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ALTERNATIVE PROTEIN SOURCES AS SUBSTITUTES FOR FISHMEAL IN THE DIET OF YOUNG TILAPIA *OREOCHROMIS NILOTICUS* (LINN.)

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Abstract

Fifteen test diets were theoretically formulated to contain 33.32% dietary protein, dry matter, using a mixture of alternative protein sources and fishmeal in various proportions. Test diets were fed to fingerlings (initial weight 3.2±0.2 g) in triplicate tanks for eight weeks at 5% body weight per day in three portions. At the end of each experimental phase, fish carcass from the feeding groups was homogenized. Freeze-dried samples of fish at the beginning and end of the experiments as well as samples of the test diets were analyzed for proximate composition. Fish fed diet 1 containing 43% fishmeal recorded the highest weight gain, highest specific growth rate (SGR, 3.46%/d) and lowest food conversion ratio (FCR, 1.11). When part of the fishmeal was substituted with 18% soybean meal and 5% blood meal (diet 8), SGR and FCR were 3.02%/d and 1.34, respectively, not significantly different from diet 1 at 0.05 probability. Results showed that proper combination of alternative protein sources can provide 42-45% of the protein required by *Oreochromis niloticus* (33.32% dietary protein, dry matter). In such a combination, soybean meal can replace up to 25% of the fishmeal as a protein source. Blood meal exceeding 6%, groundnut cake beyond 10%, soybean meal above 20%, and wheat bran beyond 10% retarded fish growth. High mortality was generally observed when these levels were exceeded, even in only one of the protein sources.

Introduction

One of the major problems confronting the fish farming industry is the increasing cost and short supply of fishmeal (an important ingredient in fish feed) and other animal protein sources. To reduce the costs of fish production, nutritionists have tried to use less expensive plant proteins to partially or totally replace fishmeal. The high protein level required by fish for maximum growth has been established. Growth of fishes and utilization of feed are

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reported to be optimal with proteins of animal origin, mainly fishmeal, with high nutritive value (Dabrowska and Wojno, 1977). The high price of fishmeal and shortage on world markets have made it necessary to look for substitutes (Tacon and Jackson, 1985; Webster et al., 1992).

Unfortunately, attempts to replace the fishmeal component of practical fish feeds with alternative protein sources have resulted in variable success and generally led to reduced feed efficiency and growth (Tacon and Jackson, 1985). Groundnut has proved to be an acceptable protein source at a low inclusion level but growth decreased rapidly as this level was increased in the diet of tilapia *Oreochromis mossambicus* (Jackson et al., 1982). Omoregie et al. (1991) reported that feeds for *O. niloticus* can contain limited amounts of cassava peelings and mango seeds as partial substitutes for fishmeal at marginal levels. In spite of limited success, the formulation of feeds containing high levels of plant proteins has become an important objective in fish nutrition research. Attempts to reach substitution levels of more than 50% of the fishmeal protein, by mixing two or more alternative protein sources, have been scarce although some of the results look promising (Jackson et al., 1982; Smith et al., 1988).

The essential amino acid compositions of alternative protein sources for fish are not comparable with that of fishmeal. The apparent chemical score data show that there is no single foodstuff that can serve as an alternative to fishmeal (De Silva and Anderson, 1995). However, combining different alternative protein sources which possess different limiting amino acids has been strongly suggested (Jackson et al., 1982; Tacon and Jackson, 1985). The objective of this study was to assess the suitability of soybean meal, groundnut cake, wheat bran, and blood meal to partially or fully replace fishmeal in the diet of *O. niloticus* (Linn.).

Materials and Methods

Fifteen test diets were theoretically formulated to yield a protein content of 33.32% dry matter (dm). Pearson squares was used for the calculation (De Silva and Anderson, 1995). The proximate compositions of the diets are presented in Table 1. The estimated optimal requirement for *O. niloticus* is 33.32% dietary protein dm (related to fishmeal; Ogunji and Wirth, 2000). Fishmeal, soybean meal (industrially processed), blood meal, groundnut cake and wheat bran at varied proportions were mixed in each diet. Cracked soybean seeds (Soja schrot from the Institute of Animal Nutrition, Leipzig, Germany) were used in diets 13, 14 and 15. The soybean was heated for 60 minutes at 105ºC (Viola et al., 1983) in an oven to deactivate trypsin inhibitors capable of interfering with protein digestion. The heated soybean was then homogenized. All dry diet components, including the vitamin and mineral mixture, were thoroughly mixed with sunflower oil. Water was added and the feed pressed into pellets of 1 mm diameter. The feeds were stored in a refrigerator at 5-7°C until used.

