Effects of Alternative Protein Blends on *Oreochromis Niloticus* (*Nile Tilapia*) Fingerlings Growth Performance, Nutrient Utilization and Carcass Composition

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Abstract

This study was conducted to evaluate blends of alternative protein sources (*Gliricidia* and *Moringa* leaves) as fishmeal supplements in the diets of *Nile tilapia* (*Oreochromis niloticus*) fingerlings. 5 experimental diets containing 30% crude protein were formulated as Fish Meal/Soybean (FMS, control), Fish Meal/*Moringa* (FMM), Fish Meal/*Gliricidia* (FMG), Fish Meal/Soybean/*Moringa* (FSM) and Fishmeal/Soybean/*Gliricidia* (FSG) and fed to *oreochromis niloticus* fingerlings for 10 weeks. A total of 150 fingerlings were distributed into 15 plastic tanks in triplicates with 10 fish per tank. The findings showed that whereas fish given diets FMS, FMM, and FMG saw similar weight gains, fish fed diet FSM experienced weight gains that were significantly different ($P<0.05$) from those of the other experimental fish. However, the experimental fish's Specific Growth Rate (SGR) did not change significantly ($P>0.05$).

Fish fed diet FSG had a Feed Conversion Ratio (FCR) of 1.12 ± 0.01, while fish fed diet FMG had an FCR of 1.23 ± 0.02. There were no appreciable variations in the experimental fish's Protein Efficiency Ratio (PER) value ($P>0.05$). The fish utilized the experimental meals effectively overall. According to the study's findings, *Nile tilapia*'s diet could be supplemented with various protein blends in addition to fishmeal.

Keywords: Protein blends, *Oreochromis niloticus*, *Gliricidia*, *Moringa*

Introduction

In order to meet demand, an additional 23 million tonnes of aquatic food would need to be produced globally [1]. Rising consumer demand for fish products has resulted in overfishing of small pelagic fish stocks and a decrease in wild fish catches, which has usually raised the cost of these items and resulted in subpar quality Veldkamp and Bosch [2]. Fishmeal is being employed in the formulation of fish feed to the tune of 10% of all fish caught worldwide this due to the nutritional makeup of fishmeal [3]. With limited access to cultivable fish species, aquaculture has increased fish supply in a number of nations and regions, typically at lower costs to offset better nutrition and food security [4]. However, the aquaculture industry faces a challenge to its growth and sustainability due to the unsustainable use of fishmeal for fish feed [1]. The production cost would be reduced if cheap plant sources were used in place of the pricey fish meal, thereby increasing profit [5]. However, before using a novel protein source in aqua feeds, it should be evaluated on several fish species since their reactions to anti-nutrients, which are plant components that hinder the body's capacity to absorb vital nutrients varies [6]. Compared to fishmeal, leave meal contains fewer amino acid components; however, this could be increased by supplementing with pure amino acids. Due to the high cost and accessibility of such additives, this is not economically feasible for fish farmers in rural areas [7]. Making meals with mixtures of various protein sources with complementary amino acid profiles is a useful strategy for raising the quality of protein [8,9].

The *Moringa* plant, *Moringa oleifera*, is a member of the *Moringaceae* family. Its fresh leaves, often known as "drumsticks," are very nutritious, especially when used as a supplement for fish that consume plants, like tilapia, barbs, and fancy carps. Because they are high in proteins, lipids, vitamins, and minerals, the aquaculture feed business frequently uses the leaves, kernels, and pods of these plants

[10]. According to Idowu, et al. and Stevens, et al., the leaves contain 26.94% crude protein [11,12]. Stevens, et al. reported 25.74% [12]. The amount of anti-nutrients in the leaves' dry matter is low because they are not known to have any negative effects on animals. These anti-nutrients include saponins, phytate, and oxalate [12]. When compared to fresh leaves, tannins can be reduced by 15%-30% through drying, fermenting, and silaging processes. African catfish and *Nile tilapia* both responded favorably to *Moringa* at inclusion levels of 10%-15% [13-15].

