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Supplemental feed formulation for the best growth performance of Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) (Pisces: Cichlidae) in pond culture system

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Abstract: Aquaculture remains virtually nonexistent in Ethiopia despite the nation's suitable natural and socio-economic conditions to support its development. One of the major setbacks to the sector's development in the country is a critical shortage of well formulated aquafeeds. Nile tilapia *(Oreochromis niloticus* (Linnaeus, 1758)) is one of the potential aquaculture species in the country. Therefore, this study was conducted to identify the best supplemental feed formulation from locally available and low-cost ingredients for the best growth performance and yield of Nile tilapia in earthen pond culture. The experiment was designed to consist of three treatments of 30 %, 35 %, and 40 % crude protein formulated feeds, and one control, each in two replicates. Juvenile fishes of known initial length and weight were stocked into the experimental ponds at a rate of 2 fish m^{-2}. The fishes in the treatment ponds were fed with the formulated feeds at 5 % of their body weight twice a day for 150 days. The growth, feeding and condition factor parameters were computed following known equations in the literature. Variations in the mean fish size were tested using one-way ANOVA. The values of growth and yield as well as feed conversion parameters and fish condition were higher for the fishes fed with the 35 % crude protein level. These parameters decreased as the amount of crude protein was increased beyond 35 %. The economic valuation suggested that large-scale earthen pond production of Nile tilapia can be economically feasible based on the feed formulated from the local ingredients experimented in the present study.

Keywords: Crude protein, supplemental feed, formulation, growth performance, Nile tilapia, pond culture

INTRODUCTION

Aquaculture provides most of the world's aquatic edible resources[1]. Nile tilapia is an important freshwater aquaculture fish species worldwide owing to its essential characteristics related to its rates of growth, breeding and feed conversion, resistance to disease and adverse environmental and human induced conditions, and its consumer acceptance[2-5]. Flexibility in feeding habits also makes Nile tilapia easy to raise in both polyculture and monoculture systems[6]. It can tolerate higher dietary fiber and carbohydrate concentrations than most other cultured fish[3]. Feed is one of the most important factors influencing performance of cultured fish[7-10]. No single feed ingredient can supply all the nutrients and energy that the fish requires for its best growth, thus calling for a formulated feed that can provide all or most of the nutritional requirements of the target fish. An increase in intensive culture of many freshwater fishes places a great demand on efficient diets. Good nutrition in production systems is essential to economically produce a healthy and high product, and thus the first consideration in formulation of feed is the quality of the feed ingredients[11]. Supplementary feeds in fish culture may consist of by-products such as wheat bran, oilseed cakes (for instance, peanuts and soybean), green fodder, maize bran, and chicken waste[12].

Aquaculture in Ethiopia (East Africa) remains underdeveloped despite the country's conducive physical and socio-economic conditions to support its development[13]. One of the major setbacks for aquaculture underdevelopment in the country is lack of sustainable formulated aqua feeds. Nevertheless, the country has a great potential for aquaculture development with many kinds of agro-industrial and abattoir by-products being available. Nile tilapia remains one of the most important potential aquaculture species in Ethiopia. Optimum feed formulation of protein requirement is one important factor for Nile tilapia pond aquaculture. Therefore, this study was conducted to identify the best feed formulation from locally available and low-cost ingredients of poultry manure and mill flour residues for the best growth performance and yield of Nile tilapia in pond culture system in Ethiopia. The economic feasibility of using these locally available feed ingredients was also assessed.

MATERIALS AND METHOD

Study area and the experimental ponds: The study was conducted at Jimma in southwestern Ethiopian highland located at an altitude of 1700 m above sea level. During the period of the experiment, the area had mean daily temperature and annual rainfall of 20.71 °C and 123.01 mm, respectively[14]. Each pond used for the experiment had an area of 15 m^2 and average depth of 75 cm. The inlet and outlet pipes were 10 cm in diameter and these were covered with wires of 2 mm mesh to prevent the escape of experimental fish from the ponds and the entry of potential predators. The ponds were filled with water from a nearby river, Awetu River. The entry and exit of water was constantly regulated to compensate for water loss through seepage and evaporation as well as to maintain water quality parameters within the optimum range for the experimental fish. The surrounding of the whole experimental ponds was fenced with wire mesh and barbed wires to keep out predators and other animals.

