

## **Revised Research Article**

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

### **Abstract**

The effects of different protein blends (gliricidia/moringa leaf meals and defatted palm weevil larvae) on Nile tilapia (*Oreochromis niloticus*) fingerlings were investigated during a 10-week feeding trial. Six experimental diets with 30 percent crude protein were formulated 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). With 10 fish each, 180 Nile tilapia fingerlings were divided into 18 plastic tanks in triplicates. The findings showed that fish fed diet PSG had final weights and protein efficiency ratios (PER) that were significantly different ( $P < 0.05$ ) from those on other diets. Fish on diet PSG gave the lowest food conversion ratio (FCR) of  $1.10 \pm 0.02$ , with those on PWS and PWG recording the least overall performance. Experimental fish's carcass protein, ash and fat contents differed significantly. This study suggests that *Oreochromis niloticus* diet could be replaced with blends of defatted palm weevil, soybean and gliricidia leaf meals.

Keywords: Growth performance, Nutrient utilization, *Oreochromis niloticus*, Defatted palm weevil larvae.

### **1.0 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 <sup>1</sup>. Due to its nutritional balance and high nutritional value for humans

and other livestock, fishmeal has been employed in the formulation of intensive fish feed <sup>2</sup>. The use of it for purposes other than feeding is limited by its economic worth, competition with human resources, and environmental sustainability.<sup>3,4</sup>. 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. <sup>5, 6</sup>.

Comment [OM1]: Add

Due to their sustainability, availability, and affordability, plant protein sources have been incorporated into the formulation of intensive feed. Aquatic plants such 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 <sup>7</sup>. Moringa leaves have been successfully used without compromising the performance of (*Oreochromis niloticus*, Linnaeus, 1758), (*Cyprinus carpio*, Linnaeus, 1758) and (*Clarias gariepinus*, Burchell, 1822) <sup>8, 9</sup>. Various studies have also reported that optimum inclusion level of 10-20% moringa leaves can be used in the formulation diet for fish <sup>6</sup>. Gliricidia leaves have shown demonstrated growth enhancement in *Clarias gariepinus*, *Oreochromis niloticus* and (*Cirrhinus mrigala*, Hamilton, 1822)<sup>10, 11, 12</sup>.

In recent years, due to its stability, ability to reduce costs, and environmental protection, researchers have recognized insect meal as a potential substitute for fishmeal in the manufacturing of aquaculture feed. <sup>5</sup>.

Comment [OM2]: What is explanation for stability? Is it due to predictable production and availability?

<sup>13</sup>. They insects are excellent sources of protein (45%-75%), balanced essential amino acid profile <sup>5</sup>, appropriate levels of minerals <sup>14</sup> and sustainable resources for nutrition. According to scientific findings, insects can serve as a viable alternative to fish meal, fish oil, and other traditional protein sources <sup>8, 15, 16</sup>, <sup>17</sup>, as since interesting results have been obtained emanated from the use of insect meals as fishmeal substitutes in the diets of some fish species.

Comment [OM3]: It is clear to add Insects here.

*Rhynchophorus phoenecis* a species of palm weevil, and is a member of the Curculionidae family. It is an important A-significant pest of that harms sugarcane (*Saccharum officinarum*), palm oils (*Elaeis guineensis*), coconut palms (*Cocos nucifera*) and date palms (*Phoenix dactylifera*)<sup>18</sup>. It contains about 25-66 % crude protein and is a source of important amino acids<sup>19, 18</sup>. The palm weevil's nutritional value makes it an ideal fish feed supplement or replacement for fishmeal. <sup>19</sup>Researches have shown that palm

Comment [OM4]: There is need for reference here

Comment [OM5]: Remove is and add "and".

Comment [OM6]: Please indicate the amino acid profile of the insect meal for comparison.

weevil larvae meal when supplemented or as complete diet, supports the healthy growth of the *Clarias gariepinus*<sup>15</sup>, *Heterobranchus longifilis* (Fakayode and Ugwumba, 2013), *Clarias gariepinus* (Fakayode and Ugwumba, 2013)<sup>20</sup>. According to<sup>15</sup>, palm weevil larvae meal can replace fishmeal up to 100% in a complete diet of African catfish without increasing the feed conversion ratio (FCR) or reduction in weight gain. In my search ~~To the best of my knowledge~~, no previous studies have been done on *Oreochromis niloticus* using the blends of defatted palm weevil meal with gliricidia or moringa(??).