Fifteen fingerlings (initial weight 3.2±0.2 g) were stocked in each of the experimental tanks (28 x 28 x 51.5 cm). Feedings of the experimental diets were carried out in triplicate. The fish were weighed every two weeks and the quantity of food adjusted accordingly. Experimental tanks were cleaned regularly. Conductivity, pH, oxygen concentration and water temperature were measured three times every week. The water was well aerated and oxygen saturation kept above 60%. Temperature was maintained at 27±1°C throughout the experiment.

Due to an insufficient number of experimental tanks, this study was carried out in phases. At the end of each experiment the fish were measured. Twenty individuals from every feeding group were taken, their intestines removed and the carcass homogenized. Freeze-dried samples of fish at the beginning and end of the experiments as well as samples of the test diets were analyzed for proximate composition. The diet ingredients were also analyzed. Every analysis was duplicated. Protein (N x 6.25) was determined by the Kjeltec System (Tecator) and crude fat by Soxtec System HT (Tecator) using petroleum ether. Ash was determined by burning in a muffle furnace at 750°C for 4 hours. Oxygen bomb

Table 1. Formulation and proximate composition (% dry matter) of experimental diets for *Oreochromis niloticus.*

	Diets														
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*
Ingredients (%)															
Fishmeal	43	26	5	–	5	–	–	26	–	–	15	8	24	15	10
Soybean meal	–	5	5	–	5	25	25	18	72	60	25	20	20	32	40
Groundnut cake	–	10	15	–	10	–	10	–	–	10	–	10	10	10	10
Blood meal	–	5	10	33	20	20	15	5	–	–	10	10	6	8	8
Wheat bran	10	10	15	14	18	20	15	10	10	10	10	20	–	–	–
Sunflower oil	10	7	6	10	7	6.4	–	6	–	–	5.2	–	3.1	2.0	0.3
Fish oil	–	–	–	–	–	–	4.5	–	–	–	–	–	–	–	–
Vitamin & mineral mix[1]	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7
Potato meal	32.3	32.3	39.3	38.3	30.3	23.9	25.8	30.3	13.3	15.3	30.1	27.3	32.2	28.3	27.0
Proximate composition (%)															
Dry matter	83.42	82.08	80.09	80.04	91.86	84.71	86.12	86.64	88.95	87.45	83.96	80.23	94.09	96.46	95.18
Protein	35.94	30.38	28.98	37.94	30.99	34.22	29.76	33.90	32.32	29.52	34.28	30.24	32.96	34.99	35.66
Fat	16.04	14.39	13.40	11.44	12.43	13.84	14.50	14.34	17.56	16.91	12.81	7.41	8.67	6.48	4.68
Ash	9.87	7.45	6.72	4.70	5.42	6.46	5.70	8.03	7.70	6.74	8.28	7.02	9.74	9.63	8.47
NFE + fiber[2]	38.15	47.78	50.90	45.92	51.16	45.48	50.04	43.73	42.42	46.83	44.63	55.33	48.63	48.91	51.19
Gross energy (kJ/g)	21.68	21.40	21.33	22.28	21.61	22.17	22.04	21.48	22.68	22.01	21.64	20.76	–	–	–

[1] Vitamin and mineral mix (Spezialfutter Neuruppin - VM BM 55/13 Nr. 7310) supplied per 100 g of dry feed: Vitamin A, 15000 IU; Vitamin D3, 2500 IU; Vitamin E, 500 mg; Vitamin K3, 23 mg; Vitamin B1, 42 mg; Vitamin B2, 18 mg; Vitamin B6, 21 mg; Vitamin B12, 59 µg; Nicotinic acid, 100 mg; Biotin, 544.65 µg; Folic acid, 13 mg; Pantothenic acid, 123 mg; Inositol, 1230 mg; Vitamin C, 66.7 mg; Antioxidants (BHT), 121.87 mg; Calcium, 20.2%

