

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

23 The effects of different protein blends (gliricidia/moringa leaf meals and defatted palm weevil
24 larvae) on Nile tilapia (*Oreochromis niloticus*) fingerlings were investigated during a 10-week
25 feeding trial. Six experimental diets with 30 percent crude protein were formulated as
26 fishmeal/soybean (FMS), palm weevil/soybean (PWS), palm weevil/moringa (PWM), palm
27 weevil/gliricidia(PWG), palm weevil/soybean/moringa(PSM), and palm weevil/soybean/gliricidia
28 (PSG). With 10 fish each, 180 Nile tilapia fingerlings were divided into 18 plastic tanks in

29 triplicates. The findings showed that fish fed diet PSG had final weights and protein efficiency
30 ratios (PER) that were significantly different ($P<0.05$) from those on other diets. Fish on diet
31 PSG gave the lowest food conversion ratio (FCR) of 1.10 ± 0.02 , with those on PWS and PWG
32 recording the least overall performance. Experimental fish's carcass protein, ash and fat
33 contents differed significantly. This study suggests that *Oreochromis niloticus* diet could be
34 replaced with blends of defatted palm weevil, soybean and gliricidia leaf meals.

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

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48 **1.0 Introduction**

49 The output of fish has increased dramatically over the past few decades, with 179 million tonnes
50 produced worldwide in 2018 through capture fisheries and aquaculture, of which 22 million tonnes were
51 used to produce fishmeal and oil ¹. Due to its nutritional balance and high nutritional value for humans
52 and other livestock, fishmeal has been employed in the formulation of intensive fish feed ². The use of it
53 for purposes other than feeding is limited by its economic worth, competition with human resources, and
54 environmental sustainability. ^{3,4}. Novel and unconventional protein sources that are cheap, readily

55 available, insect meals and other plant proteins have been identified as possible substitutes for fishmeal
56 that are currently thought to have little competition.^{5, 6}

57 Due to their sustainability, availability and affordability, plant protein sources have been incorporated into
58 the formulation of intensive feed. Aquatic plants such as azolla, water hyacinth, duckweeds, water lettuce,
59 bur-reed, and water fern as well as alfalfa, mulberry, sweet potato, cassava, cucumber, squash, broad
60 bean, papaya, moringa, leucaena, cocoyam, ipil-ipil, banana, and akee leaves have all been utilized in
61 the fish feed industry⁷. Moringa leaves have been successfully used without compromising the
62 performance of (*Oreochromis niloticus*, Linnaeus, 1758), (*Cyprinus carpio*, Linnaeus, 1758) and (*Clarias*
63 *gariepinus*, Burchell, 1822)^{8, 9}. Various studies have also reported that optimum inclusion level of 10-20%
64 moringa leaves can be used in the formulation diet for fish⁶. *Gliricidia* leaves have shown growth
65 enhancement in *Clarias gariepinus*¹⁰, the findings of Adeparusi¹¹ showed that the dietary
66 supplementation of Bambara nut with leucaena and *gliricidia* leaf meal concentrates in the diet of
67 *Oreochromis niloticus* improved fish performance and¹² also demonstrated that the inclusion of *Gliricidia*
68 *Maculata* leaf meal in (*Cirrhinus mrigala*, Hamilton, 1822) improved feed efficiency and growth.

69 In recent years, due to its availability, ability to reduce costs and environmental protection, researchers
70 have recognized insect meal as a potential substitute for fishmeal in the manufacturing of aquaculture
71 feed.^{5, 13}. Insects are excellent sources of protein (45%-75%), have a balanced essential amino acid
72 profile⁵, appropriate levels of minerals¹⁴ and are sustainable resources for nutrition. According to
73 scientific findings, insects can serve as a viable alternative to fish meal, fish oil, and other traditional
74 protein sources^{8, 15, 16, 17}, since interesting results have been obtained from the use of insect meals as
75 fishmeal substitutes in the diets of some fish species^{3, 4}.

