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Effect of different dietary protein sources on fingerling production in Nile Tilapia (*Oreochromis niloticus*)

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Abstract
Availability of quality and reliable feeds is one of the main challenges facing aquaculture development. A 12 week experiment was set where, broodstock of Nile tilapia were fed various diets with different plant protein sources for 8 weeks. The produced fingerlings were fed a common diet thereafter for 4 weeks. The diets contained Fish meal, maize meal and minerals; Moringa leaf meal, maize meal and minerals; Sunflower seedcake, maize meal and minerals; Moringa leaf meal, sunflower seedcake, maize meal and minerals. All other factors including water quality parameters were constant in all experimental units. It was noticed that, fish brood stock fed diets 1 and 2 produced highest numbers of fish fingerlings, but fish fingerlings produced by brood stock fed diet 2 showed the greatest growth and survival. This study therefore recommends diet 2 (Moringa leaf meal, maize meal and minerals) for broodstock feeding. The ingredients are readily available for use in aquaculture production.

Keywords: Protein sources, Broodstock, Fingerlings, Production

1. Introduction

Nile tilapia, (*Oreochromis niloticus)* is an extensively cultured fish because it grows and multiplies under a variety of environmental conditions and tolerates handling stress. Tilapias do well in extensive, semi-intensive and intensive culture systems [1]. Nile tilapia (*Oreochromis niloticus*) has become the predominant culture species due to its proven superior growth compared to the other species [2]. Tilapias are now the second most commonly farmed fish next to carps [3]. Currently tilapias are cultured in about 100 countries in tropical and subtropical regions.

According to [4], Aquaculture in Tanzania started in 1949 with experimental work on the culture of tilapia at Korogwe (Tanga Region) and Malya (Mwanza Region) during which many ponds were constructed. This project did not fully propergate due to common aquaculture challenges like poor managemnent strategies, low technical knowhow, poor infrastructure and physical weather conditions.

Aquaculture in Tanzania is growing rapidly over recent years though aquaculture supportive resouces are not yet fully exhausted [5]. Nile tilapia and African catfish are the common aquaculture species cultured both extensively and semi-intensively. Small fishponds of average size of 10 m x 15 m (150 m^2) are integrated with other agricultural activities such as gardening and animal and poultry production on small pieces of land. The United Republic of Tanzania is currently estimated to have 21 100 freshwater fishponds scattered across the mainland covering an estimated area of 645.5 ha [6].

Tilapia nourish on a wide range of dietary sources, including phytoplankton, periphyton, zooplanktons, larval fish, and detritus. Adult tilapia is principally herbivorous but readily adapts to complete commercial diets based on plant and animal protein sources. Fish do not have a precise requirement for crude protein per se, but rather they need a combination of essential amino acids. Therefore, the profile of dietary protein is important when formulating diets for tilapia. Fish continuously use dietary proteins for maintenance, growth and reproduction functions. When fed in excess, protein may be used as energy; however, the latter function is not desirable because of the expensive cost of proteins [7].

One of the requirements for sustainable fish farming is fish seed production from fertilized eggs that produce larvae with high survival and growth rate. Broodstock nutrition affects reproduction ability i.e. egg and larval quality in fish [8]. Some feed components like protein and lipids have been found to influence greatly the spawning excellence in several fish species [9]. Reproduction requires formation of gametes which have high protein content [10]. It is therefore imperative to provide adequate sources of dietary protein to broodstocks. This study was therefore aimed at establishing a quality reliable broodstock feed from plant protein sources which can easily be formulated and used by farmers in their tilapia hatcheries.

2. Materials and Methods

2.1 Diets Preparation

Experimental diets for this study were prepared at the Department of Animal science, Sokoine University of Agriculture. Feed materials used were; fish meal, moringa leaf meal, sunflower seedcake, maize bran and mineral premixes. Four different diets were prepared from these feed ingredients as in Table 1.