[2] Nitrogen free extract (NFE) + fiber = 100 - (% protein + % fat + % ash)

calorimeter (Framo-MK 200) was used to determine gross energy at two replications per sample. The mineral content in the samples was not analyzed. To estimate the amino acid concentrations of the samples, 5 mg of the freeze-dried samples were hydrolyzed with 6N HCl at 110°C for 24 hours. No protecting reagents were added to avoid destruction of sulphur amino acids. The hydrolyzed samples were neutralized with 6N NaOH and 200 µl of the neutralized sample was freeze dried to allow storage of the samples until amino acid analysis. Amino acids were extracted in the samples with 1000 µl extraction buffer (80% methanol, 20% water). 50 µl internal standard (2 µg homoserine; Riedel et al., 1988; Algermissen et al., 1989) was added to 500 µl extracts in a reaction vial and transferred to an Autosampler for mixing and injection. The following Merck/Hitachi HPLC equipment were used for the analysis: AS-400 intelligent Autosampler, L-6200 Intelligent pump, F-1080 Fluorescence Detector, L-5025 Column Thermostat and D-6000 HPLC Manager. Nova-Pak C-18 4 µm 3.9 mm x 300 mm Column (Waters) was used. The system was calibrated using amino acid standard solution obtained from SIGMA (Product No. A9906).

All statistical analyses were carried out by the Duncan multiple range method using SPSS for Windows (version 9). From the experimental data obtained, weight gain, specific growth rate (SGR) and food conversion ratio (FCR) were calculated. FCR = food fed/live weight gain; SGR = ($\ln W_2 - \ln W_1/T_2-T_1$) x 100 where W_2 = final weight of fish, W_1 = initial weight of fish and T_1 and T_2 = time (day); Protein to energy ratio (P/E ratio) was calculated as mg protein/kJ gross energy.

Results

Chemical compositions of the feed mixture. The nutrient contents and amino acid composition of the diet ingredients are presented in Table 2. The amino acid contents of cracked soybean seed decreased after heat treatment. The amino acid concentrations of blood meal were higher than that of fishmeal. Protein content of the test diets (formulated to yield 33.32%) ranged 28.98-37.94% at proximate analysis (Table 1), however, conclusions in this work were made based on dietary protein levels of 30.24-35.94% (33.26±1.95). The fat contents of the test diets ranged 4.68-17.56%. The diets formulated with heat treated soybean seed (13, 14, 15) had lower fat contents. The amino acid compositions of the test diets are presented in Table 3. The amino acid composition of diets 2, 8 and 13 are relatively similar but slightly lower than that of diet 1, especially regarding the essential amino acids. The composition of diet 4 appeared highest in amino acids. The energy contents of the test diets ranged 20.76-22.68 kJ/g (Table 1) while the protein to energy ratios were 13.41-17.03 mg/kJ (Table 4).

Feed performance. Growth data, FCR, protein to energy ratio and mortality of fish fed different test diets are presented in Table 4. Fish fed diet 1 (containing 43% fishmeal) performed best. They gained an average weight of 19.51 g (593.0%) in eight weeks. The SGR and FCR were 3.46%/d and 1.11, respectively, with only 2.2% mortality. Among the other treatments, diets 2, 8 and 13, containing 24-26% fishmeal, performed best. The FCRs in fish groups 2, 8 and 13 were not significantly different from group 1 while the SGR of group 1 was not significantly different from group 8 but differed from groups 2 and 13. Diet 1, with a higher proportion of fishmeal, was readily accepted and consumed by the fish. Diet 6, containing 20% blood meal and 25% soybean meal, was the least consumed (11.64 g per fish). Though the amino acid composition of diet 4 seemed best when compared with other diets (Table 3), it did not enhance fish performance. The SGR and FCR of this group were 1.11%/d and 4.43, respectively. The highest mortality (66.7%) was in fish fed diet 7.

