

Full Length Research Paper

Utilization of soya protein as an alternative protein source in *Oreochromis niloticus* diet: Growth performance, feed utilization, proximate composition and organoleptic characteristics

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Accepted 19 August, 2008

The effect of replacing fish protein with soya protein in tilapia (*Oreochromis niloticus*) diets was examined. Three isoproteic (35%) diets containing 0% (FD); 50% (MD) and 100% (SD) fish protein substituted by soya protein were formulated. Fish (initial weight = 11.56 ± 4.22 g) was fed with experimental diets for 180 days. Results showed that the best growth was achieved with fish fed FD. Growth parameters such as final body weight, body weight gain and specific growth rate decreased with increased dietary soya protein. In contrast, no significant differences were found in feed and protein utilization parameters. For carcass trait, ash, crude fat, and energy varied significantly with soya protein incorporation in fish diet. Concerning organoleptic characteristics, odour and texture in mouth were not affected by incorporation of soya protein in diet. In contrast, texture, flavour, and general appreciate note were affected by dietary treatment. Partial substitution of fish protein by soya protein gave more pronounced sweet flavour.

Key words: *Oreochromis niloticus*, fish protein, soybean meal, growth, proximate composition, organoleptic characteristics.

INTRODUCTION

Aquaculture has become the fastest-growing food production sector in which fish meal is a primary protein source for diets (El-Saidy and Gaber, 2003; Siddhuraju and Becker, 2003; Wu et al., 2004). These feedstuffs have good palatability and high nutritional quality. However, the major problems confronting the fish farming industry are the increasing cost, the competition of other livestock industries for fish meal, instability in world fish meal production and phosphorus pollution (New and Csavas, 1995; Xie et al., 2001; FAO, 2002). Therefore, it is necessary that alternative protein sources for fish diets be found. A priority area of research in aquaculture nutrition is the reduction and possible elimination of fish

meal and fish oil from practical diets (Craig, 2004). Currently, one of the challenges that fish nutritionists face is to partially or totally replace fish meal with less expensive, untraditional plant protein sources. Among the plant protein sources considered in aquaculture diets, soybean meal is the most widely used ingredient. It has been preferentially used for replacement of fish meal due to its high-protein content, relatively well-balanced amino acid profile, reasonable price and steady supply. However, soybean meal is deficient in one or more essential amino acids contain and several compounds that may disturb the digestive process (NCR, 1993; Storebakken et al., 2000).

Many studies have shown considerable success in partially or totally replacing fish meal with soybean meal in *O. niloticus* diets (Shiau et al., 1987; El Saidy and Gaber, 1997, 2002; Wilson et al., 2004). Replacement fish protein with soya protein could improve growth and reduce the cost of fish production. Replacement of fish

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Table 1. Formulation and proximate composition of the experimental diets.

Ingredients (gkg ⁻¹)	Diets		
	FD	MD	SD
Corn flour	100	100	100
Fish meal	380	190	-
Soybean meal	-	248	500
Wheat bran	340	222	120
Cottonseed meal	150	200	240
Fish oil	10	5	-
Soya oil	-	5	10
Lysine	-	5	5
Methionine	-	5	5
Vitamin and mineral premix ^a	20	20	20
Total	1000	1000	1000
Proximate analysis (% on dry matter basis)^b			
Moisture	10.8	10.7	10.5
Crude protein	35.5	35.6	35.6
Total nitrogen	5.7	5.7	5.7
Crude fat	9.2	8.3	12.9
Ash	12.1	9.5	7.4
Crude fibre	8.4	8.4	8.8
Nitrogen-free extract ^c	23.3	27.5	24.8
Gross energy (kJg diet ⁻¹) ^d	15.5	15.9	17.2
Cost (CFA kg ⁻¹) ^e	260	225	195

