0.03	1.63±0.04	1.93±0.02	2.11±0.07	2.43±0.02	2.56±0.06
20:5ω3	9.74±0.05	10.41±0.03	12.80±0.06	11.15±0.02	10.35±0.06	10.04±0.02

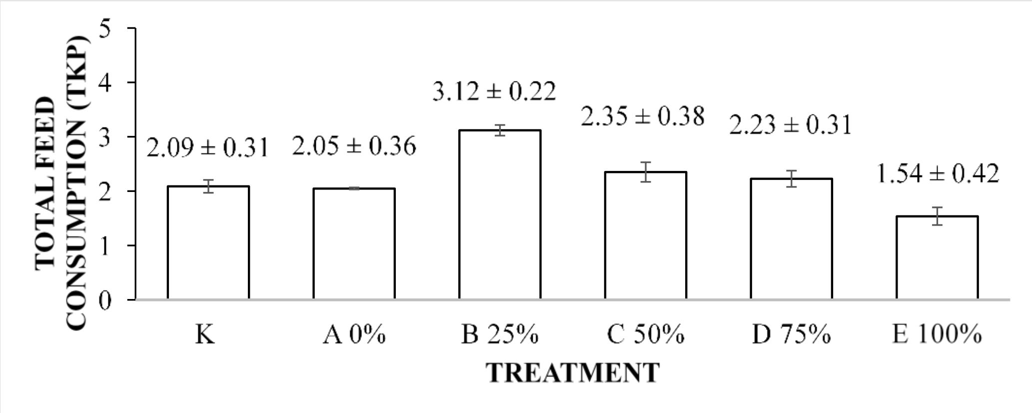

Fig. 1: Total feed consumption of Nile tilapia (*Oreochromis niloticus*) during 49 days of rearing period.

The best Feed Utilization Efficiency (FUE) result was observed in the 25% Mealworm Meal treatment group (B), where 25% of fish meal was replaced with 75% Mealworm Meal, achieving a FUE of 72.04±1.4%. The lowest FUE was recorded in the 100% Mealworm Meal treatment group (E), which used 100% Mealworm Meal with no fish meal, resulting in a FUE of 46.60±4.78%. The Feed Utilization Efficiency (FUE) values throughout the study are presented in Fig. 2.

The best Protein Efficiency Ratio (PER) was obtained in the 25% Mealworm Meal treatment group (B), where 25% of fish meal was replaced with 75% Mealworm Meal, resulting in a PER of 2.35±0.22%. The lowest PER was recorded in the 100% Mealworm Meal treatment group (E), which used 100% Mealworm Meal and no fish meal, yielding a PER of 1.54±8.42%. The Protein Efficiency Ratio (PER) values throughout the study are shown in Fig. 3. The best Specific Growth Rate (SGR) was observed in the 25% Mealworm Meal treatment (B), where 75% of fish meal was substituted with 25% Mealworm Meal, achieving an SGR of 3.12±0.14%per day. The lowest SGR was found in the 100% Mealworm Meal treatment (E), which used 100% Mealworm Meal and 0% fish meal, with an SGR of 2.38±0.22% per day. The overall Specific Growth Rate (SGR) values during the study are presented in Fig. 4.

The best absolute weight was observed in the 25% Mealworm Meal treatment (C), where 5% of fish meal was substituted with 25% Mealworm Meal, reaching 138±0.22g, while the lowest was in the 100% Mealworm Meal treatment (E), which used 100% Mealworm Meal and 0% fish meal, with a weight of 125±8.42g. The absolute weight data throughout the study are presented in Fig. 5. The results of the water quality parameter measurements showed that during the maintenance period, the water temperature ranged from 25.5 to 29ºC, pH ranged from 7.1 to 8.65, and dissolved oxygen (DO) ranged from 4.2 to 6.1mg/L. These ranges fall within the acceptable criteria according to the national quality standards (BSN, 2005), although the DO value of 4.2mg/L is below the ideal threshold but still tolerable for Nile tilapia.

