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Optimal Dietary Plant Based Lipid on Growth of *Oreochromis andersonii* (Castelnau, 1861)

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Abstract

An experiment was set to determine the optimal plant based lipid of soy bean and maize on the growth and feed utilisation of *Oreochromis andersonii*. Three isonitrogenous diets containing 30% crude protein with increasing dietary lipid levels (10, 15, and 20%) were provided at 5% live body weight to triplicate groups of 10 fish replicated four times for 55 days. There were no significant differences (P>0.05) in growth indices (final mean weight, body weight gain (BWG) and specific growth rate (SGR)) and feed utilisation (protein efficiency ratio (PER) and apparent feed conversion efficiency (AFCE)) determined. Second order polynomial regression of BWG on lipid level ($Y = -0.0043x^2 + 0.1284x + 21.499$) showed 14.9% to be optimal. The most economical dietary lipid level is below 10% lipid level as indicated by the negative linear regression ($Y = -0.1766x + 303.85$). Of the lipid levels used, 10% appears to meet the minimum requirement for this species.

Keywords: Soybean, maize, lipid, *Oreochromis andersonii*, growth.

Introduction

The use of fish meal and oil as the source of lipid is well known in fish nutrition (Caballero *et al.*, 1999). However, there are ecological, ethical and economical concerns related to the use of such products (Naylor *et al.*, 2000; Cautteau *et al.*, 2002; Bureau *et al.*, 2002). Ng *et al.* (2000) and Lim *et al.* (2001) found that 90% of fish oil can be replaced by palm oil in *Mystus nemurus* and *Clarias gariepinus* respectively without affecting any growth. However, Yilmaz and Genc (2006) did not find any growth and feed utilisation improvement in *Cyprinus carpio* fingerlings when soybean oil was used. Furthermore, it did not show any negative effects on organoleptic properties, mortality, carcass composition, blood parameters and growth in *Oreochromis niloticus* (Ochang *et al.*, 2007).

Fish growth is an important factor in fish farming since fast growth ensures large size, therefore, short culture period resulting into frequent harvests maximizing gross margins in a year. Protein is essential in the diet of fishes as a source of amino acids, the building block for fish flesh. However, it is the most expensive source of energy in the artificial feeds. Dietary protein utilization can be improved by partially replacing dietary protein with lipid or carbohydrates to benefit from protein - sparing effect (De Silva *et al.*, 1991; Du *et al.*, 2009). Lipids represent a concentrated cost effective energy source, therefore, can be used in fish feeds either partially or completely to spare the protein. They are also the sources of essential fatty acids (EFAs). However, excessive energy can reduce fish growth as a result of reduction in feed intake (Lovell, 1998) since fish eats to satisfy its maintenance requirement (Kefi, 2007) and reduction in the utilization of other nutrients (Takeda *et al.*, 1975; Shiau and Huang, 1990; Shiau and Lan, 1996). It can also result into an increase in lipid deposition in the fish muscle. It is, therefore, important to obtain an optimal protein to energy ratio for the most economical production of any fish enterprise (Shiau and Lan, 1996).

The Cichlid *O. andersonii* has been adopted as the candidate indigenous fish species for fish farming in Zambia. However, the optimal lipid level for *O. andersonii* has not been described before. The aim of the study was to determine the optimal plant based lipid sourced from soybean and maize for growth of *O. andersonii*. The cost effectiveness of these dietary lipid levels was investigated too.

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Materials and Method

The study was conducted at National Aquaculture Research and Development Centre (NARDC) for fifty five (55) days. Five hundred *O. andersonii* (4.03±1.5 g; Mean±SD) were selected and stocked in the $27m^3$ cage put in 5, $000m^2$ fish pond for 22 days for acclimatisation to the pelleted feed. In the cage the fish were provided twice a day (10:00 - 15:00 hrs) 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).

Thirty six (36) hapas fixed in a $750m^2$ semi-concrete pond were set in a Random Complete Block Design (CBD) (Gomez and Gomez, 1984) with three dietary lipid levels (10%, 15% and 20%). Hapas (0.9 x 1.5 x 0.4 m) were then set in 4 rows with each line comprising nine (9) hapas in triplicates replicated four times for each treatment. Three hundred and sixty (360) fishes were randomly distributed randomly into the thirty six (10 fishes/hapa).

To formulate the diets, 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 3.2mm metal die to make the three diets (Table 1). 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.