Quick stick, fast-growing *Fabaceae* family member *Gliricidia sepium* is a small to medium-sized browsing plant that is frequently utilized as intercropping, green manure, cattle feed and live fence [16]. On a dry matter basis, *Gliricidia sepium* leaves have a crude protein content of 20%-30% and a crude fiber content of 15%, it is a good source of protein for fish diets as a result [17]. Although the leaves are nutrient-rich, they also contain significant amounts of anti-nutrients such tannins, alkaloids, saponins, phytic and oxalic acids [18]. Methionine supplements in the diet have been shown to reduce the negative effects of antinutrients in fish by deactivating the saponin in the food and thereby improving the nutritional composition [18]. It has been reported to have a positive impact on African catfish, *Cirrhinus mrigala* and *Nile Tilapia* [14,19,20].

Therefore, this research work was conducted to evaluate the growth performance, nutrient utilization and carcass composition of *Nile Tilapia* fed different blends of plants (*Moringa* and *Gliricidia* leaf meals) protein sources as diets to supplement fishmeal. There by, enhancing fish production and overcome the problem with the relatively expensive fish meal, affording small scale farmers the ability to raise fish at low cost without compromising quality.

Materials and Methods

Experimental location

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

Preparation of and *Gliricidia* leaves

Leaves of *Moringa oleifera* and *Gliricidia sepium* were collected, cleaned, and spread out on plastic sheets in the shade to dry within the experimental setting. The dried leaves were separated from the stalks and ground in a hammer mill to a fine powder (Lab Mill, screen size 0.2 mm). Proximate analysis of the leaf meal samples was done as described by AOAC [21].

Diet formulation

Five iso-nitrogenous diets containing 30% crude protein were formulated. Blends of diets were prepared using fishmeal in combination with soybean meal (FMS), *Moringa* (FMM), *Gliricidia* (FMG), with soybean meal/ *Moringa* (FSM) and Soybean Meal/*Gliricidia* (FSG) using the trial and error method. The feed constituted yellow maize, fish meal, soybean, salt, bone meal, vegetable oil and vitamin premix. The ingredients were thoroughly mixed together with corn starch and extruded through a 2 mm die (Moulinex-HV8) mincer, which was then sundried on raised platforms until dry and put into plastic bags. Table 1 shows the gross composition of the experimental diets.

Table 1. *Gross Composition of Experimental Diets (g/100g, 30% crude protein)*

	FMS	FMM	FMG	FSM	FSG
Fishmeal (65%)	23.25	29.5	30.6	19.55	20.5
Soybean meal (45%)	23.25	-	-	19.55	20.5
Moringa (25.2%)	-	29.5	-	19.55	-
Gliricidia (22.3%)	-	-	30.6	-	20.5
Maize (10%)	43.5	31	28.8	31.35	28.5
Vit/min premix	2	2	2	2	2
Bone meal	2	2	2	2	2
Oil	4	4	4	4	4
Corn starch	2	2	2	2	2
Total	100	100	100	100	100

Feeding trial

150 fingerlings of *O. niloticus* weighing (2.8 g-3.0 g) were used for the trial. Fish were acclimated to experimental conditions and fed commercial diet for 7 days. 10 fish were randomly weighed and distributed into each of 15 plastic tanks (60 cm × 30 cm × 30 cm), each representing 5 treatments in triplicates. The fish were fed experimental diets twice daily at 5% body weight between 8 hour to 9 hour and 16 hour to 17 hour daily for 70 days. Complete water renewal was done twice a week to maintain optimal

water quality conditions. Mortality was checked daily, while the weight and feed intake were adjusted bi-weekly for the 70 days period.