Nutrient analysis: Locally available and relatively cheaper feed ingredients were used for feed formulation. These included poultry manure and three categories of mill flour residues viz. maize, barley and wheat brans. These were obtained from the poultry farm of Jimma University College of Agriculture and Veterinary Medicine and open markets selling the flour residues. The compositions of the feed ingredients were determined following the standard analytical protocols of AOAC[15]. Moisture and dry matter contents were measured by placing a sample of known weight in an oven set at 90 ^oC for 24 hours. The samples were cooled in a desiccator for 10 minutes before weighing. The weight lost from the sample was considered as the moisture content and the remaining weight as dry matter:

Moisture content (%) = $(W_F-W_D)/W_F)*100$ [1]

Where, W_F = Weight of fresh sample, W_D = Weight of dry sample

Ash content was measured by placing a sample of known weight in a furnace of 550 ^oC for 4 hours, after which the samples were cooled in a desiccator and weighed, and the remaining weight was considered the ash:

Percent of crude ash = (Weight of ash/Weight of sample)*100 [2]

Crude protein was estimated by measuring the total nitrogen content of the ingredient using the Kjeldahl method, and then by multiplying the nitrogen content with specific conversion factor:

Nitrogen content (%N) = $((V_A-V_B)*NHCL*1.4007)/(W*DM/100)$ [3]

Where, V_A = Volume, in mL, of standard HCl required for sample; V_B = Volume, in mL,of standard HCl required for blank; NHCl = Normality of HCL (a standard acid); 1.4007 = milliequivalent weight of N*100; W = sample weight (g); DM = % of dry matter

Percent of crude protein (CP) = % N*Specific conversion factor [4]

Where, specific conversion factor = 5.53 (for poultry manure); 6.31 (Wheat bran); 6.25 (Maize bran); 5.83 (barley bran)[16].

Crude fat was determined by subjecting the samples to a continuous extraction with petroleum ether method using Soxhlet apparatus:

Percent of Crude fat = (Weight of fat/Weight of sample)*100 [5]

Crude fiber was determined as that fraction remaining after digestion with standard solutions of Sulphuric acid and Sodium hydroxide under carefully controlled conditions:

Percent of crude fiber (% of fat-free dry matter) = ((WC+DR)-(WC+AR))/WS*100 [6]

Where, WC = Weight of crucible; DR= dried residue; AR= ash residue;

WS= Weight of sample

Estimate of carbohydrate (Nitrogen free extract, NFE) was made by subtracting the percentages of all the other components:

Nitrogen-free extract (NFE) (% of Carbohydrate) =

100 % **-** (%crude protein + %crude fat + %crude fiber + %ash + %moisture) [7]

Total phosphorous was determined by spectrophotometer at 430nm absorbance.

Experimental feed formulation and processing: Three levels of fish feeds were formulated based on the percentage of crude protein (CP) and energy sources as 30 %CP & 48.91 %Energy (Treatment 1, T1), 35 %CP & 24.65 %Energy (Treatment 2, T2) and 40 % CP & 0.39 %Energy (Treatment 3, T3). The proportions of the experimental feed ingredients, in the formulated feeds, were determined using the Pearson square 2 method and algebraic equations, the most commonly used methods for balancing crude protein levels[17] (**Table 1**).

Table 1: Percent proportion (%) of each feed ingredient and percent contribution of each ingredient to crude protein (%CP) for each formulated feed.

