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Effect of Dietary Soyabean [*Glycine max* (L.) Merr.] Protein Level on Growth and Feed Utilization of *Oreochromis andersonii* (Castelnau, 1861)

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Abstract: *Oreochromis andersonii* were maintained on three formulated diets based on protein levels (20, 30 and 40%) with soya bean as the source. The effect of dietary protein on growth and feed utilization were investigated. The cost effectiveness of the diets was also evaluated. Both the Body Weight Gain (BWG) and final mean weight of fish maintained on 30% protein were not significantly different ($p>0.05$) from the fish fed with 40% protein diet. The Protein Efficiency Ratio (PER) declined with protein increase ($r = -0.282$, $n = 268$, $p<0.05$). There was a linear relationship ($Y = 0.318x + 12.79$, $r^2 = 0.97$) between protein level and BWG. Gross margin for fish reared on 30% and 40% crude were insignificant ($p>0.05$) though significantly higher than the 20% crude protein fed fish and the control. The carcass protein composition was not affected ($p>0.05$) by the protein level. The 30% soyabean based crude protein is recommended for *O. andersonii*.

Key words: *Oreochromis andersonii*, weight gain, soyabean, protein, gross margin

INTRODUCTION

Protein is the main source of nitrogen and essential amino acids in animals and often the first nutritional requirement of consideration in feed formulation. Tacon and Cowey (1985) showed that there is a linear relationship between daily protein requirement per unit body weight and specific growth rate in fish. However, it is the most expensive source of energy in artificial feeds. Furthermore, excessive dietary protein may cause deterioration of water quality by increasing the ammonia excretion. Therefore, it is necessary to keep the proportion of protein down to optimum levels necessary for good growth and feed conversion ratio to reduce the cost and to reduce the nitrogen waste primarily ammonia.

Fish meal has been recognized as the best source of protein for most of fish species (Al-Kenawy *et al*., 2008) but due to increasing demand, low supply and high cost of fish meal, there has been a lot of efforts that have been directed in finding suitable substitute. Fish meal also causes phosphorus pollution (Phromkunthong and Udom, 2008; Cahu *et al*., 2009; Koumi *et al*., 2009). Several plant and animal based sources have been suggested though the latter is expensive. Soyabean meal is used most extensively as a plant source of protein, especially in human and many animal diets due to its good nutritional and commercial value. Furthermore, soyabean is an important nitrogen fixing legume to soils. Soyabean meal can replace fish meal in fresh water omnivores, such as tilapia *Oreochromis* species (Lovell, 1989). Al-Kenawy *et al*. (2008) found insignificant differences ($p>0.05$) in growth parameters on juvenile *O. niloticus* subjected to 25% soyabean and fish meal based protein.

Feed accounts for up to 60% of the total operational cost of producing fish (Virk and Saxena, 2003; Madalla, 2008). Therefore, high cost of the feed may derail the development of the sector. Availability of cost effective diet can make a difference between a profitable and unprofitable operation and may determine the economic viability of a fish farming operation (Madalla, 2008).

The study was conducted in order to determine the influence of protein sourced from soyabean (plant based) on *O. andersonii* in hapas set in an earthen pond. The cost effectiveness of the same diets was investigated too.

MATERIALS AND METHODS

Experimental fish and design: The trial was conducted at National Aquaculture Research and Development Centre (NARDC) for fifty five days. The fish (4.03±1.5 g; Mean±SD) were selected and stocked in the pond cage put in 5,000 m^2 fish pond for 22 days for acclimatization to the pelleted feed. In the pond cage the fish were provided twice a day (10:00 and 15:00hrs) with feed calculated at 5% live body weight with the 30% crude protein formulated using fish meal, maize bran, mineral premix and vitamin premix using WinFeed 2.8 version software (WinFeed (UK) Limited) after proximate analysis of the ingredients as described by AOAC (2002).