Water quality parameters (temperature, pH, dissolved oxygen concentration) were measured daily 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)

$$SGR = \frac{\text{In (Final Weight) - In (Initial Weight)}}{\text{Culture Period}} \times 100$$

Feed Conversion Ratio (FCR)

$$FCR = \frac{\text{Total Feed Intake}}{\text{Total Weight Gain}}$$

Feed Efficiency Ratio (FER)

$$FER = \frac{\text{Wet weight gain}}{\text{Dry feed intake}}$$

Protein Efficiency Ratio (PER)

$$PER = \frac{\text{Mean Weight Gain}}{\text{Mean PI}}$$

Survival rate

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

Feed samples and fish carcass were analyzed for proximate composition using AOAC methods [12]. Data obtained were expressed as mean ± standard error (S.E) and subjected to one-way analysis of variance (ANOVA). Treatment means were compared using Duncan's Multiple Range Test, using the SPSS version 22. Statistical significance difference was set at $P<0.05$.

Results

The result of the proximate composition of the experimental diets is presented in Figure 1 below. The highest ash content of 10.82 was recorded in diet FMG and diet containing FSG gave the lowest value of 9.28. The highest value of fat (11.36) was in the control diet and the lowest was recorded in diet containing FMM. The crude protein ranged between 29.64-31.75, with the highest value of 31.75 in diet FSM and the lowest value of 29.64 in diet FSG, the Nitrogen Free Extract (NFE) were between 39.72-43.06.

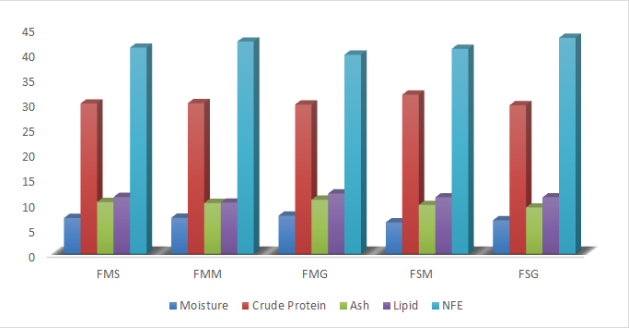

Figure 1. *Proximate composition of the experimental diets*

Water quality parameters

The temperature in the experimental tanks ranged from 25.89°C to 26.50°C, there were no significant differences among the treatments. The pH ranged from 7.07 to 7.17, there was no significant difference among the treatments. The dissolved oxygen ranged from 5.08 mg/L to 5.25 mg/L and was within the range permissible for the culture of tilapias.

Growth performance and nutrient utilization

The growth performance and nutrient utilization of *Oreochromis niloticus* fed experimental diets is presented in Table 2 below. Fish fed diet FSM gave the highest weight gain of 38.63 g and the lowest value (33.53 g) was in fish fed diet FMG, this was significantly different ($P<0.05$) from the rest of the experimental fish. The mean weight gain in the study ranged between 3.35 g to 3.86 g, with fish fed diet FSM having the highest value of 3.86 ± 0.28, this value was significantly different ($P<0.05$) from the rest of the experimental fish. The fish fed diet FSM had the highest SGR value of 1.17 ± 0.06, with those fed diet FMG recording the lowest SGR (1.09 ± 0.04). There were no significant differences in the SGR of the experimental fish. The FCR ranged between 1.12 ± 0.01-1.23 ± 0.02, these were not significantly different ($P>0.05$) from each other within the experimental units. The PER ranged between 0.97 ± 0.04 to 1.07 ± 0.01 and were not significantly different ($P>0.05$). Survival rate was 100% among the experimental units (Tables 2 and 3).