	T1 30 %CP		**T2 35 %CP**		**T3 40 %CP**	
Ingredients	**%**	**%CP**	**%**	**%CP**	**%**	**%CP**
Poultry Manure	44.93	13.48	56.80	19.88	65.72	26.29
Wheat bran	23.30	6.99	29.49	10.32	34.10	13.64
Maize bran	14.27	4.28	6.14	2.15	0.08	0.03
Barley bran	17.50	5.25	7.57	2.65	0.10	0.04
Total	100.00	30.00	100.00	35.00	100.00	40.00

Total weight of ingredients = 12.19 kg (30 %CP); 18.52 kg (35 %CP); 12.27 kg (40 %)

The processes of formulating feeds from the local ingredients are shown in **Figure 1**. Poultry manure was ground to dry in the sun to sterilize (disinfect) and to facilitate the removal of feathers and other unwanted matter. Mill flour residues (maize bran, barley bran and wheat bran) were dried, digested, and mixed by adding warm/hot water for at least 5 minutes in order to ensure even distribution of ingredients within the mixture[18]. The ingredients were then mixed according to their relative proportions (**Table 1**) in 500 ml of water per kilogram of sample to form a mash. The mash was ultimately dried in the sun and packed in water impermeable nylon/plastic bags.

Figure 1: The process of feed formulation from the local ingredients

Experimental Design: The study required eight earthen ponds for three treatments and a control, with their replications. A total of 30 Nile tilapia juveniles were stocked into each pond at the rate of 2 fish m^{-2} [19]. Fish in the treatment ponds were fed twice daily in the morning (8.00-9.00 local time) and evening before sunset (17.00-18.00 local time) at a rate of 5 % of their body weight. The quantities of formulated feeds required to feed fishes were determined based on the mean fish weight in each pond and appropriate feeding rate[20-22]. The quantities of daily feed weights were adjusted during each sampling event (i.e. every two weeks) to accommodate for fish weight changes[20, 21].

Data collection: The length and weight data were collected from 60 % of fish sampled every two weeks to evaluate the fish's growth performance and feed utilization under the different experimental setups[23-25]. Total length (from tip of snout to caudal fin tip) and total weights were measured using measuring board and triple beam balance, to the nearest 0.01 cm and 0.01 g, respectively. The initial and final numbers of fish stocked were also taken.

DATA ANALYSIS

Biological data: using one-way ANOVA in SPSS (version 16). A pair group mean difference was compared using Posthoc Tukey test. All statistical tests were considered at 5 % significance level. The various growth parameters, Feed conversion ratio and efficiency, Fulton condition factor (FCF), survival rate, and Yield were computed as follows[23, 25, 26]:

Weight gain (WG, g) = Mean W_f – Mean W_i [8]

Where, W_f = final weight (g), W_i = initial weight (g)

Daily Growth Rate (DGR, g day^{-1}) = WG/Days [9]

Where, Days = Number of culture days

Specific growth rate (SGR, % day^{-1}) = ((lnWf–lnWi)/Days))*100 [10]

Fulton condition factor (FCF, g cm^{-3}) = TW/TL^3 x 100 [11]

Where, TW is total weight (g) and TL = Total length (cm)

Survival rate (SR, %) = (Number of fish harvested/Number of fish stocked)*100 [12]

Feed Conversion Ratio (FCR) = $(W_{Feed})/W_g$ [13]

Where, W_{Feed} = Total weight of feed consumed by the fish (kg)

Wg = Total weight gain by fish from feed consumed (kg)

Feed Conversion Efficiency (FCE, %) = WG (g)/Total weight of feed (g) [14]

Net Yield (Kg $year^{-1}$) = (Biomass gain (kg)/Days)*365 days [15]

Economic data: Economic valuation of Nile tilapia production based on the current market price of the feed ingredients used for formulating the diets was made according to Engle[27] as follows:

Investment Cost Analysis (ICA) = Cost of Feeding + Cost of juveniles stocked + Construction cost + Materials cost + Labor [16]

Production Value (PV) = Mean weight gain of fish cropped (kg)*Total number of survival (n)*Price per kg [17]

Net profit Value = Production Value–Investment cost [18]

RESULTS AND DISCUSSION

The compositions of the experimental feed ingredients: The poultry manure had the highest crude protein content followed by wheat bran and barley brans, whereas maize bran had the least crude protein content. Maize bran had the highest carbohydrate content, whereas poultry manure had the least carbohydrate content (**Table 2**).