The fatty acid analysis results showed that treatment B, which involved substituting 75% fishmeal with 25% Hongkong worm meal, produced a significant fatty acid composition. In this treatment, the saturated fatty acid 16:0, known as palmitic acid, was recorded at 15.04±0.06%. Additionally, the omega-3 fatty acid 20:5ω3 or EPA (eicosapentaenoic acid) reached 12.80±0.06%. The amino acid profile of the feed used in the maintenance of Nile tilapia for 49 days is presented in Table 5.

The amino acid analysis results showed that treatment B, which involved substituting 75% fishmeal with 25% Hongkong worm meal, produced an interesting amino acid composition. In this treatment, the lysine content was recorded at 14.65±0.06%, while glutamic acid reached 12.57±0.02%. The fatty acid profile of Nile tilapia after 49 days of feeding is presented in Table 6.

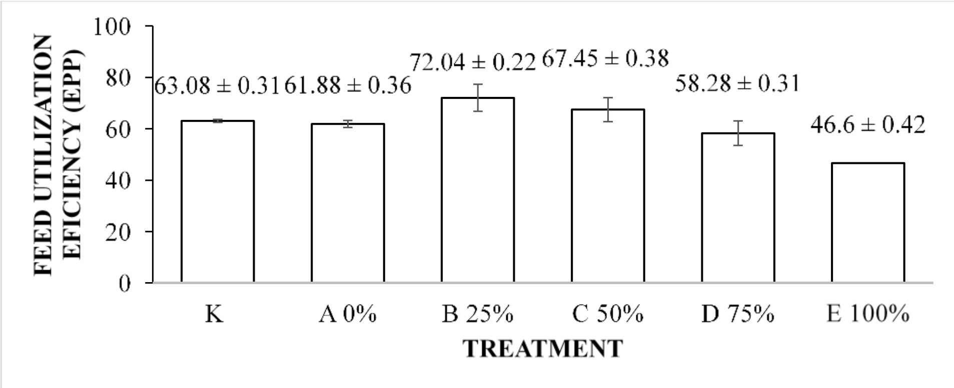

Fig. 2: Feed utilization efficiency of Nile tilapia (*Oreochromis niloticus*) during 49 days of the feeding trial.

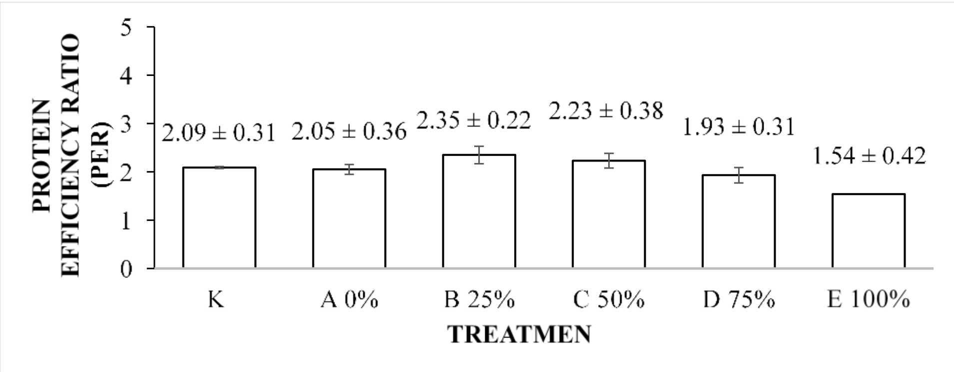

Fig. 3: Protein Efficiency Ratio of Nile tilapia (*Oreochromis niloticus*) during 49 days of the feeding trial.

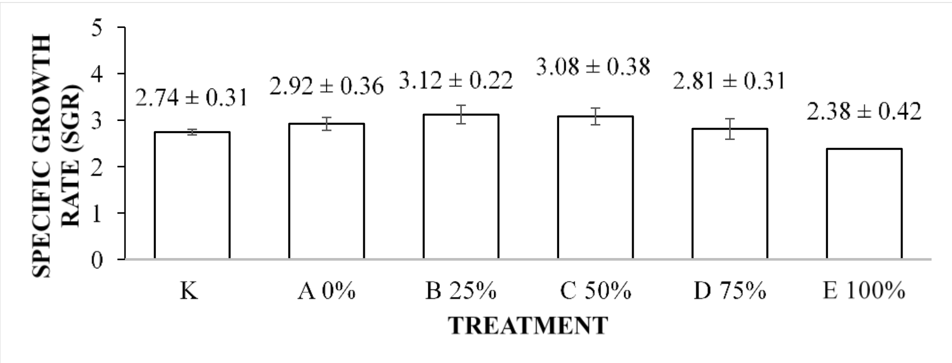

Fig. 4: Specific growth rate of Nile tilapia (Oreochromis niloticus) during the 49-day rearing period.