76 *Rhynchophorus phoenecis* is a species of palm weevil and a member of the Curculionidae family. It is an
77 important pest of sugarcane (*Saccharum officinarum*), palm oil (*Elaeis guineensis*), coconut palms (*Cocos*
78 *nucifera*) and date palms (*Phoenix dactylifera*)¹⁸. It contains about 25-66 % crude protein and is a source
79 of important amino acids^{19, 18}. The palm weevil's nutritional value makes it an ideal fish feed supplement
80 or replacement for fishmeal¹⁹. Researches have shown that palm weevil larvae meal contains amino acid
81 content such as leucine, phenylalanine+tyrosine, trptophan etc^{20, 21}. Values of 4.51 and 3.15 have been

82 recorded for lysine and methionine+cysteine respectively ²¹, which are comparable with FAO/WHO
83 reference standard ²². Palm weevil larvae meal supports the healthy growth of the *Clarias gariepinus* ¹⁵,
84 *Heterobranchus longifilis* and *Clarias gariepinus*, whether supplemented or consumed as a complete
85 diet²³. It has been demonstrated that palm weevil larvae meal can replace fishmeal up to 100% of the
86 time in an African catfish full diet without raising the feed conversion ratio (FCR) or reducing weight gain
87 ¹⁵.

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

95 2.0. Materials and methods

96 2.1. Experimental Location

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

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101 2.2. Diet preparation and formulation

102 Table 1 shows the proximate composition of Defatted palm weevil larvae meal (*Rhynchophorus*
103 *phoenicis*), Moringa (*Moringa oleifera*), Gliricidia (*Gliricidia sepium*), with crude protein contents of 65.7%,
104 25.15% and 22.3% respectively.

105

106 **Table 1: Proximate composition of Deffated African Palm Weevil Larvae Meal**
107 **(*Rhynchophorus phoenicis*), Moringa and Gliricidia**

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

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110 2. 2.1. Preparation of palm weevil larva (*Rhynchophorus phoenicis*) and leaf meals

111 Live African palm weevil (*Rhynchophorus phoenicis*) larvae were procured from a local market at Ilaje,
 112 Ondo State, Nigeria. The insect larvae were washed, blanched and oven dried at 80°C for 24 hours²⁴.
 113 The larvae were defatted by n-hexane soxhlet extraction, oven dried, and hammer milled into a fine
 114 powder (Lab Mill, screen size 0.2 mm). Proximate analysis of the defatted sample was done as described
 115 by²⁵. *Moringa oleifera* and *Gliricidia sepium* leaves were obtained within the study area. The leaves were
 116 washed and strained and spread on plastic sheets to dry under shade for a week. The dried leaves were
 117 threshed from stalks and ground into fine powder by the use of a hammer mill (Lab Mill, screen size 0.2
 118 mm) and the powder was stored in plastic bags prior to use.

119 2.2.2. Diet formulation

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

128 2-mm die (Moulinex-HV8) mincer at constant amounts of oil, vitamins, and mineral premix. The pellets
 129 were then sealed in plastic bags after being sun-dried on elevated platforms until dry.

130 **Table 2: 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

131 Vitamin premix: An animal care^R optimix Aqua product for tilapia, containing the following per 5kg of
 132 **premix**: A= 20 000 000 I U, D3= 2 000 000 I U, E= 200 000mg, K3= 10 000mg, B2= 12 000mg, B12=
 133 9mg, B1= 6 000mg, B6= 11 000mg, C= 50 000mg, Folic acid= 2 000mg, Niacin= 80 000mg, Calpan= 25
 134 000mg, Biotin= 100mg x Zinc= 30 000mg, Manganese= 50 000mg, Iodine= 1000mg, Selenium= 100mg,
 135 Antioxidant= 125 000mg. **Fishmeal and soybean meal (FMS), palm weevil meal with soybean meal**
 136 **(PWS), palm weevil with moringa (PWM), palm weevil with gliricidia (PWG), palm weevil meal with**
 137 **soybean and moringa (PSM), palm weevil with Soybean and gliricidia (PSG).**