Table 2: The compositions of the experimental feed ingredients

Ingredients	%DM	%Moisture	%CP	%CF	% CFibre	%Ash	%C	%P
Barley bran	85.72	14.28	10.73	2.35	9.9	3.52	59.22	0.35
Maize bran	93.66	6.34	8.74	4.0	5.8	1.16	73.77	0.31
Wheat bran	85.35	14.65	13.69	2.2	2.4	1.24	65.82	0.37
Poultry Manure	70.73	29.27	26.39	2.55	25.1	16.22	0.47	0.57

DM = dry matter; CP = crude protein; CF = crude fat; C = carbohydrate; P = phosphorus

Mean fish Size and Yield: Summary of the mean final length, weight and yield are presented in **Table 3**. The initial length and weight of the experimental fish ranged 10–12 cm and 20–24 g, respectively. There was no statistically significant difference in the mean initial length and weight among the treatment and control groups ($p > 0.05$). The results showed that the fish growth, both in length and weight, was affected by the variation in the supplemental formulated feed ($p < 0.05$).

Table 3: Summary of the final mean lengths, weights, total weight gain and yield of Nile tilapia during experimental period

Treatment	N_1	N_2	Mean L_f	Mean W_f	TW_g	Yield	SR (%)	FCR	FCE (%)	FCF
T1(30 %CP)	60	59	13.31 ± 0.624^a	40.06 ± 3.585^d	1.06	2.57	98.33	0.75	10.17	1.62
T2(35 %CP)	60	60	14.33 ± 0.828^b	81.00 ± 12.36^e	3.51	8.54	100	0.32	23.29	2.24
T3(40 %CP)	60	59	13.36 ± 0.723^a	40.22 ± 4.008^d	1.063	2.59	98.33	0.73	10.22	1.62
Control	60	58	12.14 ± 0.35^c	25.22 ± 0.637^f	0.16	0.397	96.67	-	-	1.42

N_1 = Number of fish stocked; N_2 = Number of fish harvested; L_f = Final length; W_f = Final weight; TW_g= Total weight gain; SR = Survival rate; FCR = Feed conversion ratio; FCE = Feed conversion efficiency; FCF = Fulton condition factor

The highest mean weight (81.00 g) was recorded for the fishes fed with 35 %CP (T2), followed by the fishes fed with the 30 %CP (T1) and 40 %CP (T3), which nearly demonstrated equivalent growth performance ($p > 0.05$). Fishes in the all treatments demonstrated better growth performance than those in the control group. Similarly, the annual total net production was higher for T2 (i.e. 8.54 kg $year^{-1}$) followed by T3 (i.e. 2.59 kg $year^{-1}$) and T1 (i.e. 2.57 kg $year^{-1}$). Feed is one of the most important factors influencing growth performance of cultured fish[8-10]. To ensure fast growth and high yield a well-balanced formulated feed with optimum levels of ingredients is essential. Particularly, dietary protein is always considered to be of primary importance among the nutrients in the formulated feeds. Biomass gain is directly influenced by good quality feed with optimum dietary protein. The present findings in relation to growth and yield of cultured Nile tilapia fed with different protein levels of formulated supplemental feed concurred with previous works. For instance, Miguel *et al.*[28] indicated that diets containing alfalfa leaf protein yielded the best growth performance when the plant protein replaced up to 35 % of the fish-meal protein in the diet, but increasing the level over 35 % decreased growth rate. Body[29] found that the crude protein requirement of Nile tilapia is size dependent and thus the proportion provided should decrease as the fish grows i.e. 45-50 % protein for smaller fish (0.02g), 40 % protein for 0.02-2g, 35 % protein for 2-35g fish, and 30-35 % protein for fish heavier than 35g. Similarly, others[9, 30] found that fish growth increased as the dietary protein level increased from 25 % to 35 % and then declined. The highest annual net yield of Nile tilapia was also reported at 35 % dietary protein[31]. This phenomenon could be explained as the fact that each size has a certain protein limit after which excess protein level could not be utilized efficiently[32].

Daily and Specific growth rates: Growth trends of Nile tilapia fed with the three differently formulated feeds and control group are given in **Figure 2**. The growth rates of the fishes varied between the sampling weeks. The trends in the daily (DGR) and specific (SGR) growth rates are given by **Figures 3 & 4**.