Forty (40) hapas fixed in a 750 m^2 semi-concrete pond were set in a Random Complete Block Design (CBD) (Gomez and Gomez, 1984) with three protein levels

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Table 1: Formulation and proximate composition of experimental diets (%) used to feed *O. andersonii*

Ingredients	20%	30%	40%
Soyabean cake	30.1	56.1	82.0
Maize bran	61.3	36.1	10.9
SoyaGold oil	6.7	5.9	5.2
[1]Vitamin	1.0	1.0	1.0
[2]DCP	1.0	1.0	1.0
Proximate analyses			
Crude protein (%)	20.0	30.0	40.0
Crude fat (%)	15.0	15.0	15.0
Crude ash (%)	3.2	3.9	4.6
Crude fibre (%)	8.2	6.4	4.7
Carbohydrates (%)	45.1	37.0	28.9
Gross energy (kcal/g)	394.6	402.3	410.0

[1]Vitamin stress pack (100 g): vitamin A 2,000,000 I.u, vitamin D3 300,000 I.u, vitamin E 3000 I.u, vitamin K3 300 mg, vitamin C 3,000 mg, riboflavin 500 mg, niacin 2,500 mg, pantothenic acid 1,000 mg, vitamin B12 3 mg, pyridoxine 200 mg, folic acid 50 mg and thiamine 200 mg.
[2]Di-calcium phosphate

(20%, 30% and 40%) and a control (no feeding). Hapas (0.9 x 1.5 x 0.4 m) were then set in 4 rows with each line comprising ten (10) hapas. For each treatment (protein level) and a control there were four replicates. Each hapa was stocked with 10 fish totaling 100 fish for every treatment.

Feed formulation procedures: The ingredients were ground and mixed thoroughly to achieve a homogenous sample. Water (5-10%) was then added before taken to a pellet making machine (BSW 330) attached with a 3.2 mm metal die to make the three diets. Since one machine was used to make the pellets the first feed from the metal die after a turn was discarded to avoid contamination from the previous feed. The feed was then spread on the sacks and sundried for two days before they were put in polythene bags and stored at 8°C till used.

Feeding regime: Fish were provided rations twice a day (10:00hrs and 15:00hrs) calculated at 5% live body weight using the formulated experimental diet. The feed allowance was adjusted only when mortality was observed in the hapa.

Chemical analysis: Dry matter of feed and fish was determined by drying samples in an electric furnace maintained at 105°C for 5 hrs. Crude protein levels were determined indirectly from the analysis of total nitrogen by the Micro-Kjeldahl method after acid digestion. The amount of protein in the sample was calculated by multiplying the amount of nitrogen by 6.25. The ash was determined as total inorganic matter by incineration of the sample in an electric furnace at 550°C for 5 hrs. The crude fat was determined by extraction with petroleum ether for 16 hrs in a Soxhlet apparatus.

After drying the ether, the flasks containing the fat were dried in an oven for 8 hrs at 85°C. The ether was evaporated and the crude fat weighed. Crude fibre was determined by subjecting the residue from ether extraction to boiling in dilute sulfuric acid (1.25%) for 30 min, followed by boiling in dilute sodium hydroxide (1.25%) for another 30 min. This nitrogen-free extracts was calculated by subtracting the percentages calculated for each nutrient from 100. Gross energy was calculated using standard factors of 23.6, 39.5 and 17.2 kJ/g for protein, lipid and carbohydrates respectively according to Jauncy (1998).

Water quality parameters: Water temperature, pH and conductivity were monitored twice a week using a Horiba U - 10 water checker. Nitrite was determined once a week analytically with samples read on a spectrophotometer (HC 1000).

Calculations: Several growth, organ and feed utilization performance parameters were calculated with the following equations:

$$\text{BWG} = \text{Final weight of fish} - \text{Initial weight of fish} \quad (1)$$

Where BWG = Body weight gain

$$\text{Specific growth rate (SGR)} = ((\ln W_f - \ln W_i)/t)*100 \quad (2)$$

Where $\ln W_f$ is the natural logarithm of final body weight, $\ln W_i$ is the natural logarithm of initial body weight of the fish and t is final time of the experiment in days.

$$\text{Condition factor (K)} = [(\text{fish weight} / (\text{length (TL)})^3]*10^5 \quad (3)$$

Where TL is total length (mm) (Ricker, 1975).

$$\text{PER} = \text{Fish weight gain/total protein fed} \quad (4)$$

Where PER: Protein Efficiency Ratio

$$\text{AFCE} = \text{Fish body weight gain/total feed intake})*100 \quad (5)$$

Where AFCE: Apparent Feed Conversion Efficiency

$$\text{Survival rate} = \frac{\text{Number of fish at the end of the experiment}}{\text{Number of fish at the start of the experiment}} \times 100 \quad (6)$$

Statistical analysis: Prior to analysis, parametric data were tested for normality using Shapiro - Wilk test and the homogeneity of variance using Levene's test for Equality of Variances. General Linear Model (GLM), univariate analysis procedure, was performed to determine the differences among treatment means deemed at $p<0.05$ followed by Turkey's multiple comparison test.