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139 2.2.3. Feeding trial

140 For this investigation, 180 Nile Tilapia (*Oreochromis niloticus*) fingerlings with an average weight of
 141 **(29.15±1.5g)** were obtained from the farm of the Federal College of Agriculture. Fish were acclimated to
 142 experimental conditions for 2weeks and placed on commercial diet. **Ten fish were randomly weighed into**
 143 **eighteen (18) plastic tanks with dimension (60 cm x 30 cm), which consisted of six treatments in**
 144 **triplicates.** Fish were fed twice daily at 5% of their body weight between 8:00 and 9:00 and 16:00 and
 145 17:00 for 70days. Water renewal was done twice a week, **while fish** were weighed and counted
 146 fortnightly. For the 70-day period, feed intake was adjusted biweekly depending on weight **gain** and daily
 147 mortality checks. **Selected** water parameters (temperature, pH, dissolved oxygen concentration) were

148 monitored **daily** to maintain optimal water quality conditions using a Yieryi Multi-parameter digital water
149 quality tester.

150 Growth Parameters were assessed using the following formulas.

151 Mean Weight Gain (MWG)

$$\text{MWG} = \text{WF} - \text{WI}$$

152 Where, WF = Final weight

153 WI= Initial weight

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

155

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

157

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

159

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

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

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163 **2. 2.4. Sample analysis**

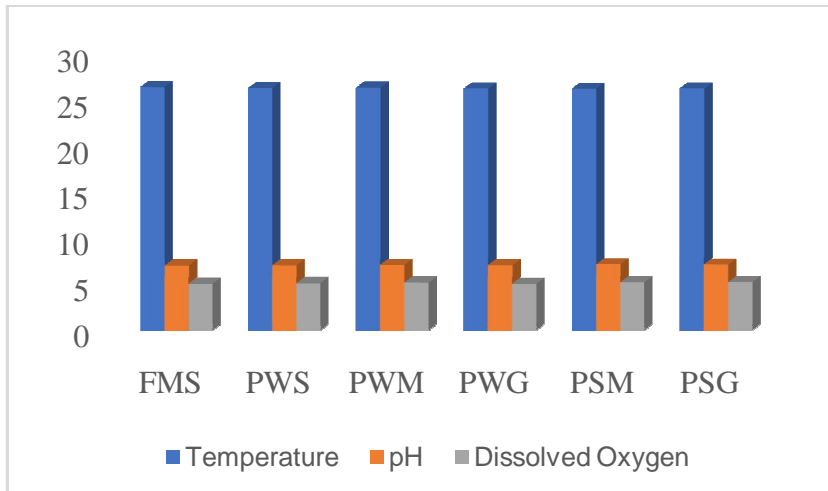
164 Experimental fish carcass and feed samples were analyzed for proximate composition using the
165 methods described by ²². Data obtained were expressed as mean ± standard error (S.E) and subjected
166 to a **one-way** ANOVA design in triplicates (SPSS 22) at statistical significance level of 95%.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

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170 **2.2.5 Results**

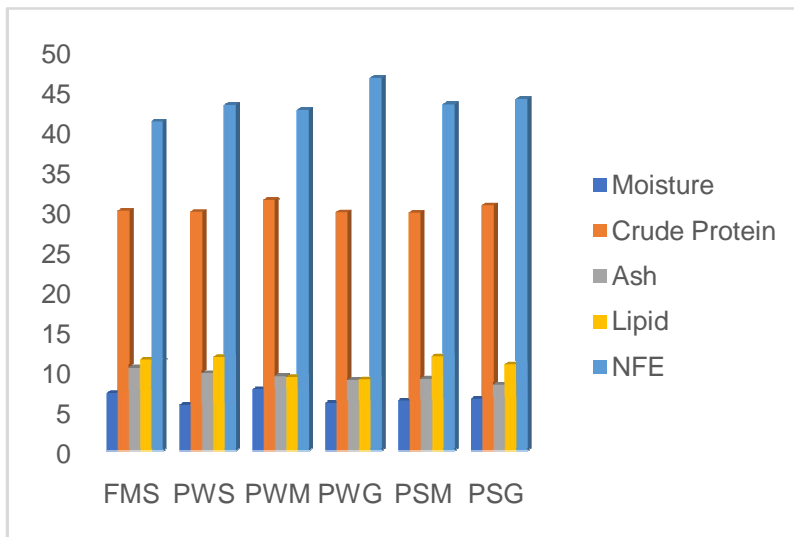
171 Fig 1 shows the water quality parameters, the temperature ranged between 25.89°C to 26.50°C, the pH
172 ranged between 7.07-7.20 and the dissolved oxygen ranged from 5.08 to 5.25 mg/L, there were no
173 significant difference in the various parameters.