Table 2. *Proximate composition of experimental diets*

Moisture	Crude Protein	Ash	Lipid	NFE
7.19 ± 0.23^{ab}	29.98 ± 0.14^{a}	10.36 ± 0.15^{a}	11.36 ± 1.31^{ab}	41.11 ± 1.26^{a}
7.22 ± 0.31^{ab}	30.04 ± 0.047^{a}	10.18 ± 0.38^{a}	10.24 ± 1.35^{b}	42.32 ± 2.08^{a}
7.63 ± 0.26^{ab}	29.78 ± 0.89^{a}	10.82 ± 0.15^{ab}	12.05 ± 1.79^{ab}	39.72 ± 2.57^{a}
6.30 ± 0.03^{a}	31.75 ± 1.01^{ab}	9.78 ± 1.1^{a}	11.29 ± 0.62^{ab}	40.88 ± 0.79^{a}
6.73 ± 0.03^{a}	29.64 ± 0.49^{a}	9.28 ± 0.18^{a}	11.29 ± 1.15^{ab}	43.06 ± 1.49^{ab}
Vitamin premix: An Animal Care ^{R} optimix Aqua product for tilapia, containing the following per 5 kg of premix: A=20,000,000 I U, D3=2 000 000 I U, E=200,000 mg, K3=10,000 mg, B2=12,000 mg, B 12=9 mg, BI=6,000 mg, B6=11,000 mg, C=50,000 mg, Folic acid=2,000 mg, Niacin=80,000 mg, Calpan=25,000 mg, Biotin=100 mg, X Zinc=30 000 mg, Manganese=50,000 mg, Iodine=1000 mg, Selenium=100 mg, Antioxidant=125,000 mg				

Table 3. *Growth performance and nutrient utilization of O.niloticus fingerlings fed experimental diets for 70 days*

Treatments	FMS	FMM	FMG	FSM	FSG
Initial weight	30.23 ± 2.04^{a}	29.47 ± 1.98^{a}	29.33 ± 1.64^{a}	30.50 ± 2.22^{a}	28.80 ± 0.30^{a}
Final weight	65.70 ± 1.71^{ab}	64.97 ± 3.72^{a}	62.77 ± 1.86^{a}	69.13 ± 4.19^{c}	65.50 ± 0.69^{ab}
Weight gain	35.47 ± 1.62^{a}	35.50 ± 1.86^{a}	33.53 ± 0.49^{a}	38.63 ± 2.75^{b}	36.70 ± 0.46^{ab}
SGR	1.11 ± 0.08^{a}	1.13 ± 0.03^{a}	1.09 ± 0.04^{a}	1.17 ± 0.06^{ab}	1.17 ± 0.01^{ab}
FCR	1.21 ± 0.05^{b}	1.17 ± 0.06^{ab}	1.23 ± 0.02^{b}	1.18 ± 0.07^{ab}	1.12 ± 0.01^{a}
FER	0.83 ± 0.04^{a}	0.86 ± 0.05^{ab}	0.81 ± 0.01^{a}	0.85 ± 0.07^{a}	0.89 ± 0.01^{ab}
PER	0.99 ± 0.04^{a}	1.03 ± 0.06^{a}	0.97 ± 0.04^{a}	1.02 ± 0.08^{a}	1.07 ± 0.01^{a}
Survival	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00

Carcass composition of experimental fish

The initial carcass Crude Protein (CP) level was lower than the levels found at the end of the feeding trial. The highest crude protein was obtained in fish fed FMM (54.24%), while fish fed diet FMG gave the lowest crude protein (50.25%). The percentage crude protein obtained in this study showed significant difference (P<0.05) between fish fed diet FMM and the rest of the diets. The ash content was highest in fish fed diet FSG (17.17) and least in diet fish that had diet FSM (13.13), the ash content of the experimental fish showed significant difference (P<0.05). The highest fat content obtained in fish fed diet FSG was (17.66), while the least was in fish fed diet FMS (12.73), fish fed diets FMG and FSM were significantly different (P<0.05) from each other. Figure 2 shows the carcass composition of the experimental fish.