Table 1: The composition of the experimental diets

Ingredient	Crude Protein (%)	Diet 1 %	Diet 2 %	Diet 3 %	Diet 4 %
Fish meal	54	35.36	-	-	-
Moringa leaves	28	-	78.37	-	-
Sunflower meal	18	-	-	85.21	-
Moringa leaves 50% + sunflower 50%	28	-	-	-	80.80
Maize bran	2	62.64	19.63	12.79	17.20
Mineral premix	0	2.00	2.00	2.00	2.00
Total	0	100.00	100.00	100.00	100.00

Dry ingredients were ground into fine powder and for each diet combination, hand mixing was done for 40 minutes. Water was added at 20-30% volume by weight to give a pelletable mixture. The diets were pelleted.

2.2 System and Experimental Design

The experiment was conducted at MAGADU research farm, Sokoine University of Agriculture, Morogoro, Tanzania. Four diets were used as experimental feeds in nine concrete tanks of equal size (21.4 m^3 each) Water depth was maintained at 1 metre thought the experiment. Each treatment was replicated twice. Fish broodstock of 70g each were stocked at a ratio of two female to one male (2:1) in each tank. Eight tanks were used for feeding broodstock and fingerlings afterwards, whereas one tank was used for reserving broodstock. Broodstock were fed for eight weeks but during this period all eggs produced were flashed out to ensure full involvement of diets in egg formation and embryology. After which they were allowed to have a single reproduction cycle. The fingerlings were fed a common type of food for four weeks.

Broodstock were provided feeds at a feeding level of 5% of their body weight and the fingerlings were fed at 10% of their body weight daily. Feeding regime was two times a day at 10:00 am and 04:00 pm for both broodstock and fingerlings. After production of fingerlings, the broodstock were removed from the tanks to prevent multiple spawning and this allowed fingerlings to grow.

2.3 Determination of the quantity of fingerlings

Fingerlings hatched from each family were counted once a week. Data collected was analyzed using SAS for windows. Complete Randomized Design (CRD) was the statistical model used.

2.4 Determination of growth rate and survival rate of fingerlings

For determination of growth rate, weight was measured every two weeks for a period of 30 days for a sample of fingerlings and specific growth rate was computed as follows

Growth rate = (final weight – initial weight) / time.

$$SGR = \frac{\ln Wf - \ln Wi \ X\ 100}{Tf - Tf}$$

Where;

SGR = specific growth rate
lnWf = natural logarithm of final weight
lnWi = natural logarithm of initial weight
Tf = final time
Ti = initial time

Complete Randomized Design (CRD) statistical model was used to analyze the data on growth rate.

Death of fingerlings was observed daily and recorded. These data was used to compute survival rate whereby, survival rate = (final count / initial count) * 100. Fingerling survival was compared among the treatment diets of the broodstock. Date was analyzed using a chi-square ($\chi 2$) test.

2.5 Statistical Analysis

Statistical analyses was performed using SAS for windows. Statistical significance between treatments was evaluated at 5% probability level. General linear model (GLM) ANOVA was used for further analysis of data. Values were expressed as means ± standard error.

3. Results

3.1 Growth performance of fingerlings produced.

Body Length of fingerlings produced

According to the observations made, the initial length for fingerlings produced by broodstock fed diet 1 (1.58 ± 0.015 cm) was longer than all other fingerlings. The length of these fingerlings was significantly different ($p<0.05$) from fingerlings from all other diets. Fingerlings produced by broodstock fed diet 3, measured (1.40 ± 0.015 cm) and this was 2nd to fingerlings produced by broodstock fed on diet 1. The length measured from the later was also significantly different ($p<0.05$) from fingerlings from all other diets. Fingerlings produced by broodstock fed diets 2 and 4 were had same initial body length (1.28 ± 0.015 cm) and they showed the shortest initial length recorded.

Broodstocks fed diets 1 and 3 produced fingerlings that showed the highest final lengths(4.24 ± 0.018 cm and 4.20 ± 0.018 cm respectively) and were not significantly different ($p<0.05$) from each other. On the other hand, Broodstock fed diets 2 and 4 showed lower final lengths (4.08 ± 0.018 cm and 4.03 ± 0.018 cm respectively) but the two were also not significantly different ($p<0.05$) from each other.

The length gained by fingerlings produced from broodstock fed diet 2 (2.80 ± 0.019 cm), diet 3 (2.80 ± 0.019 cm) and diet

4 (2.75 ± 0.019 cm) did not show a significant difference ($p<0.05$) from each other. These fingerlings showed higher length gain than broodstocks fed on diet 1 by about 0.1 cm. as shown in Figure 1.