174
175 Fig 1: Water quality parameters

176 **2.2.6. Proximate composition of the experimental diets**

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



182
183 Fig 2: Proximate composition of the experimental diets

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185 **2.2.7. Growth performance and Nutrient Utilization**

186 Table 3 below shows the growth performance and nutrient utilization of *Oreochromis niloticus* fed
 187 experimental diets. Fish fed diet PSG gained the most weight 43.57g compared to the other experimental
 188 fish and this difference was significant ($P < 0.05$). Fish fed diet PWS exhibited significantly lower weight
 189 gain (32.77g) compared to those on other experimental diets. Fish fed the PSG diet had the lowest feed
 190 conversion (FCR) and the highest SGR value (1.31 ± 0.07), which were statistically different ($P < 0.05$) from
 191 fish fed the other experimental diets. The PER ranged from 0.92 \pm 0.07 to 1.14 \pm 0.02, there was no
 192 significant difference ($P > 0.05$) in the PER of fish in all the experimental units. The FER value ranged from
 193 0.76 \pm 0.06 to 0.91 \pm 0.03 and were not significantly different ($P > 0.05$) from each other.
 194 There was no significant variation ($P > 0.05$) in the PER of fish across all experimental units; it varied from
 195 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
 196 ranged from .76 \pm 0.06 to 0.91 \pm 0.03. There were no fish mortalities during the trial as survival rate was
 197 100% among all experimental units.

198

199 **Table 3: Growth performance and Nutrient utilization of *Oreochromis niloticus* fed**
 200 **Experimental diets for 70 days (Mean \pm SE)**

Treatments	FMS	PWS	PWM	PWG	PSM	PSG
Initial Weight	30.23 \pm 2.04 ^a	28.50 \pm 1.47 ^a	28.30 \pm 1.34 ^a	30.93 \pm 1.07 ^a	27.93 \pm 1.13 ^a	28.93 \pm 1.97 ^a
Final Weight	65.70 \pm 1.71 ^{abc}	61.27 \pm 1.16 ^a	63.73 \pm 1.93 ^{ab}	66.67 \pm 1.40 ^{abc}	65.03 \pm 1.01 ^{abc}	72.50 \pm 1.55 ^c
Weight Gain	35.47 \pm 1.62 ^{ab}	32.77 \pm 2.57 ^a	35.43 \pm 1.26 ^{ab}	35.73 \pm 0.38 ^{ab}	37.10 \pm 0.51 ^{ab}	43.57 \pm 0.84 ^c
MWG	3.55 \pm 0.16 ^{ab}	3.28 \pm 0.26 ^a	3.54 \pm 0.13 ^{ab}	3.57 \pm 0.04 ^{ab}	3.71 \pm 0.05 ^{ab}	4.35 \pm 0.08 ^c
SGR	1.11 \pm 0.08 ^a	1.10 \pm 0.10 ^a	1.16 \pm 0.04 ^{ab}	1.10 \pm 0.02 ^a	1.21 \pm 0.04 ^{ab}	1.31 \pm 0.07 ^b
FCR	1.21 \pm 0.05 ^{bc}	1.25 \pm 0.09 ^{bc}	1.15 \pm 0.04 ^{ab}	1.20 \pm 0.01 ^b	1.13 \pm 0.02 ^a	1.10 \pm 0.02 ^a