Cost analysis: Simple gross margin analysis was performed to determine the cost effectiveness of the prepared diets. It was assumed that all other operating costs remained constant and only the variable cost of ingredients was used in calculations. The cost of the diets was calculated using the prevailing prices for the feed ingredients in Zambia at the time of the experiment as follows; soybean ZK2, 700/kg, maize bran ZK600/kg, SoyaGold oil ZK5, 600/L, vitamin stress pack ZK90, 000/kg and DCP ZK14, 000/kg. The final weights (FM) were assumed to be the harvest weight of fish. The key economic indicators were computed according to Jolly and Clonts (1993) as follows:

$$\text{Total Cost (TC)} = P_sS + P_mM + P_oO + P_vV + P_dD$$

Where P_s: Unit cost of Soyabean S, P_m: Unit cost of maize bran M, P_o: Unit cost of soyGold oil O, P_v: Unit cost of vitamin V and P_d: Unit cost of DCP D:

$$\text{Total Revenue (TR)} = P*FW$$

Where P: Price of fish and FW = Final fish weights.

$$\text{Gross Margin (GM)} = TR - TC$$

Where TC and TR as described above.

Statistical Package for Social Scientist (SPSS) 15.0 (SPSS Inc) and Stata 12.0 (StataCorp) softwares were used in analyzing the data. Microsoft excel was used in the production of figures and graphs. Untransformed data (mean±SEM) are presented to facilitate interpretation.

RESULTS

Growth and feed utilization: Although the final Fish Mean Weight (FMW) for fish fed with 40% protein level (30.411±0.824 g) was higher than that of fish fed with 30% level (29.501±0.753) the difference was not significant (p>0.05) (Fig. 1).

Polynomial regression analysis showed a positive linear relationship (Y = 0.318x + 12.797, F = 4773, r = 0.97) between the mean BWG (g) and the protein level with an increase in BWG associated with an increase in protein level (Fig. 2).

There were significant differences (p<0.05) in the SGR (% day^{-1}) of *O. andersonii* among the fed group. Although the 30% protein level fed fish was not significantly different (p>0.05) from the 40% protein level fed fish, the latter recorded the highest SGR (% day^{-1}) (3.235±0.097%). The control group showed the lowest SGR (% day^{-1}) (2.140±0.167%) and this was significantly (p<0.05) lower than the fed fish (Table 2).

Significant differences (p<0.05) existed in the PERs across the treatments and these declined with an increase in the protein level (r = -0.282, n = 268, p<0.05). The PER for fish fed with the least protein level exhibited the highest PER (10.633±0.539) and this was significantly (p<0.05) higher than the fish given the 30% protein level (8.468±0.567). The 40% crude protein level had significantly lower PER (6.552 ± 0.551, p<0.05). The AFCE (%) means were insignificant (p<0.05) among all the treatments (Table 2).

The carcass crude protein of the initial fish samples was significantly (p<0.05) lower than the fish carcass at the end of the experiment in all the treatments. No significant differences (p>0.05) were observed in all treatments at the end of the experiment. The carcass lipid was similar in all the treatments and was not significant different (p>0.05) from the fish carcass at the start of the experiment. However, the carcass for the initial fish sample was higher than the fish subjected to the formulated diet (Table 3).

No significant differences (p>0.05) were observed in the selected water quality parameters (Table 4).

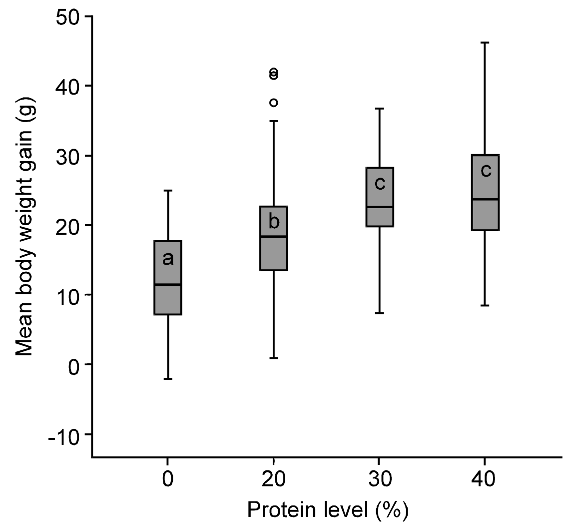

Fig. 1: Box plot showing the mean body weight gain of fish (g) according to the crude protein level (%) (different letters are significant (p<0.05))