Discussion

The crude fat content of the test diets (4.68-17.56%) did not seem to influence the performance of the fish in this study. In his study, Hanley (1991) noticed that increasing the dietary lipid level in feed for Nile tilapia (*O. niloticus*) from 5 to 12% produced no significant effects on growth rate, FCR or protein gain. According to NRC (1993), no definite percent-

Table 2. Selected nutrient content and amino acid composition (% dry matter) of diet ingredients.[1]

	Diet ingredients						
	Fishmeal	*Groundnut cake*	*Blood meal*	*Wheat bran*	*Soy meal (processed)*	*Soy seed (Soja schrot)*	*Soy Seed (heat treated)*
Nutrient content							
Dry matter	94.02	96.54	98.14	87.32	96.1	91.32	97.16
Protein	74.60	8.22	94.32	18.70	42.28	46.44	46.80
Fat	11.56	19.36	0.48	3.26	23.27	2.12	1.80
Amino acid composition							
Aspartic acid	5.66	0.45	8.86	1.34	4.48	4.47	4.19
Glutamic acid	5.62	1.48	7.53	3.56	6.79	6.81	6.32
Serine	2.16	0.28	3.45	0.74	1.80	1.80	1.54
Histidine[2]	1.40	0.10	4.81	0.40	0.89	0.85	0.71
Glycine	2.83	0.17	2.74	0.77	1.22	1.22	1.03
Threonine[2]	2.46	0.18	3.33	0.57	1.42	1.45	1.27
Arginine[2]	3.81	0.31	3.69	1.14	2.79	2.51	2.26
Carnosine	0.28	0.01	1.16	0.07	0.19	0.11	0.09
Taurine	0.45	0.40	0.04	-	0.01	0.01	0.02
Alanine	3.88	0.24	6.67	0.94	1.69	1.76	1.48
Tyrosine	0.44	0.004	0.54	0.06	0.21	0.18	0.14
Valine[2]	2.80	0.27	3.35	0.79	1.70	1.75	1.49
Phenylalanine[2]	2.45	0.31	4.74	0.73	1.94	2.01	1.74
Isoleucine[2]	2.51	0.24	1.24	0.56	1.66	1.71	1.47
Leucine[2]	4.52	0.43	5.11	1.20	3.01	3.08	2.68
Ornitine	0.08	0.01	0.10	0.02	0.07	0.07	0.05
Lysine[2]	4.88	0.13	7.16	0.74	2.25	2.27	2.01

1 Analysis was carried out using HPLC, 5 mg samples were hydrolized at 110°C for 24 hours.
2 Essential amino acids

Table 3. Selected amino acid composition (% dry matter) of the experimental diets.[1, 2]

	Diets														
Ingredients (%)	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*
Aspartic acid	2.37	2.12	2.52	4.24	2.09	2.91	3.01	2.13	2.98	3.20	2.31	2.54	2.50	1.94	2.55
Glutamic acid	3.73	3.09	3.48	3.95	2.35	3.47	4.00	3.06	4.78	5.28	3.35	3.88	3.72	2.65	3.57
Serine	0.99	0.82	1.01	1.59	0.85	1.16	1.25	0.85	1.20	1.31	1.00	1.07	1.07	0.78	1.02
Histidine[3]	0.60	0.51	0.83	1.87	0.85	1.07	1.00	0.58	0.58	0.62	0.71	0.77	0.59	0.53	0.67
Glycine	1.08	0.98	0.98	1.35	0.67	0.86	0.97	0.86	0.82	0.94	0.08	1.02	1.35	0.81	0.91
Threonine[3]	1.05	0.84	0.99	1.40	0.71	1.09	1.02	0.82	0.68	1.01	0.93	0.96	1.00	0.72	0.88
Arginine[3]	1.58	1.10	1.31	1.57	0.88	1.26	1.44	1.11	1.67	1.83	1.32	1.37	1.45	0.98	1.23
Carnosine	0.13	0.11	0.08	0.44	0.10	0.17	0.22	0.08	0.08	0.14	0.12	0.15	0.08	0.08	0.11
Taurine	0.18	0.10	0.05	0.01	0.02	0.01	0.002	0.09	0.01	0.001	0.07	0.04	0.11	0.04	0.02
Alanine	1.66	1.39	1.15	2.98	1.43	1.80	1.45	1.27	1.13	1.25	1.52	1.56	1.66	1.17	1.40
Tyrosine	0.09	0.07	0.08	0.11	0.06	0.12	0.10	0.06	0.14	0.12	0.08	0.08	0.07	0.05	0.07
Valine[3]	1.28	1.11	1.45	2.80	1.34	1.73	1.69	1.05	1.11	1.21	1.32	1.37	1.28	0.98	1.25
Phenylalanine[3]	1.07	1.0	1.31	2.45	1.19	1.59	1.61	0.99	1.26	1.39	1.22	1.37	1.21	0.95	1.25
Isoleucine[3]	1.10	0.80	0.77	0.62	0.42	0.67	0.78	0.75	1.07	1.11	0.79	0.79	0.94	0.61	0.83
Leucine[3]	2.04	1.85	2.43	4.74	2.28	2.96	2.89	1.80	2.00	2.18	2.18	2.30	2.12	1.66	2.13
Ornitine	0.03	0.03	0.03	0.04	0.02	0.04	0.04	0.07	0.05	0.06	0.07	0.04	0.08	0.03	0.06
Lysine[3]	1.83	1.55	1.84	3.20	1.44	1.92	1.94	1.57	1.51	1.64	1.57	1.59	1.70	1.24	1.50