Fish were provided rations twice a day (10:00 - 15:00 hrs) calculated at 5% live body weight using the formulated experimental diets for fifty five (55) days. Prior to use, all ingredients were analysed for proximate composition and the data obtained were the basis for feed formulation. The feed allowance was adjusted only when mortality was observed in the hapa.

Dry matter of feed and fish was determined by drying samples in an advantec electric furnace maintained at 105°C for 5 hours. 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 advantec electric furnace at 550°C for 5 hours. The crude fat was determined by extraction with petroleum ether for 16 hours in a Soxhlet apparatus. After drying the ether, the flasks containing the fat were dried in an oven for 8 hours 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 minutes, followed by boiling in dilute sodium hydroxide (1.25%) for another 30 minutes. The 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).

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

Body weight gain = final weight of fish-initial weight of fish (1)

Specific growth rate (SGR) = $((lnW_f-lnW_i)/t)*100$ where lnW_f is the natural logarithm of final body weight, lnW_i is the natural logarithm of initial body weight of the fish and t is final time of the experiment in days. (2)

Condition factor (K) = $((\text{fish weight}/ (\text{length (TL)})^3)*10^5$, where TL is total length (mm) (Ricker,

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

Ingredients	10%	15%	20%
Soybean cake	54.7	56.1	57.3
Maize bran	42.6	36	29.5
[1]SoyaGold oil	0.7	5.9	11.2
[2]Vitamin	1	1	1
[3]DCP	1	1	1
Proximate analyses			
Crude protein (%)	30	30	30
Crude Lipid (%)	15	20	15
Crude ash (%)	4.0	3.9	3.6
Crude fibre (%)	40.6	37.0	36.1
Carbohydrates (%)	7.1	6.4	6.3
Gross energy (Kcal/g)	3.7	4.0	4.3
Protein: energy ratio (g protein/Kcal)	0.1	0.1	0.1

[1]SoyaGold oil: Crude fat 100%, energy 8.84Kcal/g

[2]Vitamin stress pack (100 g): vitamin A 2, 000, 000 IU vitamin D3 300, 000 IU, vitamin E 3000 IU, 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

[3]Di-calcium phosphate

1975). (3)

Protein efficiency ratio (PER) = fish weight gain/total protein fed (4)

Apparent feed conversion efficiency (AFCE) = fish body weight gain/total feed intake)*100 (5)

Survival rate = ((Number of fish at the end of the experiment)/Number of fish at the start of the experiment)*100 (6)

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).

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. In order to determine the optimal lipid level on growth of the fish, polynomial regression was applied to determine the best regression model between the dietary lipid levels and the weight gain (g).

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 US$ 0.54/kg, maize bran US$ 0.12/kg, SoyaGold oil US$ 1.12/L, vitamin stress pack US$18/kg and DCP US$ 2.8/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; Total cost (TC) = $P_sS + P_mM + P_oO + P_vV + P_dD$, where; P_s = Unit cost of Soybean 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: Total revenue (TR) = P*FW, where; P = Price of fish and FW = final fish weights and Gross margin (GM) = TR-TC, where; TC and TR as described above. Similarly polynomial regression was utilised in order to determine the best regression model between the dietary lipid levels and the gross margin.

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 ± S.E.M) are presented to facilitate interpretation.

Results

There were no significant differences (P>0.05) in the final mean fish weight, mean body weight gain and SGR%day^{-1} among the treatments. Similarly, the AFCE (%) was not significant (P>0.05) among the dietary lipid levels (Table 2).

A polynomial regression analysis showed that a second order polynomial would describe the relationship ($Y = -0.0043x^2 + 0.1284x + 21.499$) between the lipid level and mean fish weight gain (g). A differential equation shows that maximum mean weight gain would occur at lipid level of approximately 14.9% (Figure 1).

Proximate composition of *O. andersonii* used in the experiment is presented in Table 3. With the exception of crude lipid the other parameters were significant (P<0.05) across the treatments (Table 4). The initial fish body crude protein composition was significantly (P<0.05) lower than the crude protein in the fish at the end of the experiment (Table 5).

A negative linear regression ($Y= -0.1766x + 303.85$) was found to correctly describe the relationship between dietary lipid levels and the gross margin (Figure 2).

