Evaluation of Different Protein Blends on Growth Performance, Nutrient Utilization and Carcass Composition of Nile Tilapia (*Oreochromis niloticus*) Fingerlings

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**Authors’ contributions**

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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**ABSTRACT**

The effects of different protein blends (gliricidia/moringa leaf meals, soybean meal and defatted palm weevil larvae) on Nile tilapia (*Oreochromis niloticus*) fingerlings were studied for a 10-week feeding period. Six diets with 30 percent crude protein content were formulated, the protein sources were included in equal ratios. Diets were designated as fishmeal/soybean (FMS), palm weevil/soybean (PWS), palm weevil/moringa (PWM), palm weevil/gliricidia (PWG), palm weevil/soybean/moringa (PSM) and palm weevil/soybean/gliricidia (PSG). Trials were carried out in triplicates with each having ten (10) fish in a total of 18 tanks. Fish fed diet PSG had the highest mean weight gain of 4.35±0.08 and protein efficiency ratio (PER) of 1.14±0.02. These values were significantly different (P<0.05) from those recorded for fish on other diets. Fish fed diet PSG gave the lowest food conversion ratio (FCR) of 1.10±0.02, with those on diet PWG recording the least. Fish fed diets containing soybean meal (PSM and PSG) gave better performances than those without it. The carcass protein, ash and fat contents differed significantly (P<0.05). This results showed that *Oreochromis niloticus* fingerlings fed a mix of protein blends could give good performance in terms of growth, nutrient utilization and carcass constituents.
Keywords: Growth performance; nutrient utilization; Oreochromis niloticus; defatted palm weevil larvae.

1. INTRODUCTION

The output of fish has increased dramatically over the past few decades, with 179 million tonnes produced worldwide in 2018 through capture fisheries and aquaculture, of which 22 million tonnes were used to produce fishmeal and oil [1]. Fishmeal has been employed in the formulation of intensive fish feed due to its nutritional balance and high nutritional value for humans and other livestock [2]. The use of it for purposes other than feeding is limited by its economic worth, competition with human resources and environmental sustainability [3, 4]. Novel and unconventional protein sources that are cheap, readily available, insect meals and other plant proteins have been identified as possible substitutes for fishmeal that are currently thought to have little competition [5, 6].

Due to their sustainability, availability and affordability, plant protein sources have been incorporated into the formulation of intensive fish feeds. Aquatic plants such as azolla, water hyacinth, duckweeds, water lettuce, bur-reed, and water fern as well as alfalfa, mulberry, sweet potato, cassava, cucumber, squash, broad bean, papaya, moringa, leucaena, cocoyam, ipil-ipil, banana, and akee leaves have all been utilized in the fish feed industry [7]. Moringa leaves have been successfully used without compromising the performance of (Oreochromis niloticus, Linnaeus, 1758), (Cyprinus carpio, Linnaeus, 1758) and (Clarias gariepinus, Burchell, 1822) [8, 9]. Various studies have also reported that optimum inclusion level of 10-20% moringa leaves can be used in fish feed formulation [6]. Gliricidia leaves have shown growth enhancement in Clarias gariepinus [10]. The findings of Adeparusi [11] showed that the dietary supplementation of Bambara nut with leucaena and gliricidia leaf meal concentrates in the diet of Oreochromis niloticus improved fish performance and [12] also demonstrated that the inclusion of Gliricidia Maculata leaf meal in (Cirrhinus mrigala, Hamilton, 1822) improved feed efficiency and growth.

In recent years, the availability, environmental protection and cost reduction ability of insect meals have made researchers to recognize them as potential substitutes for fishmeal in aquaculture feed manufacturing [5, 13]. Insects are excellent sources of protein (45%-75%), have a balanced essential amino acid profile [5], appropriate levels of minerals [14] and are sustainable resources for nutrition. According to scientific findings, insect meals can serve as a viable alternative to fish meal, fish oil, and other traditional protein sources [8, 15, 16, 17], since interesting results have been obtained from the use of them as fishmeal substitutes in the diets of some fish species [3, 4].