^aVitamin and mineral mixture each 1 kg of mixture contains: 4800 IU Vit A, 2400 IU cholecalciferol (Vit D), 40 g Vit E, 8 g Vit K, 4 g Vit B₁₂, 4 g Vit B₂, 6 g Vit B₆, 4 g pantothenic acid, 8 g nicotinic acid, 400 mg folic acid, 20 mg biotin, 200 mg choline, 4 g copper, 0.4 g iodine, 12 g iron, 22 g manganese, 22 g zinc, 0.04 g selenium, 1.2 mg folic acid, 12 mg niacin, 26 mg D-calcium pantothenate, 6 mg pyridoxine HCl, 7.2 mg riboflavin, 1.2 mg thiamine HCl, 3077 mg sodium chloride (NaCl, 39% Na, 61% Cl), 65 mg ferrous sulphate (FeSO₄·7H₂O, 20% Fe), 89 mg manganese sulphate (MnSO₄, 36% Mn), 150 mg zinc sulphate (ZnSO₄·7H₂O, 40% Zn), 28 mg copper sulphate (CuSO₄·5H₂O, 25% Cu), 11 mg potassium iodide (KI, 24% K, 76% I), 1000 mg celite AW521 (acid-washed diatomaceous earth moisture-silica).

^bValues represent the mean of three replicates.

^cNitrogen-free extract = 100 – (% moisture + % protein + % fat + % fibre + % ash).

^dGross energy = (22.2 x protein + 38.9 x fat + 17.2 x nitrogen-free extract).

^ePrice in CFA pound: 100 CFA = 0.15 \$ based on 2006 exchange prices in Ivory Coast.

FD = Fish diet; MD = mixture diet; and SD = soya diet.

protein by soya protein entails also replacement fish oil by soy oil in diet. Furthermore, previous studies with teleost fish have shown that nutritional factors, such as dietary protein sources (Kaushik et al., 1995), fat sources (Guillou et al., 1995), dietary fat content (Bjerkeng et al., 1997), and vitamin E (Boggio et al., 1985) can influence the physical and organoleptic flesh quality. The purpose of this study was to evaluate the growth, feed utilization, carcass composition and organoleptic characteristics of *O. niloticus* fed diets containing partial or total substitution of soya protein for the fish protein.

MATERIALS AND METHODS

Experimental diets

Three experimental diets were formulated to be isonitrogenous in

terms of crude protein (35%). The proximate composition of the experimental diets was given in Table 1. Fish meal protein is replaced by soya protein on the basis of crude protein as follows: fish diet (FD) = 0% soya protein replaced fish protein; mixture diet (MD) = 50% soya protein replaced fish protein; soya diet (SD) = 100% soya protein replaced fish protein in diet. To balance for lysine and methionine, 0.5% of each amino acid was added in soya diets, and 2% premix vitamin and mineral supplemented each diet formulated. The energy values were calculated using the gross energy values for the macro nutrients (Luquet and Moreau, 1989). The cost of each diet was determined by multiplying the contributions of each diet ingredients by their cost per kilogram and summing the values obtained for all the ingredients. The diets were dried, broken into suitable sizes and stored at -20°C until use.

Fish feeding and experimental condition

Juveniles of *O. niloticus* used in this study were obtained from culture pond of Layo Aquaculture Station (5°19'N, 4°19'W; Ivory

Coast). Fish were acclimated for two weeks into tanks and fed at 3% of their body weight with commercial diet containing 30% crude protein. At the end of acclimated period, fish were counted and stocked at density of 50 fish per tank (12 fish m⁻³). Three replicate tanks were constituted for each diet and fish were fed at ration of 3% fresh weight three times a day (08:00, 12:00 and 17:00 h). Once a month, fish were bulk weighed and ration was adjusted.

Sample collection

At the beginning of the experiment, fish were individually weighed and every month fish in each tank were weighed. At the end of the experiment, survival fish were collected, counted from each replicate. Thus individual body weight was recorded and 10 fish were removed from each replicate to chemical composition determination. After collection of chemical analyses samples, fish were pooled and stored at -20°C for organoleptic characteristics analyse.

The survival rate, growth performance and nutrient utilization were evaluated as follows:

Body weight gain (BWG) = [(final weight - initial weight) / initial weight] x 100

Specific growth rate (SGR) = (ln final weight - ln initial weight) / number of day

Feed conversion ratio (FCR) = dry feed intake (g) / weight gain (g)

Feed efficiency ratio (FER) = [wet weight gain (g) / dry feed intake (g)] x 100

Protein efficiency ratio (PER) = weight gain (g) / protein intake (g)

Protein productive value (PPV) = [(final carcass protein - initial carcass protein) / protein feed] x 100

Energy retention (ER) = [retained carcass energy / energy intake] x 100

Daily lipid gain (DLG) = retained lipids (g) / biomass gain (kg) / number of day

Cost benefit analyses of the diets were performed according to El-Sayed (1990).