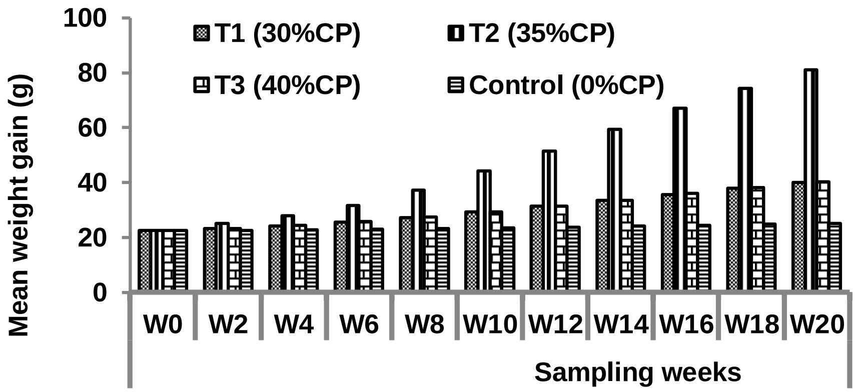

Figure 2: Mean weight (g), during the successive sampling weeks, of fishes in the treatments and control group.

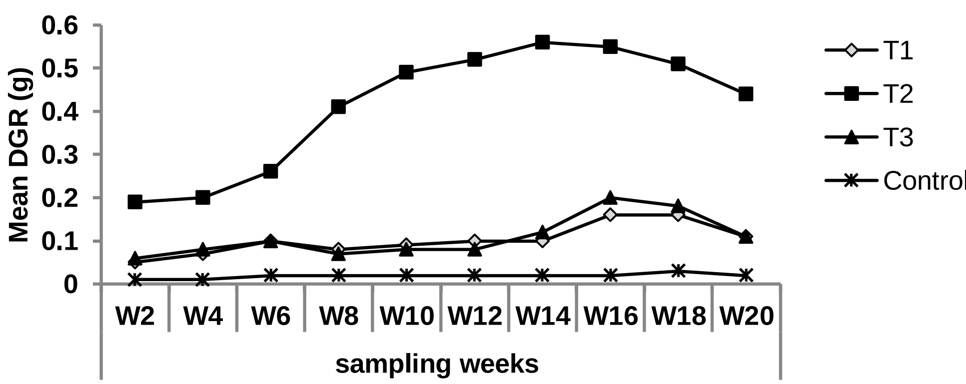

Figure 3: Mean daily growth rates (g day^{-1}), during the successive sampling weeks, of fishes in the treatments and control group.

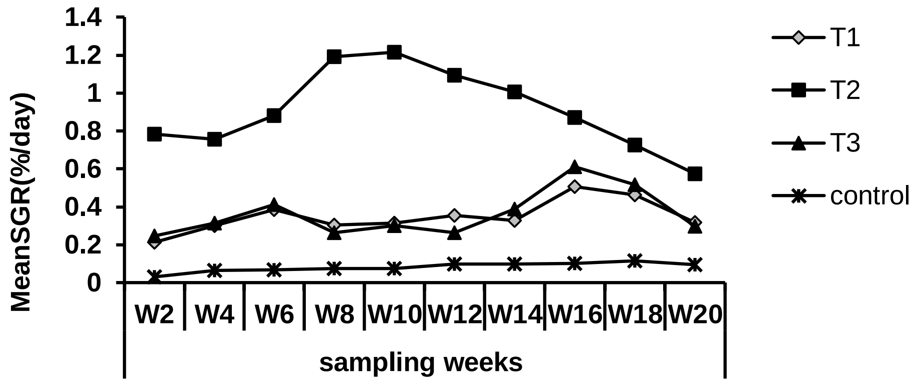

Figure 4: Mean specific growth rate (% day^{-1}), during the successive sampling weeks, of fishes in the treatments and control group.

Though the fishes generally demonstrated an increasing trend in both daily and specific growth rates, the fishes in the control group grew at much lower rate as compared to the fishes in the treatment groups. Fishes in the 35 %CP treatment (T2) attained the highest mean DGR (i.e. 0.56 g day^{-1}) and mean SGR (1.22 % day^{-1}) while growth rates for the fishes in the 30 %CP (T1) and 40 %CP (T3) were more or less comparable. Both the mean size (i.e. length and weight) and growth rate parameters showed that a supplemental feed formulated with 35 %CP and 24. 65 % Energy promoted the best growth performance for Nile tilapia pond culture. The DGR and SGR of Nile tilapia in the present study are comparable with the values reported previously from elsewhere[31, 33-38].