Discussion

Polynomial regression has been recommended in approximating the optimal requirements of the nutrients of fish (Zeitoun *et al.*, 1976; Chou and Shiau, 1996; Ryan, 1997; Shearer, 2000). A polynomial regression equation ($Y = -0.0043x^2 + 0.1284 x + 21.499$) to describe the effect of dietary lipid on body weight gain showed 14.9% as optimal. However, an increase in lipid level was found to negatively affect gross margin. This could be attributed to the fact that an increase in lipid level did not correspond to the somatic growth of the fish that ultimately affected the difference between feed cost and revenue. Both PER and AFCE (%) were highest at 15% lipid. Studies by De Silva *et al.* (1991) on the hybrid *O. mossambicus* X *O. niloticus* found 18%

Table 2. Growth performance and feed utilization of plant based lipid by *O. Andersonii*

Treatment	10%	15%	20%
Final weight (g)	27.772 ± 0.716^a	27.921 ± 0.800^a	28.179 ± 0.944^a
SGR (% day^{-1})	3.097 ± 0.102^a	3.111 ± 0.102^a	3.022 ± 0.103^a
BWG (g)	22.371 ± 0.857^a	22.573 ± 0.857^a	22.645 ± 0.862^a
K	2.438 ± 0.060^a	2.372 ± 0.061^a	2.224 ± 0.051^a
PER	8.916 ± 0.606^a	9.212 ± 0.606^a	8.555 ± 0.609^a
AFCE (%)	251.543 ± 135.774^a	260.869 ± 167.272^a	241.072 ± 147.205^a
*Survival rate (%)	96.67	100	96.67

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

*No statistical analysis was possible as determinations were performed on pooled samples

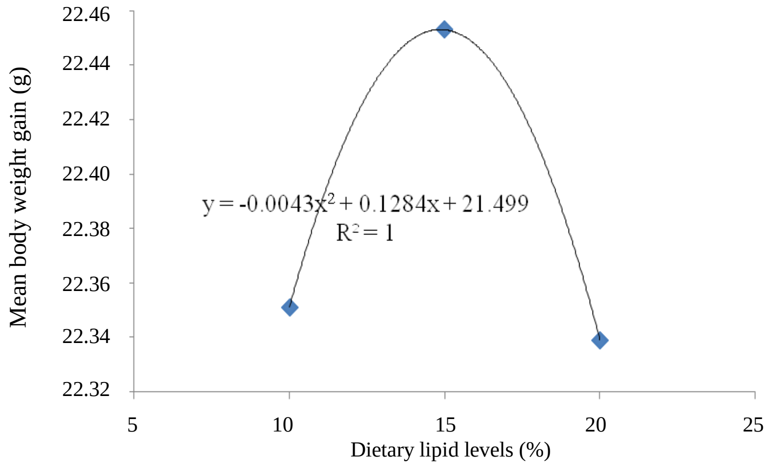

Figure 1. Relationship between the lipid level and mean fish body weight gain (g).

Table 3. Proximate composition of the *O. andersonii* at different levels of lipid

Treatment	Initial	10%	15%	20%
Ash (%)	5.538 ± 0.528^{a}	11.588 ± 1.701^{b}	15.235 ± 0.610^{bc}	16.041 ± 0.916^{c}
Crude Protein (%)	48.000 ± 0.866^{a}	54.317 ± 1.001^{b}	55.217 ± 1.126^{b}	55.317 ± 1.178^{b}
Crude Lipid (%)	17.750 ± 3.377^{a}	19.550 ± 1.434^{a}	14.133 ± 1.389^{a}	16.417 ± 1.775^{a}
Crude Fibre (%)	11.520 ± 0.298^{b}	11.286 ± 0.126^{ab}	11.109 ± 0.069^{ab}	10.975 ± 0.060^{a}
NFE (%)	17.192 ± 3.431^{b}	3.259 ± 1.286^{a}	4.307 ± 1.767^{a}	1.250 ± 1.478^{a}
Gross Energy (KJ/g)	2129.635 ± 94.866^{b}	2110.158 ± 48.674^{b}	1935.456 ± 28.560^{a}	1975.437 ± 39.591^{ab}

Different superscripts in a row indicate significant difference ($P < 0.05$).