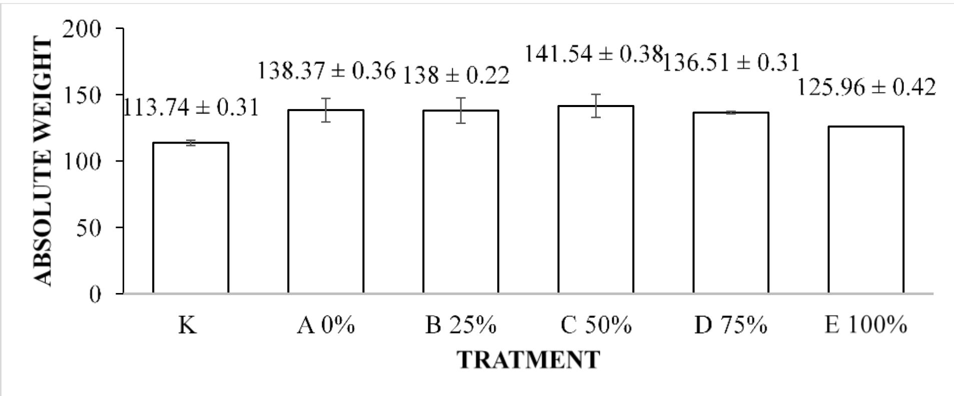

Fig. 5: Absolute weight gain of Nile tilapia (Oreochromis niloticus) during the 49-day rearing period.

The fatty acid analysis showed that treatment B, which involved substituting 75% fishmeal with 25% Hongkong worm meal, produced the best results. The feed from this treatment was given to the test fish and resulted in palmitic acid content of 5.52±0.06% and DHA content of 6.15±0.06%. The amino acid profile of Nile tilapia after 49 days of feeding is presented in Table 7.

The amino acid analysis also showed that treatment B provided the best outcomes. The feed given to the test fish resulted in a significant lysine content of 4.70±0.06%. These findings confirm the potential of Hongkong worm meal as an effective nutritional source that not only improves feed quality but also supports the overall growth and health of the fish.

In recent years, there has been a growing trend in exploring the inclusion of novel ingredients to replace fish meal, particularly in studies involving insect meal. The incorporation of insect meal as a substitute for fish meal

Table 5: Amino acid profile of the feed used in the maintenance of Nile tilapia during 49 days

Asam Amino (%)	K	A (0%)	B (25%)	C (50%)	D (75%)	E (100%)
Arginine	2.90±0.04	2.85±0.05	2.80±0.05	2.75±0.02	2.87±0.05	2.05±0.09
Histidine	2.21±0.02	2.19±0.04	2.30±0.08	2.23±0.05	2.43±0.08	2.13±0.04
Isoleucine	2.40±0.09	2.45±0.08	1.36±0.02	1.36±0.09	1.96±0.02	2.16±0.02
Leucine	8.87±0.02	8.85±0.09	8.96±0.08	8.61±0.03	8.07±0.08	8.77±0.08
Lysine	10.28±0.04	10.93±0.02	14.65±0.06	12.25±0.09	10.,99±0.09	10.57±0.01
Methionine	10.15±0.07	10.12±0.01	11.06±0.03	10.52±0.09	10..60±0.01	10.57±0.05
Phenylalanine	6.99±0.08	6.95±0.09	6.96±0.01	6.75±0.05	7.60±0.05	6.90±0.05
Threonine	4.09±0.02	4.03±0.03	4.23±0.08	4.17±0.08	4.53±0.03	4.10±0.02
Cystine	5.90±0.07	5.97±0.09	6.27±0.03	5.88±0.02	5.07±0.06	5.17±0.01
Valine	7.57±0.04	7.50±0.08	9.05±0.05	8.77±0.06	8.04±0.02	798±0.08
Alanine	0.93±0.09	0.99±0.04	2.06±0.03	0.69±0.02	1.52±0.03	1.89±0.08
Aspartic acid	6.47±0.01	6.52±0.02	6.99±0.09	7.22±0.09	6.52±0.06	6.89±0.02
Glutamic acid	10.03±0.05	10.55±0.07	12.57±0.02	11.25±0.02	10.49±0.02	9.85±0.06
Glycine	4.82±0.05	4.78±0.021	5.07±0.09	4.67±0.08	5.25±0.01	4,99±0.02
Serine	3.69±0.08	3.66±0.09	3.52±0.05	3.48±0.01	3.69±0.07	3,88±0.08
Tyrosine	4.19±0.03	4.23±0.01	4.88±0.01	4.65±0.08	4.23±0.09	4,35±0.05