FER	0.83±0.04 ^{ab}	0.80±0.06 ^a	0.87±0.03 ^{ab}	0.84±0.01 ^{ab}	0.89±0.03 ^{ab}	0.91±0.03 ^c
PER	0.99±0.04 ^{ab}	0.96±0.06 ^{ab}	1.04±0.07 ^b	1.00±0.05 ^{ab}	1.07±0.03 ^b	1.14±0.02 ^{bc}
Feed intake	79.5±0.13 ^{ab}	76.6±0.12 ^a	73.3±0.03 ^a	73.3±0.01 ^a	73.5±0.06 ^a	79.8±0.01 ^{ab}

201 MWG – Mean Weight Gain, SGR- Specific Growth Rate, FCR- Feed Conversion Ratio, FER- Feed
 202 Efficiency Ratio, PER- Protein Efficiency Ratio- FER, PER- Protein Efficiency Ratio. Different letters within a
 203 row indicate significant differences (P< 0.05). Fishmeal and soybean meal (FMS), palm weevil meal with
 204 soybean meal (PWS), palm weevil with moringa (PWM), palm weevil with gliricidia (PWG), palm weevil
 205 meal with soybean and moringa (PSM), palm weevil with Soybean and gliricidia (PSG).

206 3.0. Discussion

207 Results from this study showed that the various experimental diets increased fish weight gain (WG), fish
 208 given diet PSG gained the most weight (WG) among the various experimental diets. Similarly, fish on the
 209 PSG diet also showed the best nutrient utilization in terms of feed conversion ratio, feed efficiency ratio,
 210 and protein efficiency ratio. When compared to the other experimental fish, there were appreciable
 211 differences in the growth performance of fish fed diet PSG. The documented variances in growth
 212 performance may result from variations in the protein blends used in the experimental diets. Fish fed the
 213 PWS diet gave the least performance in terms of weight gain and feed efficiency, but this was not
 214 significantly different from the other experimental fish, except those fed diet PSG, which could be
 215 adduced to the feed composition and/or palatability. This agrees with the report of ¹⁴, who stated that
 216 adding Black Soldier fly meal and soybean to a Nile Tilapia diet stunted growth as the inclusion level rose,
 217 but disputes the report of ¹⁵, who reported that the blend of defatted palm weevil and soybean increased
 218 fish performance. The utilization of protein blends may be constrained due to the presence of anti-
 219 nutrients and variations in feed consumption have been shown to reduce fish weight gain ²⁶.

220 Our findings also imply that a diet in which defatted palm weevil completely replaced fishmeal produced
 221 superior results to the control diet. Studies have demonstrated that using a variety of protein blends
 222 increased fish performance over using a single source of protein. The findings in our study showed that
 223 the blends that contained three protein sources gave better performances compared to those that had
 224 two. This has been ascribed to the complementary effects of amino acids from the different protein

225 sources^{27,28}. Fish fed diets comprising mixtures of FSM, PSM, and PSG all performed better than fish fed
226 diets with just two protein sources, supporting this pattern. This effect corroborates those of¹², where
227 gliricidia was used to replace FM up to 40% in *Cirrhinus mrigala* without compromising growth.⁸ also
228 reported that moringa supplementation in *Clarias gariepinus* improved growth. This contrast earlier reports
229 in which fish fed diets supplemented or replaced with fishmeal exhibited reduced growth. The works of²⁹
230 revealed that feeding diets containing Black soldier fly larvae to *Lates Calcarifer* inhibited its growth. Nile
231 Tilapia growth was also reduced when fed diet containing *Tenebrio molitor*¹⁴.
232 The FCR of the experimental diets was low and comparable, showing good utilization of the experimental
233 diets. Fish fed diet PSG recorded the best growth performance while having the lowest FCR (1.10), this is
234 comparable to the works of²³, where *C. gariepinus* fed on palm grub-containing diets showed low FCR.
235 The study's findings that Turbot given BSF Larvae meal had a high FCR due to the diet's poor palatability
236 were contradicted by finding³⁰. The protein efficiency ratio (PER) values of fish fed experimental diets
237 were comparable between the experimental fish, this is an indication that the dietary protein were similar
238 and effectively used by fish, this is in agreement with the work of²³, where palm grub meal was fed to *C.*
239 *gariepinus*, but contrary to those reported by³⁰, where Turbot (*Psetta maxima*) fed diets containing
240 defatted BSF larvae had poor feed utilization in comparison to the control.
241 The protein efficiency ratio (PER) values of fish fed experimental diets were comparable between the
242 experimental fish, indicating that the dietary protein were similar and effectively used by fish. This is in
243 agreement with work by²³, where palm grub meal was fed to *C. gariepinus*. This is in contrast to findings
244 by³⁰, who found that turbot (*Psetta maxima*) fed diets containing defatted BSF larvae had poor feed
245 utilization in comparison to the control.
246 In all of the experimental units, the feed efficiency ratio was not significant ($P>0.05$), however the fish fed
247 PSG containing diets used their feed more effectively than other fish did. This might be a result of the
248 diets' incorporation of various protein blends.