Biochemical analysis

The approximate composition of experimental diets and the fish carcasses were analysed using standard methods (AOAC, 1995). Moisture content of each sample was determined through a hot-air oven set at 105°C for 24 h, and ash was determined by incineration at 550°C in a muffle furnace for 24 h. Crude protein (nitrogen x 6.25) was determined using micro-Kjeldahl method; crude fat was extracted (hexane extraction) by using the Soxhlet method and crude fibre was quantified by acid digestion followed by ashing the dry residue at 550°C in muffle furnace for 4 h. The gross energy of samples was determined using the gross energy values for the macronutrients (Luquet and Moreau, 1989).

Organoleptic evaluation

Sensory profiling (Stone et al., 1974; ISO 11035) described by Regost et al. (2003) was performed 2 weeks after the end of growth trial. 14 regular fish eating and non smoking individuals was selected

for their interest, availability and sensorial capacities of discriminating intensities. All were volunteers and received regular training sessions to develop their sensory performances and knowledge of marine products. Sessions were conducted in an air-conditioned room according to AFNOR (1995).

The average weight of the fish used in the evaluation was 223.675 ± 27.15 for FD; 180.08 ± 8, 98 for MD; and 156.67 ± 34.13 for SD. Fish of each diet were defrosted overnight at 5°C, and were cleaned, washed without the heads. Samples were placed in covered foil dishes, cooked with skin in a fan forced electric oven at 200°C for 20 min before analyses. Products were assigned alphabetical letters (A, B, and C) randomised and simultaneously served. The samples were evaluated using a continuous scale presented on a computer screen from 0 (low intensity) to 5 (high intensity) for odour, aspect, texture, flavour, and texture in mouth attributes. The odour and aspect attributes were assessed immediately after opening the bowl, while the flavour, texture, and texture in mouth attributes were evaluated after cutting and eating the samples. Distilled water was freely available for palate cleansing prior to and during tasting. The sensory panellists have the opportunity to record additional descriptors and comments and give one general appreciate note.

Statistical analysis

All percentage and ratio values were transformed to arcsin values and data of weight to logarithm values before analyse. Growth data (weight and data of growth performance), fish carcass composition and organoleptic characteristics were analysed by using one way analysis of variance (ANOVA). Duncan multiple range test was used to compare the differences among the individual means. The treatment effects were considered to be significant at p < 0.05.

RESULTS

Growth and feed utilization

At the end of the study, fish fed FD showed the higher values (p < 0.05) of final body weight (FBW), body weight gain (BWG) and specific growth rate (SGR), and fish fed SD showed the lower values. Survival rates (SR), feed conversion ratio (FCR) and feed efficiency ratio (FER) were similar for all diets. Protein utilization indices such as protein efficiency ratio (PER) and protein productive value (PPV) were not differed significantly between fish fed different diets. Contrary, the higher values (p < 0.05) of energy retention (ER) and daily lipid gain (DLG) were obtained with fish fed SD followed by MD and FD. The cost for kg fish production was lower and production time was higher (p<0.05) in fish fed soya protein diets (Table 2).

Carcass composition

No significant differences were found in carcass moisture of different fish fed the diets. Fish carcass ash content showed a decreasing trend with increasing soya protein level in diets. The higher values of ash content were given by fish fed FD followed by the fish fed MD; the lower values were obtained with those of fish fed SD. The

Table 2. Growth performance and nutrient utilization of *Oreochromis niloticus* fed the experimental diets.