**Comment [OM7]:** You can remove and connect the statement as I have suggested without losing the message.

**Comment [OM8]:** Why did you isolate these plants for this study?

**Comment [OM9]:** You would normally not start a statement with "So"

~~So~~, 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.

## 2.0. 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.

#### 2.1.1. Preparation of Palm Weevil Larva (*Rhynchophorus phoenicis*)

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 according<sup>21</sup>. 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<sup>22</sup>.

#### 2.1.2. Diet formulation

Blends of fishmeal/soybean (FMS) and defatted palm weevil/soybean (PWS) served as controls for the six isonitrogenous diets, each of which contained 30% crude protein. 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), palmweevil mealwith soybean and moringa (PSM), and palm weevil with Soybean and gliricidia (PSG). The feed composition included ingredients

**Comment [OM10]:** There are several steps to diet formulation:  
a) Indicate nutrient requirement of the fish  
b) Select ingredients and their composition  
c) Show basal or fixed portion of the formulation according to minimum needs  
d) Include vitamins, minerals and other supplements.

that were thoroughly combined with maize starch as a binder and extruded through a 2-mm die (Moulinex-HV8) mincer at constant amounts of oil, vitamins, and mineral premix. The pellets were then sealed in plastic bags after being sun-dried on elevated platforms until dry.

**What was the protein/energy balance?? Better still DE/DP should be known for the rations.**

**Comment [OM11]:** Since this is done for the first initial steps should followed as follows: Selection of ingredients and their composition, 2) basal and fixed part of the formulation, 3) vitamins, minerals etc.

**Table 1: Gross composition of the experimental diets (g/100g)**

	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<sup>®</sup> 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, B1= 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

### 2.1.3. Feeding trial

For this investigation, 180 Nile Tilapia (*Oreochromis niloticus*) fingerlings with an average weight of (3.02±0.13g) 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. **Eighteen** (18) plastic tanks (60 cm x 30 cm) containing ten (10) fish each and six treatments in triplicates were filled with randomly weighed

**Comment [OM12]:** An accepted practical arrangement statement.

fish. 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 ~~in each tank~~ were weighed and counted fortnightly. For the 70-day period, feed intake was adjusted biweekly depending on weight gain and daily mortality checks ~~were made~~. Selected Water parameters (temperature, pH, dissolved oxygen concentration) were monitored ~~continuously~~ to maintain optimal water quality conditions using a Yieryi Multi-parameter digital water quality tester.

**Comment [OM13]:** I suggest that you remove these words.

**Comment [OM14]:** Is it possible to give time when records were conducted?

Growth Parameters were assessed using the following formulas.

Mean Weight Gain (MWG)

$$MWG = WF - WI$$

Where, WF = Final weight

WI = Initial weight

$$\text{Specific Growth Rate (SGR)} = \frac{\ln(\text{final weight}) - \ln(\text{initial weight})}{\text{Culture period}} \times 100$$

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Total Feed Intake}}{\text{Total Weight}}$$

$$\text{Feed Efficiency Ratio (FER)} = \frac{\text{Weight gained}}{\text{protein fed}}$$

$$\text{Protein Efficiency Ratio (PER)} = \frac{\text{Mean Weight Gain}}{\text{Mean PI}}$$

$$\text{Survival rate} = \frac{\text{Number of fish stoked} - \text{mortality}}{\text{Initial number of fish}} \times 100$$

#### 2.1.4. Sample analysis

Experimental fish carcass and feed samples were analyzed for proximate composition using the methods described by<sup>22</sup>. 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.

## 2.2. Results

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. The data obtained were within the range permissible for the culturing of Nile tilapia.