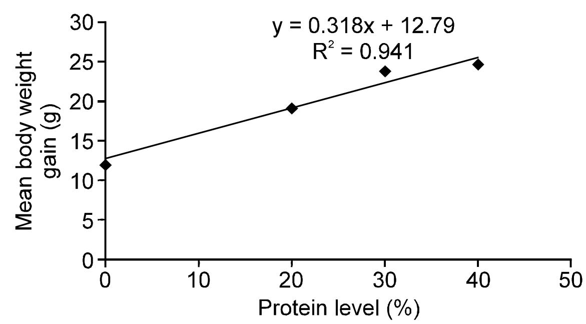

Fig. 2: Effect of dietary protein level on the mean body weight of *O. andersonii*

Table 2: Growth performance of the *O. andersonii* at different levels of protein

Treatment	0%	20%	30%	40%
[a]FMW (g)	17.467±1.162[a]	24.537±0.735[b]	29.501±0.753[c]	30.411±0.824[c]
[b]SGR (% day^{-1})	2.140±0.167[a]	2.840±0.095[b]	3.157±0.100[c]	3.235±0.097[c]
[c]*K*	2.515±0.181	2.299±0.055	2.333±0.045	2.410±0.071
[d]PERs	-	10.633±0.539[c]	8.468±0.567[b]	6.552±0.551[a]
[e]AFCE (%)	-	210.897±14.942[a]	254.514±15.713[ab]	274.779±15.356[b]
[f]Survival rate (%)	96.670	96.670	100.000	96.670

[a,b,c,d,e]Values (mean±SEM) in the same row not sharing a common superscript are significantly (p<0.05) different.
[f]No statistical analysis was possible as determinations were performed on pooled samples

Table 3: Carcass composition of *O. andersonii* (on dry matter basis) at different levels of protein

Proximate composition	Initial	0%	20%	30%	40%
Ash (%)	5.538±0.528[a]	3.464±0.181[a]	12.382±2.033[b]	15.277±0.666[b]	15.205±0.501[b]
Crude protein (%)	48.000±0.866[a]	56.000±0.0577[b]	53.483±0.277[b]	55.817±0.952[b]	55.550±1.534[b]
Crude lipid (%)	17.750±3.377	15.850±2.569	16.050±1.817	16.983±1.666	17.067±1.682
Crude fibre (%)	11.520±0.298[c]	10.170±0.470[a]	11.209±0.127[cd]	11.186±0.079[cd]	10.975±0.063[b]
NFE (%)	17.192±3.430[c]	14.517±3.032[c]	6.877±1.114[b]	0.737±1.479[a]	1.203±1.102[a]
Gross energy (KJ/g)	2129.635±94.866	2197.360±50.720	2014.458±71.012	2000.784±28.817	2005.809±31.015

Different superscripts in a row indicate significant difference (p<0.05)

Table 4: Water quality parameters of protein experiment

Treatment	0%	20%	30%	40%
Temperature (°C)	23.678±0.132	23.679±0.076	23.679±0.076	23.679±0.076
pH	7.780±0.060	7.760±0.040	7.770±0.030	7.770±0.030
Conductivity (µmho/cm)	0.776±0.082	0.758±0.044	0.758±0.042	0.760±0.043
Nitrite (NO_2^-) (mg/L)	0.169±0.740	0.145±0.029	0.131±0.027	0.167±0.028

Different superscripts in a row indicate significant difference (p<0.05)

Table 5: Cost effectiveness of protein level in the diet fed to *O. andersonii*

Treatment	0%	20%	30%	40%
Cost of soybean (ZK)	0	9.120±0.76[a]	17.230±0.80[b]	24.780±0.78[c]
Cost of maize bran (ZK)	0	4.120±0.12[c]	2.480±0.13[b]	0.720±0.12[a]
Cost of soyaGold oil (ZK)	0	4.340±0.20[b]	3.680±0.21[a]	3.340±0.20[a]
Cost of Vitamin (ZK)	0	10.080±0.43[a]	10.260±0.45[a]	10.070±0.44[a]
Cost of DCP (ZK)	0	1.570±0.07[a]	1.600±0.07[a]	1.570±0.07[a]
FMW (g)	19.727±4.496[a]	25.772±1.459[ab]	29.076±1.28[b]	28.831±2.414[b]
TC (ZK)	0	29.500±2.67[a]	35.320±1.51[b]	40.490±1.55[b]
TR (ZK)	187.150±16.44[a]	293.230±8.67[b]	354.020±9.31[c]	364.940±9.19[c]
GM (ZK)	187.150±16.44[a]	264.010±8.82[b]	319.390±9.47[c]	325.310±9.35[c]