Table 6: Fatty acid profile in Nile tilapia after 49 days of feeding

Saturated fatty acid	Sample						
	Req	K	(A) 0%	(B) 25%	(C)50%	(D)75%	(E)100%
Methyl Butyrate	< 0.1	0.55±0.04	0.63±0.09	2.88±0.06	1.66±0.02	0.88±0.09	0.76±0.02
Methyl Hexanoate	< 0.1	1.47±0.07	1.59±0.03	1.93±0.02	3.52±0.07	1.89±0.03	3.62±0.07
Methyl Undecanoate	< 0.1	0,89±0.02	1.09±0.02	2.25±0.09	3.47±0.03	3.09±0.02	3.70±0.03
Methyl Laurate	0.23	1.78±0.06	1.83±0.02	1.90±0.08	2.82±0.04	1.83±0.02	2.80±0.04
Methyl Tridecanoate	0.89	2.45±0.08	3.82±0.06	4.78±0.08	2.75±0.03	3.82±0.06	4.75±0.03
Methyl Pentadecanoate	2.27	2.88±0.09	3.46±0.08	4.99±0.09	3.50±0.01	3.86±0.08	4.83±0.01
Methyl Palmitate	0.73	3.74±0.01	3.85±0.02	5.52±0.06	4.75±0.03	3.85±0.02	3.59±0.03
Methyl Heptadecanoate	0.97	1.19±0.08	1.28±0.07	1.80±0.09	2.15±0.05	1.28±0.07	2.40±0.05
Methyl Arachidate	4.75	3.69±0.09	3.73±0.07	3.45±0.03	4.65±0.02	4.37±0.07	4.90±0.02
Methyl Tricosanoate	1.26	1.29±0.08	1.35±0.02	1.93±0.06	2.09±0.03	1.85±0.02	2.39±0.03
Unsaturated Fatty Acid							
Linoleic	< 0.1	1.89±0.05	3.36±0.02	5.97±0.06	4.55±0.04	3.17±0.02	3.10±0.04
Linolenic	< 0.1	2.66±0.06	2.74±0.05	3.18±0.09	3.26±0.06	3.54±0.05	3.50±0.06
Erucate	2.93	1.63±0.03	1.63±0.02	2.62±0.05	3.05±0.01	2.83±0.02	3.17±0.01
Eicosapentaenoic	0.93	1.03±0.08	1.08±0.04	3.17±0.03	2.67±0.01	1.98±0.04	2.87±0.01
Docosahexaenoic	< 0.1	0.65±0.09	5.62±0.02	6.15±0.06	5.79±0.07	4.22±0.02	4.38±0.07