Parameters*	Diets		
	FD	MD	SD
IBW (g)	11.6 ± 4.2	11.6 ± 4.2	11.6 ± 4.2
FBW (g)	221.9 ± 50.4 ^c	169.7 ± 32.6 ^b	140.7 ± 30.3 ^a
SR (%)	71.3 ± 12.2	76.0 ± 9.2	67.3 ± 6.4
BWG (%)	1861.9 ± 353.3 ^b	1375.9 ± 106.7 ^a	1140.8 ± 192.2 ^a
SGR (% day ⁻¹)	1.6 ± 0.1 ^b	1.5 ± 0.4 ^a	1.4 ± 0.1 ^a
FCR	1.1 ± 0.2	1.2 ± 0.04	1.2 ± 0.1
FER (%)	95.7 ± 14.5	84.1 ± 2.9	82.8 ± 8.9
PER	2.7 ± 0.4	2.4 ± 0.1	2.3 ± 0.2
PPV (%)	52.1 ± 5.7	46.7 ± 1.4	45.9 ± 4.6
ER (%)	22.2 ± 0.8 ^a	25.2 ± 1.1 ^b	31.9 ± 0.9 ^c
DLG (gkg ⁻¹ day ⁻¹)	1.1 ± 0.03 ^a	1.2 ± 0.02 ^a	1.6 ± 0.04 ^b
PC (F.CFA)	278.1 ± 46.2 ^b	270.9 ± 9.6 ^b	235.1 ± 25.8 ^a
PT (dayskg ⁻¹)	26 ± 1 ^a	31 ± 1 ^a	43 ± 5 ^b

*Values are means ± SD. Values in the same row with same superscripts are not significantly different ($p \geq 0.05$).

FD = Fish diet; MD = mixture diet; and SD = soya diet.

IBW = Initial body weight; FBW = Final body weight; SR = Survival rate; BWG = Body weight gain; SGR = specific growth rate; FCR = feed conversion ratio; FER = feed efficiency ratio; PER = protein efficiency ratio; PPV = protein productive value; ER = energy retention; DLG = daily lipid gain; PC = cost per fish kg produced; PT = time per fish kg produced.

Table 3. Final carcass composition (% on dry matter basis) of *O. niloticus* fed experimental diets.

Parameters*	Diets		
	FD	MD	SD
Moisture	69.6 ± 1.4	69.1 ± 0.1	69.1 ± 0.3
Ash	17.7 ± 0.6 ^c	15.3 ± 0.5 ^b	13.0 ± 0.01 ^a
Crude protein	63.1 ± 0.03	63.0 ± 0.1	62.6 ± 0.5
Crude fat	19.7 ± 0.2 ^a	20.5 ± 0.5 ^a	26.6 ± 0.6 ^b
Energy (KJg ⁻¹)	21.6 ± 0.1 ^a	22.2 ± 0.2 ^b	23.9 ± 0.2 ^c

*Values are mean ± SD of triplicate analysis. Values in the same row with same superscripts are not significantly different ($p \geq 0.05$).

FD = Fish diet; MD = mixture diet; and SD = soya diet.

carcass fat content was significantly higher ($p < 0.05$) with fish fed SD followed by MD and FD. The carcass energy content showed a similar trend as the carcass fat content (Table 3).

Organoleptic appraisal

The result of organoleptic characteristics of *O. niloticus* is presented in Table 4. Odour and texture in mouth of fish were not significantly affected by the dietary treatment. In contrast aspect, texture, flavour, and general appreciate note were differed ($p < 0.05$) with dietary treatment. Concerning aspect, fish fed FD had a whiteness aspect significantly more pronounced than those fed MD and

SD. Fatter aspect was significantly different among groups ($p < 0.05$); fish fed FD and SD had the highest values while those of fish fed MD had the lowest value. For the texture, firm and sticky attributes differed significantly ($P < 0.05$) with the dietary treatment. The firm texture of fish fed FD and SD were significantly more pronounced than those of fish fed MD. Texture of fish fed FD was stickier than those of fish fed MD and SD. The flavour, sweet and fat attributes differed significantly ($P < 0.05$) among groups. Fish fed MD had a better sweet flavour than those fed FD in which more pronounced fatty flavour was obtained. For the general appreciate note, the higher and the lower values were respectively obtained in fish fed MD and FD.