**Comment [OM15]:** It is even better to compute oxygen saturation which affects feeding efficiency.

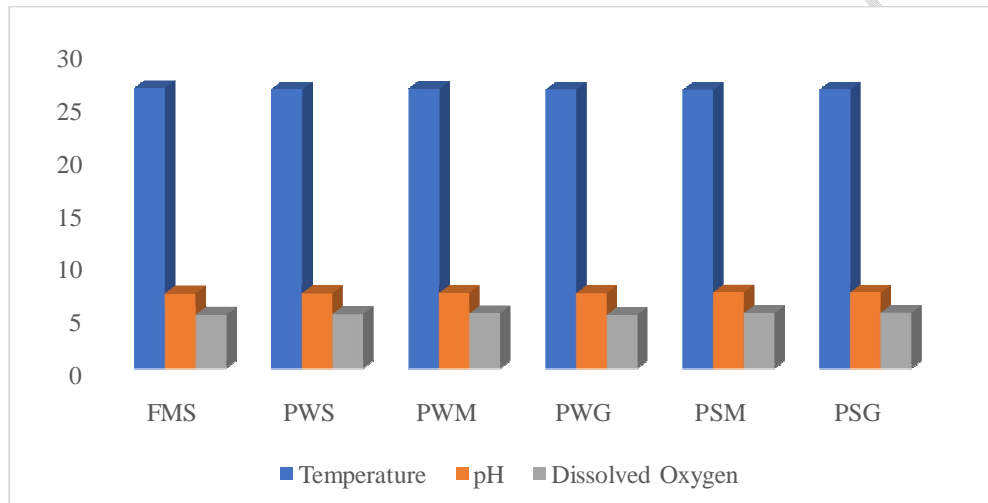


Fig 1: Water quality parameters

### 2.2.1. Proximate composition of the experimental diets

The result of the proximate composition of the experimental diets is presented in figure 2 below. 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

**Comment [OM16]:** Moisture content was relatively high. Was this a dry or moist diet?

PWG. There were no appreciable variations ( $P>0.05$ ) across the dietary regimens

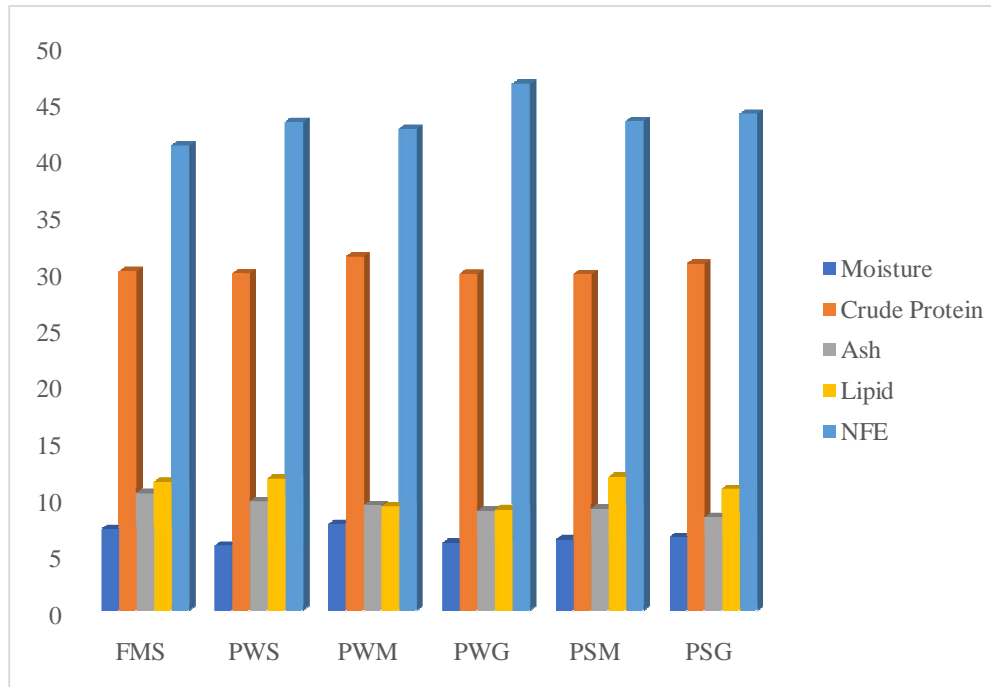


Fig 2: Proximate composition of the experimental diets

### 2.2.2. Growth performance and Nutrient Utilization

Table 2 below shows the growth performance and nutrient utilization of *Oreochromis niloticus* fed experimental diets. Fish fed diet PSG gained the most weight 43.57g compared to the other experimental fish and this difference was significant ( $P<0.05$ ). Fish fed diet PWS exhibited significantly lower weight gain (32.77g) compared to those on other experimental diets. Fish fed the PSG diet had the lowest feed conversion (FCR) and the highest SGR value ( $1.31\pm 0.07$ ), which were statistically different ( $P<0.05$ ) from fish fed the other experimental diets. The PER ranged between  $0.92\pm 0.07$  to  $1.14\pm 0.02$ , there was no significant difference ( $P>0.05$ ) in the PER of fish in all the experimental units. The FER value ranged between  $0.76\pm 0.06$  to  $0.91\pm 0.03$  and were not significantly different ( $P>0.05$ ) from each other.