Rhynchophorus phoenicis (palm weevil) is a species of palm weevil and a member of the Curculionidae family. It is an important pest of sugarcane (Saccharum officinarum), palm oil (Elaeis guineensis), coconut palms (Cocos nucifera) and date palms (Phoenix dactylifera) [18]. It contains about 25-66% crude protein and is a source of important amino acids [19, 18]. The palm weevil's nutritional value makes it an ideal fish feed supplement/replacement for fishmeal [19]. Researches have shown that palm weevil larvae meal contains amino acid content such as leucine, phenylalanine-tyrosine, trptophan etc. [20, 21]. Values of 4.51 and 3.15 have been recorded for lysine and methionine-cysteine respectively [21], these values are comparable to FAO/WHO reference standard [22]. Palm weevil larvae meal supports the healthy growth of the Clarias gariepinus [15], Heterobranchus longifilis and Clarias gariepinus, whether supplemented or consumed as a complete diet [23]. It has been demonstrated that palm weevil larvae meal can replace fishmeal up to 100% in the diet of African catfish without raising the feed conversion ratio (FCR) or reducing weight gain [15].

In our search, no previous studies have been done on Oreochromis niloticus using the blends of defatted palm weevil meal with gliricidia or moringa leaf meals as these feed ingredients are easily sourced and economical. Therefore, in order to increase fish production and solve the issue of the relatively expensive fish meal, this research was done to evaluate the growth performance, nutrient utilization and carcass composition of Nile Tilapia fed different blends of defatted palm weevil larvae and/or gliricidia/moringa leaf meals. Thereby affording small-scale farmers the ability to raise fish (tilapia) at low cost without compromising quality.
2. MATERIALS AND METHODS

2.1 Experimental Location

The experiment was carried out at the Department of Fisheries Technology, Teaching and Research Farm, Federal College of Agriculture, Akure, Ondo State, Nigeria.

2.2 Diet Preparation and Formulation

Table 1 shows the proximate composition of defatted palm weevil larvae meal (Rhynchophorus phoenicis), moringa (Moringa oleifera), gliricidia (Gliricidia sepium), with crude protein contents of 65.7%, 25.15% and 22.3% respectively.

| Compositions       | Gliricidia | Moringa | Palm weevil |
|--------------------|------------|---------|-------------|
| Moisture           | 9.51       | 8.51    | 9.72        |
| Crude protein      | 22.3       | 25.15   | 65.7        |
| Ash                | 8.24       | 11.65   | 12.67       |
| Lipid              | 1.41       | 2.91    | 3.9         |
| Fibre              | 14.46      | 14.95   | 1.55        |

2.2.1 Preparation of palm weevil larva (Rhynchophorus phoenicis) and leaf meals

Live African palm weevil (Rhynchophorus phoenicis) larvae were procured from a local market at Ilaje, Ondo State, Nigeria. The insect larvae were washed, blanched and oven dried at 80°C for 24 hours [24]. The larvae were defatted by n-hexane soxhlet extraction, oven dried, and hammer milled into a fine powder (Lab Mill, screen size 0.2 mm). Proximate analysis of the defatted sample was done as described by [25]. Moringa oleifera and Gliricidia sepium leaves were obtained within the study area. The leaves were washed, strained and spread on plastic sheets to dry under shade for a week. The dried leaves were threshed from stalks and ground into fine powder by the use of a hammer mill (Lab Mill, screen size 0.2 mm) and the powder was stored in plastic bags prior to use.

2.2.2 Diet formulation

A 30% iso-nitrogenous diet was formulated using the trial and error method. Ingredients as oil, binder, vitamin and mineral premix (fixed ingredients) was kept constant for all the diets and maize was used as a filler to balance up the quantity of each diet to 100%. Blends of fishmeal/soybean (FMS) and defatted palm weevil/soybean (PWS) served as controls for the six iso-nitrogenous diets. The six diets comprised of fishmeal with soybean meal (FMS control), defatted palm weevil meal with soybean meal (PWS control), palm weevil with moringa (PWM), palm weevil with gliricidia (PWG), palm weevil meal with soybean and moringa (PSM), and palm weevil with Soybean and gliricidia (PSG). The feed composition included ingredients that were thoroughly combined with corn starch as a binder and extruded through a

| Compositions       | FMS | PWS | PWM | PWG | PSM | PSG |
|--------------------|-----|-----|-----|-----|-----|-----|
| Fishmeal           | 23.25 | -   | -   | -   | -   | -   |
| Soybean meal       | 23.25 | 23  | -   | -   | -   | 19.5 | 20.3 |
| Palm weevil meal   | -   | 23  | 29.5| 30.6| 19.5| 20.3 |
| Moringa            | -   | -   | 29.5| 30.6| 19.5| -   |
| Gliricidia         | -   | -   | -   | -   | -   | 20.3 |
| Maize              | 43.5| 44  | 31  | 28.8| 32.5| 29.1 |
| Vit/Min premix     | 2   | 2   | 2   | 2   | 2   | 2   |
| Bone Meal          | 2   | 2   | 2   | 2   | 2   | 2   |
| Oil                | 4   | 4   | 4   | 4   | 4   | 4   |
| Corn Starch        | 2   | 2   | 2   | 2   | 2   | 2   |
| Total              | 100 | 100 | 100 | 100 | 100 | 100 |