249 3.1. Carcass composition

250 In line with the findings of Opiyo³¹, the initial carcass crude protein (CP) level in this study was lower than
251 the CP levels found at the end of the feeding trial. Fish fed PSG had the highest crude protein content

252 (60.80%), while fish fed diet PWG had the lowest crude protein content (50.59%). In this study, the
253 percentage of crude protein found revealed a significant difference ($P < 0.05$) between fish fed diet PSG
254 and the other diets. Fish fed diet PWS (17.95) had the highest ash level, while diet PWM (13.13) had the
255 lowest. Fish fed diet FSG (17.66) had the highest fat content, while fish fed diet FMS had the lowest
256 (12.73). The fish carcass composition varied, which may have been caused by variations in the quality of
257 the feed, the rate at which muscle was deposited, the amount of nutrients in the diet, and the capacity of
258 the fish to convert food into absorbable nutrients³². Fish on the experimental diets had greater values for
259 crude protein, crude fat, ash, and NFE than the baseline fish. This suggests that the experimental fish's
260 carcass quality may have been impacted by dietary treatment. The same patterns have been noted by³³,
261¹² at the conclusion of the feeding trial and lower in others³⁴.

262 The marked reduction in the protein composition of fish fed diet PWS and PWG could be due to the feed
263 composition or imbalance EAA profile. This is in agreement with those reported by¹⁶ when FM was
264 replaced with black soldier fly meal in Rainbow Trout, but disagrees with those of²⁸ where maggot meal
265 was fed to *O. niloticus* fingerlings. There was no relationship between the dietary protein content and the
266 carcass fat composition of *O. niloticus* in this study, this is in contrast with those reported by³⁵ who
267 reported that carcass lipid content correlated with dietary lipid level in tilapia.

268 **Conclusion**

269 Over the years, the search for alternative protein sources as fishmeal replacement in aquaculture feed
270 has been a subject of extensive research. The use of fishmeal in livestock feed has dwindled fish
271 population in the wild due to overexploitation. Alternative and novel feed ingredients that meets the
272 nutritional requirements of fish are being looked into, that would increase production and invariably
273 reduce overdependence on fishmeal, which has been overused to the detriment of wild fish population.

274 The results of this study indicate that the use of alternative protein sources had positive impacts on the
275 growth performance, nutrient utilization and carcass composition of Nile Tilapia (*Oreochromis niloticus*).
276 This indicates that defatted palm weevil larvae which have a crude protein content similar to fishmeal can
277 totally replace the FM without causing any negative effect on the health of the fish especially in

278 combination with soybean and gliricidia meals. The diet that contained the blends of PSG was the most
279 suitable for the successful culture of the Nile tilapia fingerlings. However, further research should be
280 carried out to know the synergy between soybean meal and gliricidia leaf meal and their combined effect
281 on fish. It could be recommended that fish farmers be encouraged to culture palm weevil as feed
282 supplement.

283

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287 **Competing Interests**

288 “Authors have declared that no competing interests exist.”.

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