There was no significant variation ( $P>0.05$ ) in the PER of fish across all experimental units; it varied from  $0.92\pm 0.07$  to  $1.14\pm 0.02$ . The FER values were not substantially different from one another ( $P>0.05$ ) and

ranged from  $.76 \pm 0.06$  to  $0.91 \pm 0.03$ . There were no fish mortalities during the trial as survival rate was 100% among all experimental units.

**Table 2: Growth performance and Nutrient utilization of *Oreochromis niloticus* fed Experimental diets for 70 days (Mean  $\pm$  SE).**

Treatments	FMS	PWS	PWM	PWG	PSM	PSG
Initial Weight	30.23 $\pm$ 2.04 <sup>a</sup>	28.50 $\pm$ 1.47 <sup>a</sup>	28.30 $\pm$ 1.34 <sup>a</sup>	30.93 $\pm$ 1.07 <sup>a</sup>	27.93 $\pm$ 1.13 <sup>a</sup>	28.93 $\pm$ 1.97 <sup>a</sup>
Final Weight	65.70 $\pm$ 1.71 <sup>abc</sup>	61.27 $\pm$ 1.16 <sup>a</sup>	63.73 $\pm$ 1.93 <sup>ab</sup>	66.67 $\pm$ 1.40 <sup>abc</sup>	65.03 $\pm$ 1.01 <sup>abc</sup>	72.50 $\pm$ 1.55 <sup>c</sup>
Weight Gain	35.47 $\pm$ 1.62 <sup>ab</sup>	32.77 $\pm$ 2.57 <sup>a</sup>	35.43 $\pm$ 1.26 <sup>ab</sup>	35.73 $\pm$ 0.38 <sup>ab</sup>	37.10 $\pm$ 0.51 <sup>ab</sup>	43.57 $\pm$ 0.84 <sup>c</sup>
MWG	3.55 $\pm$ 0.16 <sup>ab</sup>	3.28 $\pm$ 0.26 <sup>a</sup>	3.54 $\pm$ 0.13 <sup>ab</sup>	3.57 $\pm$ 0.04 <sup>ab</sup>	3.71 $\pm$ 0.05 <sup>ab</sup>	4.35 $\pm$ 0.08 <sup>c</sup>
SGR	1.11 $\pm$ 0.08 <sup>a</sup>	1.10 $\pm$ 0.10 <sup>a</sup>	1.16 $\pm$ 0.04 <sup>ab</sup>	1.10 $\pm$ 0.02 <sup>a</sup>	1.21 $\pm$ 0.04 <sup>ab</sup>	1.31 $\pm$ 0.07 <sup>b</sup>
FCR	1.21 $\pm$ 0.05 <sup>bc</sup>	1.25 $\pm$ 0.09 <sup>bc</sup>	1.15 $\pm$ 0.04 <sup>ab</sup>	1.20 $\pm$ 0.01 <sup>b</sup>	1.13 $\pm$ 0.02 <sup>a</sup>	1.10 $\pm$ 0.02 <sup>a</sup>
FER	0.83 $\pm$ 0.04 <sup>ab</sup>	0.80 $\pm$ 0.06 <sup>a</sup>	0.87 $\pm$ 0.03 <sup>ab</sup>	0.84 $\pm$ 0.01 <sup>ab</sup>	0.89 $\pm$ 0.03 <sup>ab</sup>	0.91 $\pm$ 0.03 <sup>c</sup>
PER	0.99 $\pm$ 0.04 <sup>ab</sup>	0.96 $\pm$ 0.06 <sup>ab</sup>	1.04 $\pm$ 0.07 <sup>b</sup>	1.00 $\pm$ 0.05 <sup>ab</sup>	1.07 $\pm$ 0.03 <sup>b</sup>	1.14 $\pm$ 0.02 <sup>bc</sup>

**Comment [OM17]:** Table 2 does not tally with section 2.1.3, please explain the discrepancy in weights.

MWG – Mean Weight Gain, SGR- Specific Growth Rate, FCR- Feed Conversion Ratio, FER- Feed Efficiency Ratio, PER- Protein Efficiency Ratio- FER, PER- Protein Efficiency Ratio. Different letters within a row indicate significant differences ( $P < 0.05$ ).