Vitamin premix: An animal care "optimix Aqua product for tilapia, containing the following per 5kg of premix: A=20 000 000 I U, D3= 2 000 000 I U, E= 200 000mg, K3= 10 000mg, B2= 12 000mg, B12= 9mg, Bl= 6 000mg, B6= 11 000mg, C= 50 000mg, Folic acid= 2 000mg, Niacin= 80 000mg, Calpan= 25 000mg, Biotin= 100mg x Zinc= 30 000mg, Manganese= 50 000mg, Iodine= 1000mg, Selenium= 100mg, Antioxidant= 125 000mg. Fishmeal and soybean meal (FMS), palm weevil meal with soybean meal (PWS), palm weevil with moringa (PWM), palm weevil with gliricidia (PWG), palm weevil meal with soybean and moringa (PSM), palm weevil with Soybean and gliricidia (PSG)
2-mm die (Moulinex-HV8) mincer. The pellets were then sealed in plastic bags after being sun-dried on elevated platforms.

2.2.3 Feeding trial

For this investigation, 180 Nile Tilapia (*Oreochromis niloticus*) fingerlings with an average weight of (29.15±1.5g) were obtained from the farm of the Federal College of Agriculture. Fish were acclimated to experimental conditions for 2 weeks and placed on commercial diet. Ten fish were randomly weighed into eighteen (18) plastic tanks with dimension (60 cm x 30 cm), which consisted of six treatments in triplicates. Fish were fed twice daily at 5% of their body weight between 8:00 and 9:00 and 16:00 and 17:00 for 70 days. Water renewal was done twice a week, while fish were weighed and counted fortnightly. For the 70-day period, feed intake was adjusted biweekly depending on weight gain and daily mortality checks. Selected water parameters (temperature, pH, dissolved oxygen concentration) were monitored daily to maintain optimal water quality conditions using a Yieryi Multi-parameter digital water quality tester.

Growth Parameters were assessed using the following formulas.

**Mean Weight Gain (MWG)**

\[ MWG = WF - WI \]

Where, \( WF = \) Final weight

\( WI = \) Initial weight

**Specific Growth Rate (SGR)**

\[ SGR = \frac{\ln(\text{final weight}) - \ln(\text{initial weight})}{\text{culture period}} \times 100 \]

**Feed Conversion Ratio (FCR)**

\[ FCR = \frac{\text{Total Feed Intake}}{\text{Total Weight}} \]

**Feed Efficiency Ratio (FER)**

\[ FER = \frac{\text{Weight gained}}{\text{protein fed}} \]

**Protein Efficiency Ratio (PER)**

\[ PER = \frac{\text{Mean Weight Gain}}{\text{Mean Pi}} \]

**Survival rate**

\[ = \frac{\text{Number of fish stoked} - \text{mortality}}{\text{Initial number of fish}} \times 100 \]

2.2.4 Sample analysis

Experimental fish carcass and feed samples were analyzed for proximate composition using the methods described by [22]. Data obtained were expressed as mean ± standard error (S.E) and subjected to a one-way ANOVA design in triplicates (SPSS 22) at statistical significance level of 95%. The variance was separated using Duncan’s multiple range test.

3. RESULTS

Fig. 1 shows the water quality parameters, the temperature ranged between 25.89°C to 26.50°C, the pH ranged between 7.07-7.20 and the dissolved oxygen ranged from 5.08 to 5.25 mg/L, there were no significant difference in the various parameters.

![Bar chart showing water quality parameters](image-url)

**Fig. 1. Water quality parameters**
3.1 Proximate Composition of the Experimental Diets

The result of the proximate composition of the experimental diets is presented in Fig. 2. The highest moisture content value was recorded in diet PWM and lowest in diet containing PWS respectively. The Crude protein ranged between 29.73-31.31 in diets PSM and PWM respectively. Fish fed diet PSM got the highest lipid content value of 11.78 and the lowest value of 8.91 was recorded in those fed diet PWG. There were no appreciable variations (P>0.05) across the dietary regimens.