Survival rate, Feed conversion rate and efficiency, Fulton Condition Factor: The percent survival rate (%SR), feed conversion rate (FCR), Feed conversion efficiency (%FE) and the Fulton condition factor (FCF) values for the present study are summarized in **Table 3.** Though there was 100 % survival rate in T2, there was no marked variation in the survival rate among the treatment groups as well as between the treatments and the control group. The feed conversion ratio is an index for the amount of feed used to produce one kilogram of fish, and as such it shows whether the fishes are overfed or underfed[30]. FCR decreased with increasing dietary protein from 30 % to 35 %CP, and then increased at 40 %CP in agreement with the previous findings[37, 39]. The lowest FCR at 35 % dietary protein is indicative of the most efficient feed utilization of the feed at this protein level. Fishes in this feed treatment (T2) also had the highest feed conversion efficiency values. The values of FCR, FCE and FCF for the T1 and T3 were comparable and remained subordinate to the values for T2. These findings are in agreement with the previous reports who found, for instance, FCF values ranging from 2.34 to 5.18[36, 38, 40-42]. Similar result was also observed at 35 % dietary protein level (1.77-2.92 g cm^{-3}) by other investigators[31]. Furthermore, the best mean FCF (2.24±0.11g cm^{-3}) at 35 % dietary protein level is close to 3 indicating the proportional growth with respect to weight and length. Therefore, healthy Nile tilapia can be obtained at this formulated dietary crude protein level with efficient utilization of protein. There was no significant variation in survival rate between formulated feeds.

Economic Analysis: A total of 24.05 kg of poultry manure, 12.48 kg of wheat bran, 2.89 kg of maize bran and 3.55 kg of barley bran, totaling 42.97 kg was used for the present experiment. The prices of poultry manure, wheat bran, maize bran and barley bran during the experiment period were 0.40, 0.70, 0.50 and 0.60 Ethiopian Birr (ETB)[An average exchange rate for 1Ethiopian Birr (ETB) ≈ 0.0434USD] per kg, respectively. Thus, only 21.95 ETB was paid to buy the whole ingredients of the fish feed for this study. Thus, with the free collection of juveniles from wild with the minimum labor cost, 150 ETB for the water canal maintenance, and 100 ETB for the others material cost, ICA was estimated as 271.95 ETB for the experiment period. With 35 Birr kg^{-1} of fish price, the total PV and NPV were estimated as 387.10 ETB and 115.15 ETB, respectively (**Table 4**). Thus, large-scale production of Nile tilapia based on the outcome of the present finding, i.e. a feed with 35 %CP and formulated from locally available low-cost feed ingredients such as poultry manure and mill flour residues, can be economically feasible as it can produce 2,833.33 kg of fish ha^{-1} worth of total value of 99, 166.55 ETB.

Table 4: The economic analysis parameters of feed used in this study

	Cost or Value (ETB)			
Treatment	**Feed**	**ICA**	**PV**	**NPV**
30 %CP (T1)	6.35	73.85	82.75	8.90
35 %CP (T2)	9.40	76.95	170.10	93.15
40 %CP (T3)	6.20	73.65	83.05	9.40
0 %CP (Control)	0.00	47.50	51.20	3.70
Total (ETB)	**21.95**	**271.95**	**387.10**	**115.15**

ICA = Investment cost analysis; PV = Production value; NPV = Net production value

CONCLUSION

The results of the present study demonstrated that affordable and feasible fish feed can be formulated from locally available low cost ingredients for Nile tilapia culture production. Though the crude protein requirement of Nile tilapia was shown to be size dependent, on average the fish shows better growth performance, feed utilization, fish condition and survival rate at 35 % CP level. The feed composition of 35 % CP included 75 % from protein supplements and 25 % from energy sources. The application of the outcome of the present study for large-scale production of Nile tilapia is economically feasible.

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