1 Analysis was carried out in replicates and the mean concentration is presented.
2 The concentration of all essential amino acids except threonine are higher than the established EAA requirement of this species (Santiago and Lovell, 1988).
3 Essential amino acids

Table 4. Growth data, food conversion ratio (FCR), protein/energy ratio (P/E) and mortality of *Oreochromis niloticus* fingerlings fed experimental diets.

Diets	*Protein (% dry matter)*	*Initial weight (g)*	*Final weight (g)*	*Weight gain (g)*	*Food fed (g/fish)*	*P/E[1] ratio*	*SGR[2]*	*FCR[3]*	*Mortality (%)*
1	35.94	3.29^{bc}	22.80^{i}	19.51^{g}	21.63	16.58	3.46^{h}	1.11^{a}	2.2
2	30.38	3.46^{c}	17.51^{h}	14.04^{f}	19.85	14.20	2.89^{g}	1.41^{a}	2.2
3	28.98	3.24^{bc}	13.63^{fg}	10.39^{e}	16.55	13.59	2.56^{fg}	1.60^{a}	17.8
4	37.94	3.42^{c}	6.56^{a}	3.14^{a}	13.91	17.03	1.11^{a}	4.43^{c}	42.2
5	30.99	3.29^{bc}	8.61^{abc}	5.32^{abc}	13.68	14.34	1.72^{bcd}	2.57^{ab}	6.7
6	34.22	3.03^{ab}	7.57^{ab}	4.54^{ab}	11.64	15.44	1.63^{abc}	2.56^{ab}	22.2
7	29.76	3.04^{ab}	10.92^{bcdef}	7.88^{cde}	13.30	13.50	2.28^{def}	1.69^{a}	66.7
8	33.90	3.03^{ab}	16.47^{gh}	13.45^{f}	17.96	15.78	3.02^{gh}	1.34^{a}	2.2
9	32.32	3.01^{ab}	9.52^{abcd}	6.51^{bcd}	14.05	14.25	2.02^{cdef}	2.16^{ab}	33.1
10	29.52	3.03^{ab}	8.80^{abc}	5.78^{abc}	13.65	13.41	1.90^{bcde}	2.36^{ab}	40.0
11	34.28	2.83^{a}	12.23^{def}	9.39^{de}	14.35	15.84	2.58^{fg}	1.53^{a}	20.0
12	30.24	2.88^{a}	8.74^{abc}	5.86^{abc}	12.39	14.51	1.98^{cde}	2.12^{ab}	0.0
13	32.96	3.46^{c}	13.91^{fg}	10.45^{e}	17.93	-	2.48^{efg}	1.72^{a}	17.8
14	34.99	3.47^{c}	12.70^{ef}	9.23^{de}	16.05	-	2.31^{ef}	1.74^{a}	11.1
15	35.66	3.47^{c}	10.36^{bcde}	6.90^{bcd}	15.54	-	1.93^{bcde}	2.25^{ab}	26.7

Figures in the same row with different superscripts are significantly different ($p<0.05$) from each other.