3.2 Growth Performance and Nutrient Utilization

Table 3 below shows the growth performance and nutrient utilization of Oreochromis niloticus fed experimental diets. Fish fed diet PSG had the highest weight gain of 43.57g, this was significantly different (P< 0.05) compared to the other experimental fish. Fish fed diet PWS exhibited a lower weight gain (32.77g) than those on other experimental diets. Fish fed the PSG diet had the lowest feed conversion (FCR) and the highest SGR value (1.31±0.07), which were statistically different (P< 0.05) from fish fed other experimental diets. The PER ranged from 0.92±0.07 to 1.14±0.02, there was no significant difference (P>0.05) in the PER of fish in all the experimental units, except in fish fed diet PSG. The FER value ranged from 0.76±0.06 to 0.91±0.03 and were not significantly different (P>0.05) from each other.

There were no fish mortalities during the trial as survival rate was 100% among all experimental units.

4. DISCUSSION

Results from this study showed that the various experimental diets increased fish weight gain (WG), with fish fed diet PSG recording the best weight gain (WG) among the various experimental fish. Similarly, fish on the PSG diet also showed the best nutrient utilization in terms of feed conversion ratio, feed efficiency ratio and protein efficiency ratio. There were appreciable differences in the growth performance of fish fed diet PSG compared to those on other diets. The documented variances recorded in growth performance could have resulted from variations in the protein blends used in the diets. Fish fed the PWS diet gave the least performance in terms of growth performance. However, this did not significantly differ from the other experimental fish, except those fed diet PSG. This could be adduced to the feed composition and/or palatability. This agrees with the report of [24], who stated that adding Black Soldier fly meal and soybean to a Nile Tilapia diet stunted growth as the inclusion level rose, but disputes the report of [25], who reported that the blend of defatted palm weevil and soybean increased fish performance.
Table 3. Growth performance and nutrient utilization of *Oreochromis niloticus* fed experimental diets for 70 days (Mean ± SE)

| Treatments       | FMS          | PWS          | PWM          | PWG          | PSM          | PSG          |
|------------------|--------------|--------------|--------------|--------------|--------------|--------------|
| **Initial Weight** | 30.23± 2.04a | 28.50±1.47a  | 28.30±1.34a  | 30.93±1.07a  | 27.93±1.13a  | 28.93±1.97a  |
| **Final Weight**  | 65.70±1.7abc | 61.27±1.6a   | 63.73±1.93ab | 66.67±1.40abc| 65.03±1.01abc| 72.50±1.55c  |
| **Weight Gain**   | 35.47±1.62ab | 32.77±2.57a  | 35.43±1.26ab | 35.73±0.38ab | 37.10±0.51ab | 43.57±0.84c  |
| **MWG**           | 3.55± 0.16ab | 3.28±0.26a   | 3.54±0.13ab  | 3.57± 0.04ab | 3.71± 0.05ab | 4.35± 0.08c  |
| **SGR**           | 1.11± 0.08a  | 1.10± 0.10a  | 1.16± 0.04ab | 1.10± 0.02a  | 1.21± 0.04ab | 1.31± 0.07b  |
| **FCR**           | 1.21±0.05bc  | 1.25±0.09bc  | 1.15±0.04ab  | 1.20±0.01b   | 1.13±0.02a   | 1.10±0.02a   |
| **FER**           | 0.83±0.04ab  | 0.80±0.06a   | 0.87±0.03ab  | 0.84±0.01ab  | 0.89±0.03ab  | 0.91±0.03c   |
| **PER**           | 0.99±0.04ab  | 0.96±0.06ab  | 1.04±0.07a   | 1.00±0.05ab  | 1.07±0.03ab  | 1.14±0.02bc  |
| **Feed intake**   | 79.5±0.13ab  | 76.6±0.12a   | 73.3±0.03a   | 73.3±0.01a   | 73.5±0.06a   | 79.8±0.01ab  |

MWG– Mean Weight Gain, SGR– Specific Growth Rate, FCR– Feed Conversion Ratio, FER– Feed Efficiency Ratio, PER– Protein Efficiency Ratio. Different letters within a row indicate significant differences (P< 0.05). Fishmeal and soybean meal (FMS), palm weevil meal with soybean meal (PWS), palm weevil with moringa (PWM), palm weevil with gliricidia (PWG), palm weevil meal with soybean and moringa (PSM), palm weevil with Soybean and gliricidia (PSG)
The utilization of protein blends may be constrained due to the presence of anti-nutrients and variations in feed consumption which have been shown to reduce fish weight gain [26]. However, the processing methods employed in this study (defatting, drying and sieving) could have reduced some of the anti-nutrients present in the diets, thereby enhancing feed utilization.