### 3.0. Discussion

Results from this study showed that the various experimental diets increased fish weight gain (WG), fish given diet PSG gained the most weight (WG) among the various experimental diets. 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. When compared to the other experimental fish, there were appreciable differences in the growth performance of fish fed diet PSG. The documented variances in growth performance may result from variations in the protein blends used in the experimental diets. Fish fed the

PWS diet gave the least growth among the experimental fish, this could be attributed to feed composition or palatability, as the least feed intake was recorded for fish on this diet. The utilization of protein blends may be constrained due to the presence of anti-nutrients and variations in feed consumption have been shown to reduce fish weight gain<sup>23</sup>. This agrees with the report of<sup>14</sup>, 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<sup>15</sup>, who reported that the blend of defatted palm weevil and soybean increased fish performance.

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. [This has been ascribed to the complementary effects of amino acids from the different protein sources]<sup>24, 25</sup>. Fish fed diets comprising mixtures of FSM, PSM, and PSG all performed better than fish fed diets with just two protein sources, supporting this pattern. This effect corroborates those of.....<sup>12</sup>, where glirescins was used to replace FM up to 40% in *Cirrhinus mrigala* without compromising growth.<sup>8</sup> 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<sup>26</sup> 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*<sup>14</sup>.

The FCR of the experimental diets was low and comparable, showing good utilization of the experimental diets. Fish fed diet PSG recorded the best growth performance while having the lowest FCR (1.10), this is comparable to the works of<sup>20</sup>, where *C. gariepinus* fed on palm grub-containing diets showed low FCR. The study's findings that Turbot given BSF Larvae meal had a high FCR due to the diet's poor palatability were contradicted by finding<sup>27</sup>. The protein efficiency ratio (PER) values of fish fed experimental diets were comparable between the experimental fish, this is an indication that the dietary protein were similar and effectively used by fish, this is in agreement with the work of<sup>20</sup>, where palm grub meal was fed to *C. gariepinus*, but contrary to those reported by<sup>27</sup>, where Turbot (*Psetta maxima*) fed diets containing defatted BSF larvae had poor feed utilization in comparison to the control.

Comment [OM18]: Without your own results, it is difficult to assure us of this>

Comment [OM19]: Author?

The protein efficiency ratio (PER) values of fish fed experimental diets were comparable between the experimental fish, indicating that the dietary protein was similar and effectively used by fish. This is in agreement with work by <sup>20</sup>, where palm grub meal was fed to *C. gariepinus*. This is contrast to findings by <sup>27</sup>, who found that turbot (*Psetta maxima*) fed diets containing defatted BSF larvae had poor feed utilization in comparison to the control,

In all of the experimental units, the feed efficiency ratio was not significant ( $P > 0.05$ ), however the fish fed PSG-containing diets used their feed more effectively than other fish did. This might be a result of the diets' incorporation of various protein blends.

### 3.1. Carcass composition

In line with the findings of <sup>28</sup>, the initial carcass crude protein (CP) level in this investigation was lower than the CP levels found at the end of the feeding trial. Fish fed PSG had the highest crude protein content (60.80%), while fish fed diet PWG had the lowest crude protein content (50.59%). In this study, the percentage of crude protein found revealed a significant difference ( $P < 0.05$ ) between fish fed diet PSG and the 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 may have been caused by variations in the quality of the feed, the rate at which muscle was deposited, the amount of nutrients in the diet, and the capacity of the fish to convert food into absorbable nutrients <sup>29</sup>. 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 dietary treatment. The same patterns have been noted by <sup>30</sup> <sup>12</sup> at the conclusion of the feeding trial and lower in others <sup>31</sup>.

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 <sup>16</sup> when FM was replaced with BSF meal in Rainbow Trout, but disagrees with those of <sup>25</sup> where maggot meal was fed to *O. niloticus* fingerlings. 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 <sup>32</sup> who reported that carcass lipid content correlated with dietary lipid level in tilapia.

**Comment [OM20]:** It is advisable to mention at least the first author!

The results of this study indicates 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. However, further research should be carried out to know the synergy between soybean meal and gliricidia leaf meal and their combined effect on fish. The result of this study indicates that the diet that contained the blend of PSG was the most suitable for the successful culture of the Nile tilapia fingerlings.

### Consent

A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal."

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UNDER PEER REVIEW