[1] Protein to energy ratio = mg protein/kJ gross energy

[2] Specific growth rate (%/d) = $(\ln W_2 - \ln W_1/T_2 - T_1) \times 100$

[3] Food conversion ratio = food fed/live weight gain

age of dietary lipids can be given for fish diets without considering the type of lipid as well as the protein and energy content of the diet. It may therefore be concluded that only the dietary protein content, and not the dietary fat or energy content, influenced the fish performance in this study.

After heat treatment, amino acids in the cracked soybean seed (Soja schrot) decreased in concentration. Lysine, leucine and arginine, among other amino acids, were lost in the range of 11.45%, 12.98%, and 9.96% (dry matter), respectively (Table 2). This agrees with results of Viola et al. (1983) who observed that soybean meals heated at 105°C and 17% moisture for 60 and 120 minutes lost about 10% lysine.

In diet 9, where industrially processed soybean meal provided the entire dietary protein, fish food intake and fish growth were low, the FCR was poor and mortality was high (Table 4). Similar results were observed in diet 10. It has been reported that growth tends to be low in fish fed diets with soybean meal replacing all the fishmeal (Jackson et al., 1982; Webster et al., 1992). According to Shiau et al. (1989), male tilapia (*O. niloticus* x *O. aureus*) fed diets in which 100% of the fishmeal was replaced with soybean meal either with or without methionine supplementation had significantly lower weight gain, FCR, P/E ratio and protein digestibility than that of the groups fed diets containing fishmeal as the sole source of protein. The essential amino acid (EAA) contents of all the diets, except for threonine, were sufficient to satisfy the EAA requirements of this species (Santiago and Lovell, 1988). The amino acid composition (Table 3) reveals no great difference between diet 9 and diet 1 (which was formulated with 43% fishmeal and resulted in the best fish performance). However, there may be a suboptimal amino acid balance. The biological value of amino acids from soybean meal may be lower than indicated (Murray, 1982). Dabrowski et al. (1989) stated that amino acid availability, especially methionine, was reduced if soybean meal protein was used in excess of 50% of the diet. It may be possible that diets 9 and 10 were deficient in phosphorous. Substitution of animal meals, which contain bones, by seed proteins creates a deficiency in phosphorous, the only really critical nutrient when fishmeal is replaced by soybean meal (Viola et al., 1988). Watanabe et al. (1997) suggest that adding high percentages of soy products in fish diets can cause unpalatability and unacceptability, leading to diminished growth. The texture and taste of test diets are bound to differ with increasing levels of plant material, also affecting the acceptability of the diets by the fish (De Silva et al., 1989). In this study, when the soybean meal constituted more than 20% of the diet, food intake, weight gain, FCR and survival were negatively affected (diets 6, 7, 9, 10, 11, 14, 15).

Diet 4, formulated with 33% blood meal, resulted in reduced fish growth, low feed intake and poor FCR The blood meal used in this study contained 94.32% protein, 98.14% dry matter and a better amino acid profile than the other feed ingredients. While the amino acid composition of diet 4 seems better than diet 1, it was lower in isoleucine. Close and Menke (1986) observed that blood meal protein is of low quality, has low digestibility, and the amino acid composition is rather biased, being low in isoleucine and methionine. Cullison (1979) noted that blood meal is not very palatable to most livestock and hence it is not popular as a protein supplement in livestock feeds. In this study also, diet 4 was not well accepted. This may be due to the unpalatable nature of the blood meal component. At a high dietary inclusion of blood meal, poor performance of fish was observed (diets 3-7, 11, 12, 14, 15). A dietary blood meal inclusion rate, not exceeding 6%, may therefore be recommended for *O. niloticus.* Fish mortality increased when this level was exceeded. A high mortality of 42.2% was recorded in the group fed diet 4 with 33% blood meal, underscoring the fatal effect of a high dietary inclusion of blood meal in the fish diet. Contrary to the results of this study, Otubusin (1987) obtained good performance in the same species with a feed containing 10% blood meal in a cage environment.