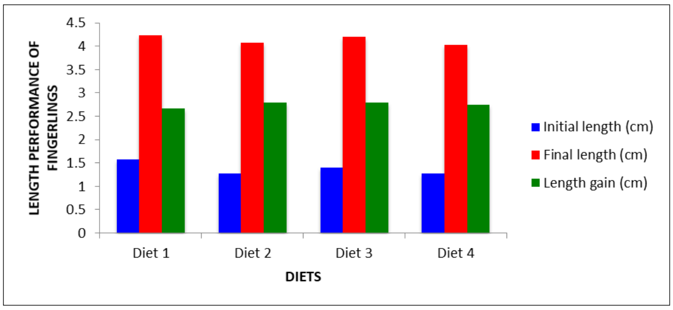

Fig 1: Body length of fingerlings produced by broodstock fed different diets

Body Weight of fingerlings produced

From the observations made, broodstock fed on diet 2 produced fingerlings with higher initial weight (0.29 ± 0.009 g) and significantly different ($p<0.05$) from all other diets' fed broodstock. Broodstock fed on diet 1, 3 and 4 produced fingerlings that were not significantly different ($p<0.05$).

Broodstock fed on diet 2 also produced fingerlings with higher final weight (1.19 ± 0.03 g) and the weight was significantly different ($p<0.05$) from all other fingerlings. Broodstock fed on diet 1, 3 and 4 produced fingerlings that were not significantly different ($p<0.05$) from each other in their final weights.

Broodstock fed on diets 2 and 3 produced fingerlings that gained higher weights (0.90 ± 0.03 g and 0.82 ± 0.03 g respectively) these weights were not significantly different($p<0.05$) from each other. Weights gained by fingerlings produced from brooders fed on diets 1 and 4 (0.71 ± 0.03 g and 0.72 ± 0.03 g respectively) did not show a significant difference ($p<0.05$) among themselves but were lower by about 0.2 g compared to weight gained by fingerlings produced by broodstock fed on diets 2 and 3.

From the observations made, broodstock fed on diets 2 and 3, produced fingerlings with higher growth rates in grams per day (0.04±0.001 g/d). Their rates were significantly different from those of fingerlings produced by Broodstock fed on diets 1 and 4 (0.03±0.001 g/d). The difference in growth rate was 0.01 g/d.

Broodstock fed on diet 3 produced fingerlings with the highest specific growth rate (7.57 ± 0.24) followed by fingerlings produced by brooders fed on diet 4 (7.28 ± 0.24). Broodstock fed on diet 2, produced fingerlings with the least specific growth rate (6.75 ± 0.24). The specific growth rate for fingerlings produced by brooders fed on diet 2 was significantly different from that of diet 3. Growth performance of fingerlings produced by broodstock fed different diets is summarized in Table 2 and Figure 2, Growth rate of fingerlings produced by broodstock fed different diets is illustrated in Figure 3.

Table 2: Growth performance of fingerlings produced by broodstock fed different diets

Parameters	Diet 1	Diet 2	Diet 3	Diet 4
Initial weight (g)	0.21 ± 0.009^b	0.29 ± 0.009^a	0.21 ± 0.009^b	0.20 ± 0.009^b
Final weight (g)	0.92 ± 0.03^b	1.19 ± 0.03^a	1.03 ± 0.03^b	0.92 ± 0.03^b
Weight gain (g)	0.71 ± 0.03^b	0.90 ± 0.03^a	0.82 ± 0.03^a	0.72 ± 0.03^b
Growth rate (%)	9.5 ± 0.001^b	14.3 ± 0.001^a	7.9 ± 0.001^a	14.3 ± 0.001^b
Specific growth rate	7.07 ± 0.24^{ab}	6.75 ± 0.24^b	7.57 ± 0.24^a	7.28 ± 0.24^{ab}