Our findings also imply that a diet in which defatted palm weevil completely replaced fishmeal produced superior results to the control diet. Studies have demonstrated that using a variety of protein blends increased fish performance over using a single source of protein. The findings in our study showed that the blends that contained three protein sources gave better performances compared to those that had two. This has been ascribed to the complementary effects of amino acids from the different protein sources [27, 28]. Supporting this pattern, fish fed diets comprising mixtures of PSM and PSG performed better than fish fed diets with just two protein sources. This effect corroborates those of [12], where gliricidia was used to replace FM up to 40% in *Cirrhinus mrigala* without compromising growth [8] also reported that moringa supplementation in *Clarias gariepinus* improved growth. This contrast earlier reports in which fish fed diets supplemented or replaced with fishmeal exhibited reduced growth. The works of [29] revealed that feeding diets containing Black soldier fly larvae to *Lates Calcarifer* inhibited its growth. Nile Tilapia growth was also reduced when fed diet containing *Tenebrio molitor* [14].

The FCR of the experimental diets was low and comparable, showing good utilization of the experimental diets. Fish fed diet PSG recorded the best nutrient utilization while having the lowest FCR (1.10), this is comparable to the works of [23], where *C. gariepinus* fed on palm grub-containing diets showed low FCR. The findings of [30] showed that Turbot given BSF Larvae meal had a high FCR due to the diet's poor palatability, this contradicts those gotten in our study. The protein efficiency ratio (PER) values of fish fed experimental diets were comparable between the experimental fish, indicating that the dietary protein were similar and effectively used by fish. This is in agreement with work by [23], where palm grub meal was fed to *C. gariepinus*. This contrasts the findings of [30], who found that turbot (*Psetta maxima*) fed diets containing defatted BSF larvae had poor feed utilization in comparison to the control.

### 4.1 Carcass Composition

In line with the findings of Opiyo [31], the initial carcass crude protein (CP) level in this study was lower than the CP levels found at the end of the feeding trial. Fish fed diet PSG had the highest crude protein content (60.80%), while fish fed diet PWG had the lowest crude protein content (50.59%). The percentage crude protein found in this study revealed a significant difference (P<0.05) between fish fed diet PSG and those fed other diets. Fish fed diet PWS (17.95) had the highest ash level, while diet PWM (13.13) had the lowest. Fish fed diet FSG (17.66) had the highest fat content, while fish fed diet FMS had the lowest (12.73). The fish carcass composition varied, which could have been caused by variations in the quality of the feed, the rate at which the muscles deposits it, the amount of nutrients in the diet and the capacity of the fish to convert food into absorbable nutrients [32]. Fish on the experimental diets had greater values for crude protein, crude fat, ash, and NFE than the baseline fish. This suggests that the experimental fish's carcass quality may have been impacted by the dietary treatments. The same patterns have been noted by [33, 12] at the conclusion of the feeding trial and lower in others [34].

The marked reduction in the protein composition of fish fed diet PWS and PWG could be due to the feed composition or imbalance EAA profile. This is in agreement with those reported by [16] when FM was replaced with black soldier fly meal in Rainbow trout, but disagrees with those of [28] where maggot meal was fed to *O. niloticus* fingerlings improving the carcass quality. There was no relationship between the dietary protein content and the carcass fat composition of *O. niloticus* in this study, this is in contrast with those reported by [35] who reported that carcass lipid content correlated with dietary lipid level in tilapia.

### 5. CONCLUSION

Over the years, the search for alternative protein sources as fishmeal replacement in aquaculture feed has been a subject of extensive research. The use of fishmeal in livestock feed has dwindled fish population in the wild due to overexploitation. Alternative and novel feed ingredients that meets the nutritional requirements of fish are being looked into, that would increase production and invariably reduce...
overdependence on fishmeal, which has been overused to the detriment of wild fish population. The results of this study indicate that the use of alternative protein sources had positive impacts on the growth performance, nutrient utilization and carcass composition of Nile Tilapia (*Oreochromis niloticus*). This indicates that defatted palm weevil larvae which has a crude protein content similar to fishmeal can totally replace the FM without causing any negative effect on the health of the fish especially in combination with soybean and gliricidia meals. The diet that contained the blends of PSG was the most suitable for the successful culture of the Nile tilapia fingerlings. However, further research should be carried out to know the synergy between soybean meal and gliricidia leaf meal and their combined effect on fish. It could be recommended that fish farmers be encouraged to culture palm weevil as feed supplement.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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