When 15% groundnut cake was included in diet 3, fish growth was relatively depressed. Inclusion of 5% fishmeal in the diet did not improve growth. The reduced fish performance may be a result of the inadequate protein content of the diet (28.98%), rather than the effect of the groundnut cake. However, the amino acid profile of the groundnut cake used in this study was extremely poor. Wu and Jan (1977) reported a very low specific growth rate of *Tilapia aurea* fed an all groundnut protein diet. They associated the poor fish performance to the amino acid profile of groundnut. Jackson et al. (1982) reported a rapid decrease in growth as the level of groundnut inclusion was increased in the diet of *O. mossambicus,* a problem blamed on the low methionine level in the sample used. Considering the results of the current study, inclusion of groundnut cake at about 10% is recommended.

The reason for the high mortality in the group fed diet 7 (66.7%) is unclear. However, spoilage of the diet in the course of the experiment (after which a replacement was made) may have been the cause. Aflatoxin (a toxic component of groundnut) has been linked to increased mortality in animal feeding (Lovell, 1989). Adequate storage of fish diets is required when groundnut or any of its byproducts is included in the formulation to prevent spoilage and the development of aflatoxin. According to Jackson et al. (1982), the limited information concerning deleterious levels of aflatoxin to fish species, especially tilapia, makes it difficult to eliminate the possibility that this factor may produce sublethal effects during growth trials.

None of the foodstuffs under investigation, except for fishmeal, can solely provide all the nutrients needed by *O. niloticus.* This agrees with the observation of De Silva and Anderson (1995). Fish fed diets 2, 8 and 13 performed better than other groups when compared with group 1. From this observation, it appears that a proper combination of soybean meal, blood meal, groundnut cake and wheat bran can provide the 42-45% protein needed by *O. niloticus.* In this combination, soybean alone is capable of replacing up to 25% of the fishmeal in the diet. Jackson et al. (1982) tried to substitute different plant proteins for fishmeal. At low levels of replacement (25%), growth rates were similar; at higher inclusion rates, the performance was considerably reduced. Viola et al. (1986) showed that tilapia responded very well to feeds containing plant proteins when they were supplemented with phosphorus.

When replacing fishmeal, the amino acid composition of the diet determines the performance of the fish at any dietary protein level. Attention should therefore be paid to the amino acid profile of alternative protein sources and the resulting test diets. More investigations are needed to further this direction of research. Many alternative protein sources are yet to be investigated and preliminary results need to be harmonized. Results of this study have shown that alternative protein sources, when combined properly, are capable of replacing fishmeal in diets of *O. niloticus*.

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References

Algermissen B., Nuendel M. and E. Riedel, 1989. Analytik von Aminosäuren mit Fluoreszenz-HPLC. *GIT Fachz. Lab.,* 33:783-790.

Close W. and K.H. Menke, 1986. *Selected Topics in Animal Nutrition*. Manual prepared for 3rd Hohenhiem course on animal nutrition in the tropics and semitropics. 2nd ed. Dt. Stiftung Fuer Internat. Entwicklung, Zentrastelle fuer Ernaehrung und Landwirtschaft. 255 pp.

Cullison A. E., 1979. *Feeds and Feeding.* 2nd ed. Reston Publ. Co., Reston, VA. 595 pp.

Dabrowska, H. and T. Wojno, 1977. Studies on the utilisation by rainbow trout (*Salmo gaidneri* Rich.) of feed mixtures containing soybean meal and addition of amino acid. *Aquaculture,* 10:297-310.

Dabrowski K., Poczyczynski P., Kock G. and B. Berger, 1989. Effect of partially or totally replacing fish meal protein by soybean meal protein on growth, food utilisation and proteolytic enzyme activities in rainbow trout (*Salmo gairdneri*). New *in vivo* test for exocrine pancreatic secretion. *Aquaculture,* 79:29-49.

De Silva S.S. and T.A. Anderson, 1995. *Fish Nutrition in Aquaculture*. Chapman and Hall London. 319 pp.

De Silva S.S., Rasanthi M. and R.M. Gunasekera, 1989. Effect of dietary protein level and amount of plant ingredient (*Phaseolus aureus*) incorporated into the diets on consumption, growth performance and carcass composition in *Oreochromis niloticus* (L) fry. *Aquaculture,* 80:121-133.