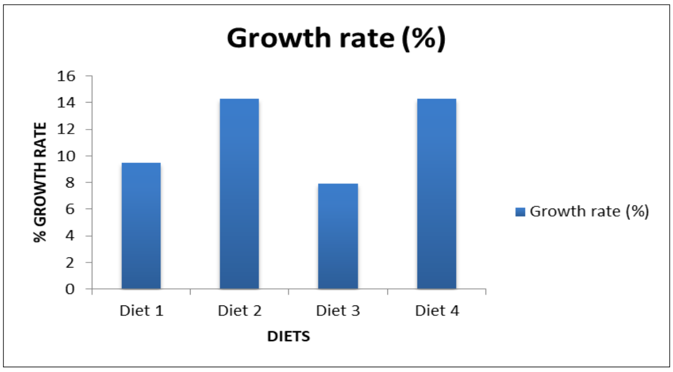

Fig 2: Growth rate of fingerlings produced by broodstock fed different diets

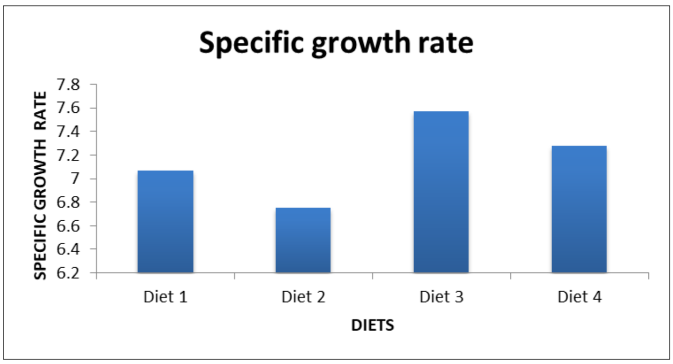

Fig 3: Growth rate of fingerlings produced by broodstock fed different diets

3.2 Number of fingerlings produced by broodstock fed different protein sources

The quantity of fingerlings was determined by counting their number every week in every diet.

Broodstock fed on diets 1 and 2 produced a higher initial count of fingerlings (532.5 ± 15.6 and 525.0 ± 15.6) the counts were not significantly different ($p<0.05$) from each other. These counts ware significantly different ($p<0.05$) from the initial counts experienced in fingerlings produced by brooders fed on diets 3 and 4. The difference between the highest initial count i.e. diet 1 (533 fingerlings) and the lowest initial count diet 4 (424 fingerlings) was so big (109 fingerlings).

The final counts also showed the same trend of results as the initial weights. Diet 1 produced the highest final count of fingerlings (520 fingerlings), while diet 4 produced the lowest final count of fingerlings (412 fingerlings). The difference of the lowest final count from the highest final count is the same as that of initial count (108 fingerlings).

Fingerlings produced by Broodstock fed on diet 2 had a higher survival rate (99.4 ± 0.2%) and significantly different from survival rates for all other fingerlings. Broodstock fed on diets 1, 3 and 4 had no significant difference in their survival rates. Fingerlings produced by broodstock fed on diet 4 had the poorest survival rate than all others (97.1 ± 0.2%). Fingerlings count produced by broodstock fed different diets is summarized in Table 3, Figure 4 illustrates the number and survival of fingerlings produced by broodstock fed different diets.

Table 3: Fingerlings count produced by broodstock fed different diets

Parameters	Diet 1	Diet 2	Diet 3	Diet 4
Initial Count	532.5 ± 15.6[a]	525.0 ± 15.6[a]	440.5 ± 15.6[b]	424.0 ± 15.6[b]
Final Count	519.5 ± 15.9[a]	522.0 ± 15.9[a]	429.5 ± 15.9[b]	411.5 ± 15.9[b]
Survival Rate (%)	97.6 ± 0.2[b]	99.4 ± 0.2[a]	97.5 ± 0.2[b]	97.1 ± 0.2[b]

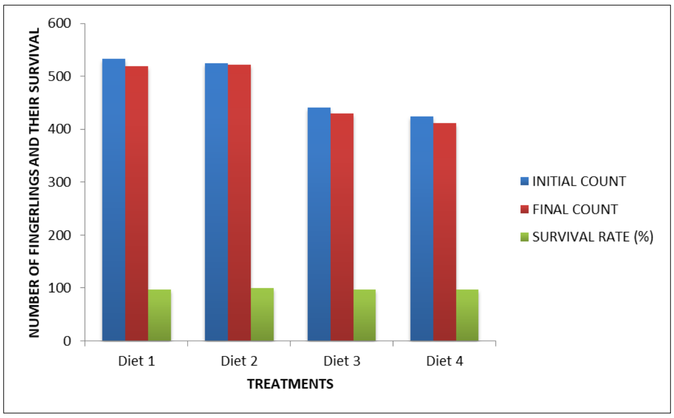

Fig 4: Number and survival of fingerlings produced by broodstock fed different diets