Table 7: Amino acid profile in Nile tilapia after 49 days of feeding

Amino acid	Req*	K	(A) 0%	(B) 25%	(C) 50%	(D) 75%	(E)100%
Arginine	1.74	1.73±0,06	1.78±0,05	2.12±0.09	2.15±0,05	2.09±0,07	2.20±0,09
Histidine	0.78	1.08±0,09	1.19±0,08	1.76±0.02	2.04±0,08	1.70±0,01	2.15±0,08
Isoleucine	0.99	0.85±0,04	0.75±0,03	1.33±0.03	1.19±0,07	1.06±0,09	1.57±0,05
Leucine	1.01	1.26±0,07	1.30±0,09	1.86±0.06	2.03±0,05	1.80±0,07	2.15±0,01
Lysine	2.16	3.19±0,05	3.56±0,04	4.93±0.02	3.85±0,06	3.55±0,08	3,05±0,08
Methionine	1.07	3.06±0,08	3.20±0,07	3.88±0.05	3.70± 0,09	3.54±0,03	2.05±0,10
Phenylalanine	1.64	1.42±0,05	1.40±0,04	1.9±0.04	2.05±0,03	2.12±0,09	2.49±0,07
Threonine	0.90	0.66±0,08	0.75±0,09	1.35±0.02	1.20±0,08	1.18±0,01	1.59±0,03
Tryptophan	1.91	1.10±0,07	1.15±0,06	1.62±0.02	1.80±0,09	1.90±0,07	2.05±0,08
Valine	0.96	1.28±0,08	1.39±0,07	1.58±0.03	1.75±0,05	1.38±0,08	2.05±0,09

may lead to more efficient utilization of fish meal. Although the number of studies reporting 100% substitution of fish meal using insect-based ingredients without compromising fish growth has increased, most research still recommends partial replacement (Hua, 2021). Yellow mealworm meal, as an insect-based ingredient, shows a comparable nutrient profile to fish meal (Hameed et al., 2022), but further evaluation is still required. This experiment aimed to evaluate the benefits and limitations of using yellow mealworm meal as a replacement for fish meal in terms of fish growth performance in Nile tilapia.

DISCUSSION

Feed intake is influenced by several factors including environment, genetics, feed composition, and others. Feed nutrients and additives can affect feed intake and utilization in aquaculture species (Marimuthu et al., 2022). In this experiment, there was a shift in lipid sources due to the gradual increase in yellow mealworm meal inclusion. To the authors' knowledge, the effect of yellow mealworm oil inclusion is still not well understood. However, from various sources of insect oil, black soldier fly (BSF) oil is well-studied and has been reported to have no significant effect on feed intake. Previously, BSF prepupal oil was used in Nile tilapia (*Oreochromis niloticus),* and 100% replacement of fish oil did not affect feed intake (Goda et al., 2024). Similar findings in rainbow trout also showed that BSF oil substitution for fish oil had no effect on feed intake (Fawole et al., 2021).

In line with those experiments, the change in lipid source in this trial diet did not affect feed intake in Nile tilapia. However, contrasting results were observed when the control treatment was taken into account, as the results showed a significant effect ($p<0.05$) on FI, which might be highly influenced by the control treatment value. Further studies on fish oil replacement using yellow mealworm oil may still be required to clarify and elaborate on this phenomenon. Additionally, changes in lipid sources may influence the fatty acid profile, which can affect fish metabolism.

The ability to utilize feed for body structure synthesis is related to the nutritional value of aquatic feed, particularly protein (Teles et al., 2020). As protein is considered the most valuable macronutrient (Aragao et al., 2022; Thiviya et al., 2022), achieving higher protein efficiency is desirable. In this study, feed efficiency, protein efficiency ratio, and specific growth rate showed a similar trend with increasing substitution levels. Diet B (25% substitution of fish meal with yellow mealworm meal) and diet C (50% substitution) achieved better FUE, PER, and SGR values compared to the zero inclusion treatment and higher inclusion levels, indicating better amino acid balance in the diet. A well-balanced amino acid profile in aquafeeds can enhance growth performance and profitability in the aquaculture industry (Li et al., 2008). Furthermore, the control treatment overall did not show significant differences ($P \geq 0.05$) in FUE, PER, and SGR compared to other experimental treatments. We assume the nutrient content in the experimental diets met the nutritional requirements similarly to the control.

Diets D (75% substitution) and E (100% substitution) showed lower performance in growth parameters. Previously, the addition of yellow mealworm as a fish meal replacement was reported to reduce growth performance due to changes in amino acid composition. Higher inclusion levels of yellow mealworm were reported to decrease lysine, methionine, and histidine levels in the diet (Lin et al., 2023). A study substituting yellow mealworm meal with soybean meal also observed increased hepatic crude lipid and mesenteric fat, indicating amino acid imbalance (Zhang et al., 2023). These fat increases may have influenced feed utilization efficiency. Herawati et al., (2023a) reported increases in FUE, PER, and SGR in common carp (Cyprinus carpio) when substituting fish oil with similar insect fat, namely BSF maggot oil. Thus, to some extent, the lipid source shift may have positively influenced PER and SGR in this study.