US$1 = ZK4, 800. Different superscripts in a row indicate significant difference (p<0.05)

Cost analysis of the experimental diets: The GM of the fish fed with the 20% protein level was significantly lower (p<0.05) than the 30% and 40%. However, the control group had a significant lower (p<0.05) GM than the fed group.

Specific Growth Rate (%day^{-1}) and PER (%) contributed significantly (GM = 76.22 - 3.963(PER) + 84.850(SGR); r^2 = 0.47, p<0.05) to the model.

DISCUSSION

The growth of the control fish (unfed) was significantly lower than the fish that was subjected to treatment diets. According to De Graaf (2004), a reduction in somatic growth can lead to stunted growth of *O. niloticus*, resulting in fewer marketable sized fish at harvest. This is because real stunting takes place at low feeding and can be avoided by higher feeding levels. In the current study, the control group might have been stunted due to lack of feed.

Protein requirement is the first nutritional parameter to be determined for formulated feed production for newly established cultured fish species (Kim and Lee, 2009) due to cost implications and avoidance of water pollution through ammonia. Therefore, the commercial viability of an aquaculture enterprise is closely related to the supply and cost of the feed protein (Hoffman *et al*., 1997; Diyaware *et al*., 2009). It must then be kept to the minimum consistent with good fish growth and feed utilization. In the current experiment, there was an increase in the growth indices with an increase in the protein level. The 40% CP gave the highest final body weight and body weight gain although this was not significantly different (p>0.05) from the 30% CP. In a study conducted by Gunasekera and Lam (1997) on *O. niloticus* broodstock they found no significant difference (p>0.05) in the growth between the fish fed with the 20% CP and 30% CP. In the current study, the final body weights between the 20% CP and 30% CP were significant ($p<0.05$) although the latter was not significant different ($p>0.05$) from the 40% CP level. The difference would be attributed to the variances in the size and species used the experiments. In the current study the experimental animal used was *O. andersonii* at juvenile stage while in their study they used brooding *O. niloticus*. This is because the protein requirement for juvenile fish is higher than that of old fish.

Polynomial regression equation ($Y = 0.318x + 12.797$, $F = 4773$, $r = 0.97$) showed that the relationship between the protein level and body weight gain was linear. This shows that the highest protein level (40%) used was not high enough to impose detrimental effects on the growth parameters of *O. andersonii*.

The total cost of producing fish at 40%CP was significantly higher ($p<0.05$) than the 30% and 20% CP feed. The study reveals that 40%CP resulted into the highest final fish weight and economical returns. Gross Margin was highest too at the highest protein level although not significant different from the 30%CP. This could be accredited to the fact that high quality feed cost more but produce better yield resulting into higher revenue. Oladejo (2010) reported similar results when the cost of *C. gariepinus* fingerlings was found to be proportionally related to revenue.

Data on the body composition of fish allows assessment of the efficiency of transfer of nutrients from feed to fish and helps in predicting the overall nutritional status (Ali *et al*., 2000). In most cases retention of energy and deposition of new tissue results in an increase in the weight of an animal and the weight of young fish is usually a reliable indicator of adequacy of the nutritional and management regimes. In the current study, the body protein of fish increased at the end of the experiment showing that fish growth was as a result of protein synthesis and tissue production and not only to fish weight only due to lipid deposition. Similar results were observed in *C. gariepinus* (Fafioye *et al*., 2005). In the current studies, crude protein of up to 40% did not affect the body crude protein. This is contrary to the study conducted on Tiger puffer (*Takifugu rubripes*) by Kim and Lee (2009). In their experiment there was a dose dependent response to dietary protein levels.

Biologically and economically, therefore, the 30% crude protein would be ideal in *O. andersonii* when using soya bean as the source of protein. This is because the growth and gross margin indices were higher than the control and 20% crude protein fed fish but not significant different ($p>0.05$) from the 40% crude protein treatment.

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