Hanley F., 1991. Effects of feeding supplementary diets containing varying levels of lipids on growth, food conversion, and body composition of Nile tilapia, *Oreochromis niloticus* (L.) *Aquaculture*, 93:323-334.

Jackson A.J., Capper B.S. and A.J. Matty, 1982. Evaluation of some plant proteins in complete diets for the tilapia (*Sarotherodon mossambicus*). *Aquaculture,* 27:97-109.

Lovell R.T., 1989. *Nutrition and Feeding of Fish*. Van Nostrand Reinhold, New York. 260 pp.

Murray M.G., 1982. *Replacement of Fishmeal with Soybean Meal in Diets Fed to Channel Catfish in Ponds.* M.Sc. Thesis, Auburn Univ., Auburn, AL. 40 pp.

NRC, 1993. *Nutrient Requirements of Warm Water Fishes and Shell Fishes*. Natl. Res. Council, Natl. Acad. Sci., Washington, DC.

Ogunji J.O. and M. Wirth, 2000. Effect of dietary protein content on growth, food conversion and body composition of *Oreochromis niloticus* fingerlings, fed fishmeal diet. *J. Aqua. Trop.,* 15(4):381-389.

Omoregie E., Ufodike E.B.C. and M.S. Umaru, 1991. Growth and feed utilisation of the tropical Cichild, *Oreochromis niloticus* fingerlings fed with diets containing cassava peelings and mango seeds. *Aquabyte*, 4(2):6-7.

Otubusin S.O., 1987. Effects of different levels of blood meal in pelleted feeds on tilapia, *Oreochromis niloticus* production in floating bamboo net-cage. *Aquaculture*, 65:263-266.

Riedel E., Algermissen B. and M. Nuendel, 1988. Improved high-performance liquid chromatographic (HPLC) determination of free amino acids and amines in biological fluids by pre-column fluorescence derivative. *J. Pr. Ch.,* 7(3):278-279.

Santiago C. B. and T. Lovell, 1988. Amino acid requirements for the growth of Nile tilapia. *J. Nutr.*, 118:1540-1546.

Shiua S.Y., Kwok C.C., Kwang J.Y., Chen C.M. and S.L. Lee, 1989. Replacement of fishmeal with soybean meal in male tilapia (*Oreochromis niloticus* x *O. aureus*) fingerling diets at a sub-optimal protein level. *J. World Aqua. Soc*., 20:230-235.

Smith R.R., Kincaid H.I., Regenstein J.M. and G.L. Rumsey, 1988. Growth, carcass composition and taste of rainbow trout of different strains fed on diets containing primary plant or animal proteins. *Aquaculture,* 70:309-321.

Tacon A.G. and A.J. Jackson, 1985. Utilisation of conventional and unconventional protein sources in practical fish feeds. pp. 119-145. In: C.B. Cowey, A.M. Mackie and J.G. Bell (eds.). *Nutrition and Feeding in Fish*. Academic Press, London.

Viola S., Mokady S. and Y. Arieli, 1983. Effects of soybean processing methods on the growth of carp (*Cyprinus carpio*). *Aquaculture*, 32:27-38.

Viola S., Zohar G. and Y. Arieli, 1986. Phosphorous requirements and its availablility from different sources for intensive pond culture species in Israel. Part I. Tilapia. *Bamidgeh*, 38(1):3-12.

Viola S., Arieli Y. and G. Zohar, 1988. Animal-protein-free feeds for hybrid tilapia (*Oreochromis niloticus* x *O. aureus*) in intensive culture. *Aquaculture*, 75:115-125.

Watanabe T., Verakunpiriya V., Watanabe K., Kiron V. and S. Shuichi, 1997. Feeding rainbow trout with non-fish meal diets. *Fish. Sci.,* 63(2):258-266.

Webster C.D., Yancey D.H. and J.H. Tidwell, 1992. Effect of partially or totally replacing fish meal with soybean meal on growth of blue catfish (*Ictalurus furcatus*). *Aquaculture,* 103:141-152.

Wu J.L. and L. Jan, 1977. Comparison of the nutritive value of dietary proteins in *Tilapia aurea*. *J. Fish Soc. Taiwan,* 5(2):55-60.