Another factor potentially affecting feed utilization and growth performance is the presence of chitin. Chitin is a complex carbohydrate typically found in insect-based ingredients (Sankian et al., 2018). Chitin can influence the gut microbiota as some bacteria utilize it as a prebiotic, promoting the growth of heterotrophic bacteria such as Bifidobacterium and Lactobacillus (Imathiu, 2020). LAB bacteria (including Bifidobacterium and Lactobacillus) can improve growth performance by producing digestive enzymes that enhance nutrient digestibility and feed conversion (Wang et al., 2023).

Despite its prebiotic properties, chitin may also have adverse effects when inclusion levels exceed certain thresholds. Chitin is characterized by nitrogen bound in polysaccharides present in insect exoskeletons, leading to overestimated protein values (Pascon et al., 2024; Hong et al., 2020). Excessive chitin may affect digestibility and nutrient bioavailability by binding digestive enzymes, reducing feed utilization (Belghit et al., 2018). Previous studies indicated that 5% chitin in aquatic feed did not affect the growth of Atlantic cod and Atlantic halibut (Karlsen et al., 2015). There are also reports of lower chitin levels improving digestibility in insect-protein-based aquafeeds (Eggink et al., 2022). Although this study lacks direct chitin content data, we assume that Diets B (25%) and C (50%) contained tolerable levels of chitin. Further research on chitin inclusion in aquafeeds is needed to determine acceptable inclusion thresholds.

In this study, mealworm meal had no effect on survival rate. The observed survival rate may have been affected by extreme weather changes that caused temperature drops in the early phase of the trial. A similar condition was reported in the study of Herawati et al. (2023b), where no effect on survival rate of vannamei shrimp (*Litopenaeus vannamei*) was observed. Temperature fluctuations can stress Nile tilapia and lead to mortality (Sherif et al., 2024). Smaller Nile tilapia may also be more susceptible to sudden temperature drops than larger individuals (El-Hack et al., 2022).

To some extent, substitution of fish meal with insect meal improved lysine and glutamic acid availability in the feed. In this study, Diet B (25% substitution) had the highest lysine and glutamic acid contents compared to the other diets, including the control. Lysine plays a vital role in metabolism and physiological processes in fish. It is absorbed in the intestines as L-lysine, deposited in tissues and organs, and is actively involved in protein synthesis and muscle growth. Without lysine, the utilization of other amino acids is limited (Yu et al., 2023). Moreover, glutamic acid improves feed utilization efficiency by aiding the synthesis of arginine, proline, and glutathione, which support intestinal health and enhance nutrient absorption (Indriati et al., 2023). In this study, the high lysine content in Diet B led to higher lysine levels in fish from the B treatment group compared to others, including the control.

The inclusion of yellow mealworm meal also improved the fatty acid profile to some extent. Diet C (50% substitution) showed the best fatty acid profile with higher omega-3 (ALA, EPA, and DHA) content than other diets, including the control. According to Lawal et al., (2021), yellow mealworms can synthesize linoleic acid (LNA) and alpha-linolenic acid (ALA), but EPA and DHA are not present in this insect. This is supported by the observed increase in alpha-linolenic acid levels in both feed and fish as mealworm inclusion increased. In fish, ALA contributes to growth, protein deposition, immune resistance, and reduction of inflammation and lipid oxidation (Behairy et al., 2024). Thus, changes in fatty acid content due to fish meal substitution with yellow mealworm meal had a positive effect on the fatty acid profile and overall health condition of Nile tilapia.

Conclusion

Fish meal substitution using yellow mealworm meal at a certain level did not impair growth performance. A 25% replacement level is recommended as the best treatment in this study. The effects of fish meal substitution on FUE, PER, and SGR may be attributed to the amino acid profile and chitin content. The substitution did not affect Nile tilapia's feed intake and survival rate. The feed influenced the amino acid and fatty acid profiles in Nile tilapia, with the 25% fish meal replacement resulting in the highest amino acid and fatty acid values.

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