Table 4. Water quality parameters for different lipid levels

Treatment	10%	15%	20%
Temperature (°C)	23.677 ± 0.076^{a}	23.682 ± 0.079^{a}	23.676 ± 0.076^{a}
pH	7.77 ± 0.03^{a}	7.77 ± 0.03^{a}	7.77 ± 0.03^{a}
Conductivity (µmho/cm)	0.760 ±0.042^{a}	0.753±0.045^{a}	0.765 ±0.042^{a}
Nitrite (NO_2^-) (mg/L)	0.159±0.074^{a}	0.129±0.028^{a}	0.155±0.028^{a}
Dissolved Oxygen (mg/L)	7.966±0.184^{a}	7.983±0.182^{a}	7.965±0.188^{a}

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

Table 5. Cost effectiveness of lipid level in the diet fed to *O. Andersonii*

Treatment	10%	15%	20%
Cost of soybean (ZK)	16.73 ± 0.81^{a}	16.28 ± 0.78^{a}	18.01 ± 76^{a}
Cost of maize bran (ZK)	2.79 ± 0.13^{b}	2.50 ± 0.12^{ab}	2.02 ± 0.12^{a}
Cost of SoyaGold oil (ZK)	0.40 ± 0.21^{a}	3.70 ± 0.20^{b}	7.27 ± 0.19^{c}
Cost of Vitamin (ZK)	10.02 ± 0.45^{a}	9.94 ± 0.44^{a}	10.44 ± 0.43^{a}
Cost of DCP (ZK)	1.56 ± 0.07^{a}	1.55 ± 0.07^{a}	1.624 ± 0.07^{a}
TC (ZK)	31.439 ± 1.665^{a}	33.653 ± 1.662^{a}	38.54 ± 1.68^{a}
TR	333.27 ± 9.64^{a}	335.05 ± 9.64^{a}	338.15 ± 9.70^{a}
GM	301.94 ± 9.65^{a}	301.44 ± 9.65^{a}	300.19 ± 9.71^{a}

Different superscripts in a row indicate significant difference ($P<0.05$).
Exchange rate: US$1 = ZK5

lipid as optimal although they did not indicate the source of the lipid. The optimal dietary lipid for tilapia has been estimated to be less than 10% (Guillaume *et al.*, 2001). Uys (1989) observed 10-12% as optimum lipid level for *C. gariepinus*. This is similar to what has been found in the current study on *O. andersonii* since the 10% lipid was not significantly different ($P>0.05$) from the 15% and 20% lipid levels. In the current study no differences ($P>0.05$) were observed in the growth parameters

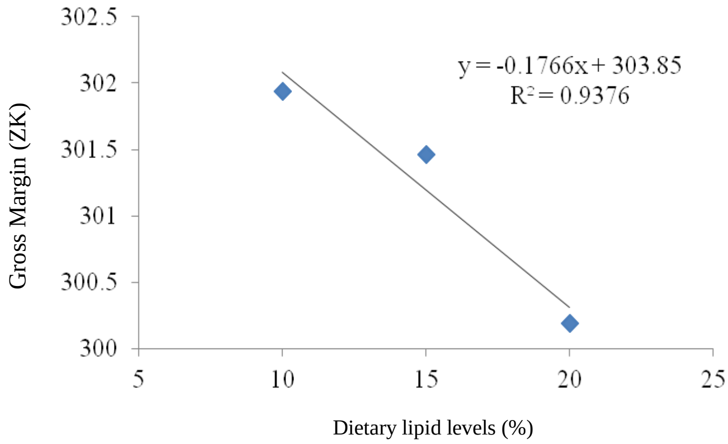

Figure 2. Relationship between the lipid level and gross margin (g) (Exchange rate: US$1 = ZK5).

among the lipid levels in the fed groups. Similar results were observed by Hanley (1991) in supplementary feeds at levels of 5, 9 and 12% lipid. Therefore, soybean and maize based lipid requirements can be maintained as low as 10%. This confirms the low requirement of non protein energy sources requiring the high dietary protein (Jauncey, 1998).

Data on the body composition of fish allows assessment of the efficiency of transfer of nutrients from feed to fish and also 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, data on whole body composition indicated significant differences (P>0.05) among treatments. 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). This is consistence to the feed utilisation data which showed high deposition of lipid at lower lipid inclusion level than at high levels. In the current study, there is no evidence to show that the levels up to 20% lipid sourced from soya bean and maize to have an effect on the body lipid.

In conclusion, the 10% soybean lipid appears to meet the minimum lipid requirements in *O. andersonii* since no significant differences (P>0.05) were observed even at higher levels used. Biologically, 14.9% seems to be optimal as attested by second order polynomial regression. It appears too that the most economical lipid level is below 10% lipid level as indicated by the negative linear regression. A further study is recommended that will include dietary lipid level below 10% in order to determine the most economical level in *O. andersonii*.

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