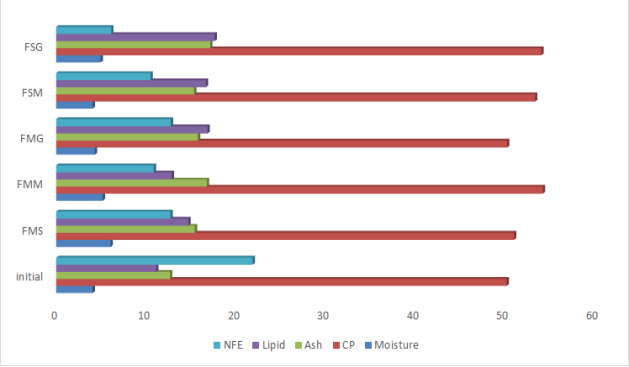

Figure 2. *Carcass composition of experimental fish*

Discussion

The growth performance and nutrient utilization of the experimental fish were not compromised; this indicates that the experimental diets were acceptable for *O. niloticus* fingerlings in this study. Studies have established the use of plant based protein sources as alternatives to fish meal in regards to their cheaper cost and availability [6,11,12]. The results of the current study, which reveal that *O. niloticus* fingerlings given the various protein blends gained weight, support the findings of Billah, et al., who found that *Moringa* supplementation enhanced growth in *Clarias gariepinus* [15]. The results of Vhanalakar and Muley, who employed *Gliricidia* to replace FM in *Cirrhinus mrigala* up to 40% without affecting growth, further support this impact [19].

There were similarities in the weight gain of fish fed the control diet (FMS), FMM and FSG, while fish on diet FMG gave a lower performance, this could be adduced to reduced feed intake due to low palatability resulting in reduced growth. It was observed that fish fed blends of fishmeal/ *Moringa* performed better than those fed the blends of fishmeal/*gliricidia*. This scenario is in tandem with the works of Billah, et al., whose report showed that fishmeal supplementation with *Moringa* improved the growth of *Nile Tilapia* [15]. This was in contrast to the findings reported by Tiiniub, et al., who found that performance was negatively impacted by feeding all male tilapia a *Moringa* feed supplement [22]. The best results in this trial came

from the partial replacement of fishmeal in the fish-fed diet FSM with soybean meal and *Moringa* leaf meal. This supports the findings of Adeshina, et al. and El-Gawad, et al., whose studies showed that adding *Moringa* to the diet of *Cyprinus carpio* and *Nile tilapia* significantly improved the performance of the fish as a whole [23,24].

According to our research, fish given mixtures of fishmeal/ *Moringa* and/or fishmeal/*Gliricidia* didn't grow as much as those fed blends that also contained soybean. Observing a lower growth rate in *Clarias gariepinus* fed *Moringa* leaf meal at inclusion levels higher than 15%, Gbadamosi and Osunbamiro hypothesized that larger substitutions of fishmeal had an effect on fish performance due to the presence of anti-nutrients [25]. This might be enhanced by combining different protein blends, which, in accordance with Djissou, et al. [9]. Could be related to the synergy between the various protein sources in the diets complementing one another.

The nutrient utilization markers were in line with what was previously reported for *Nile tilapia* culture, and the FCR of the experimental diets were generally low and comparable. The fact that the FCR obtained in this study is lower than that of Nadia, et al., who supplemented fishmeal with *Moringa* leaf powder, suggests that the fish made good use of the feeds. This study's SGR is consistent with Bello and Nzeh's findings indicating the protein sparing impact improves growth performance and nutrient utilization [26]. Makori, et al., reported a higher SGR (3.7%-4.4%) than the current study when *Nile Tilapia* was fed commercial diets [27]. 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 [28,29].

Conclusion

The experimental diets improved fish body composition as several studies have similarly reported variations in fish carcass composition between the initial and final stages that were seen in the current study. When *Moringa* was fed to *Cyprinus carpio*, they had better body compositions, as reported by Adeshina, et al. Fish fed diet FMG showed a noticeably reduced protein carcass composition. The carcass's moisture levels fell within the range that was suggested for enhancing the fish's quality and shelf life. There was no correlation between the carcass fat composition and the amount of protein in the diet. In contrast, claimed that the amount of dietary lipids in tilapia showed a favorable connection with carcass lipid content. Survival results demonstrated that *Oreochromis niloticus* fingerlings thrived under the study's culture conditions.

The findings of this study suggest that the use of alternative protein sources had favorable impact on *Nile Tilapia* (*Oreochromis niloticus*) performance, utilization and carcass characteristics. This shows that plant protein blends can completely replace fishmeal without having any harmful effects on the wellbeing of the fish. According to this findings, a diet rich in FSM seems to be the best choice for successfully raising tilapia fingerlings.

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Conflict of Interest

The author declares there is no conflict of interest in publishing this article.

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