3.3 Survival rate of fingerlings produced by broodstock fed different protein sources

During the experiment, there was fingerling mortality in every treatment, though in treatment number 2 (diet 2), mortality was negligible therefore diving the highest survival rate. The calculated chi square value was 16.5. Degree of freedom was 3 and the tabulated chi square value was 7.8 for level of significance $p<0.05$. Under this level of significance, it showed that only treatment 1 (diet1) had significant effect on survival of fingerlings.

4. Discussion

Seed availability is one of the main challenges for small-scale fish farmers [11]. For maintenance, growth, and reproduction functions, fish uses dietary proteins continuously [12]. Increased plant ingredients in fish diets decrease diet digestibility due to increased fiber and other ant nutritional factors [13]. According to the Proximate analysis that was done at the Department of Animal, Aquaculture and Range Sciences, Sokoine University of Agriculture, crude protein for the main protein sources in each diet were; Fish meal 54%, Moringa leaf 28%, Sunflower seedcake 18%.

Brooders fed on diet 1 (with fishmeal as the protein source) produced fingerlings with highest final lengths of i.e. 4.24 ± 0.018 cm, Diet 2 (contained moringa leaf meal as the protein source) produced fingerlings of highest final weight i.e. 1.19 ± 0.03 g, diets 1 and 2 recorded the highest count of fingerlings i.e. 519.5 ± 15.9 fingerlings. Diet 2 had the highest survival rate i.e. 99.4 ± 0.2.

Generally, Diet 2 produced fingerlings with highest survival rate, highest count and highest weight. Diet 1 produced fingerlings with highest length and count. Diet 3 produced fingerlings with highest length, length gain and weight gain. Many studies have been conducted on suitability of fishmeal, as protein source for fish feed and it has proved to have no competent in promoting growth and reproduction of fish. However, to the best of my knowledge, fishmeal has not yet been compared with other protein sources for quality and quantity fingerling production. This study has compared the three different protein sources in four different diets and it has shown that diet 2 produce the best fingerlings that weighed higher and had a greater survival rate. Diet 1 comes after diet 2.

4.1 Properties of *Moringa oleifera* that may have led to these findings

Moringa protein is made of 82-91 % pepsin soluble nitrogen 1–2% of acid-detergent insoluble protein [14], thus a high digestibility. Similar *Moringa* Protein digestibility results in the red strain of tilapia at Stirling University but originally from Egypt, reported by Madalla 2018 [15] using the Guelph system but lipid was only 65% digested. *Moringa oleifera* is reported to be a good source of vitamins and amino acids [16]. *Moringa oleifera* is claimed to boost immune systems [17, 18].

4.2 Anti-nutrients

Moringa oleifera leaves contained tannins 21.19%, phytates 2.57%, trypsin inhibitors 3.0%, saponins 1.6%, oxalates 0.45% and cyanide 0.1%. The levels of these anti-nutrients were low.

5. Conclusion and Recommendations

Though fishmeal is mentioned as a good protein source for growth performance in tilapia [19], the Tanzania local communities farming tilapia have a perception that fishmeal is expensive and indeed it is because its highly used as human food but not animal feed. Therefore, availability and affordability of moringa leaves is higher than fishmeal and other feed ingredients competitive for both human and animal consumption [20]. According to this study, Moringa leaves produce the best protein source for broodstock diet. Fingerlings produced by brooders fed on diet 2 showed better performance in parameters assessed followed by fingerlings produced from brooders fed on diet 1. In order to have sustainable aquaculture, farmers should adapt the use of moringa leaves for reliable fingerling production. Adoption of this feed in aquaculture hatcheries will help in releasing the overexploitation burden on wild fish. Further studies should be done on other plant protein sources as broodstock feeds, perhaps there are others that are more efficient than moringa leaves.

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