DISCUSSION

Incorporation of 50 and 100% soya protein in diet decreased the growth of *O. niloticus*. This decrease of fish growth can be due to the quantity and the quality of soya protein in diet. Shiau et al. (1987) had reported that soybean meal could fully substitute fish meal without a significant reduction in tilapia growth if the diet contained sub-optimal (24%) levels of protein. However, growth reductions were observed when soybean meal substituted fish meal at 32% protein level. These reports clearly demonstrate that substituting animal protein with plant protein at higher levels than the optimal dietary protein reduces the growth of tilapia. Similarly, in the pre-

Table 4. Sensory analyses of fish at the end of growth trial.

Sensory parameter	Attributes	Diets			ANOVA P values
		FD	MD	SD	
Odour	Fishy	2.64	3.21	3.00	0.378
	Meaty	0.43	0.93	0.50	0.394
	Weedy	0.21	0.36	0.36	0.924
	Earthy	0.86	0.43	0.50	0.571
	Milky	0.29	0.14	0.21	0.876
	Rancid	0.86	0.64	0.50	0.779
Aspect	Whiteness	2.36 ^b	0.71 ^a	0.71 ^a	0.000
	Pale	1.43	0.93	0.93	0.560
	Fatness	3.36 ^b	2.57 ^a	3.5 ^b	0.021
Texture	Firm	3.43 ^b	2.57 ^a	3.57 ^b	0.019
	Fibrous	1.64	1.57	1.21	0.684
	Sticky	2.21 ^b	0.71 ^a	0.71 ^a	0.000
	Moist	2.14	1.86	2.00	0.866
	Fat	2.07	1.36	1.36	0.329
	Juicy	2.36	2.21	2.36	0.711
Flavour	Fishy	3.14	3.21	3.5	0.757
	Meaty	0.36	0.64	0.21	0.437
	Weedy	0.21	0.21	0.14	0.450
	Earthy	0.7	0.64	0.50	0.956
	Sweet	2.50 ^a	3.43 ^b	3.00 ^{ab}	0.015
	Fatty fish	2.29 ^b	1.21 ^a	1.14 ^a	0.025
	Bitter aftertaste	0.79	0.50	0.71	0.774
Texture in mouth	Firm	3.50	3.29	3.64	0.480
	Fibrous	1.00	1.14	0.57	0.531
	Sticky	1.43	0.57	0.64	0.065
	Fat	1.07	0.79	1.36	0.306
	Juicy	2.29	1.50	1.64	0.060
General note		2.14 ^a	4.00 ^c	3.21 ^b	0.000

The values represented the means of all notes and values in the same row with same superscripts are not significantly different ($p \geq 0.05$).
FD = Fish diet; MD = mixture diet; and SD = soya diet.

sent study fish were fed sub-optimal 35% levels of protein, and thus significant growth reduction would be when fish meal protein was partially and totally substituted with the soya protein. In fact, soybean meal contains antinutritional factors, so adding high percentage of this product in fish diets (35% protein) had delay growth of fish in ours study. However higher values of the nutritional utilization indices of protein (PER and PPV) in all diets showed that diets formulated had good protein quality and palatability.

The result of proximate composition showed that carcass ash content increased with highest level of ash and fish meal content in diet. In fact, fish meal has high levels of minerals including phosphor associated with the bone fraction (Ketola and Richmond 1994; Sugiura et al., 2000), which were highly available and retained for *O. niloticus*. Similar results were obtained with *Symphysodon aequifasciata* HECKLEL (Chong et al., 2003), and

Sarotherodon galileus (Goda et al., 2007) fed with plant protein-based diets. The increase of soya protein level in diet did not affected proteins of fish; this may be because all diets tested had a similar protein contain. Ofojekwu (1993) also did not find any effects of the dietary protein source on the fish protein content in *O. niloticus*. The lipid and energy contents in fish carcass increased with dietary lipid and soya protein level. The maximum values of lipid and energy obtained with fish fed SD is one consequence of higher values of ER and DLG obtain with this diet. The high lipid and energy values in this group clearly suggest that there were increased lipogenesis with increasing levels of fish meal replacement. So the diets containing high levels of soybean meal were not efficiency utilised for growth of *O. niloticus* but increased body fat deposition as observed by Abdelghany (2003) in the same species and Kaushik et al. (2004) in European Seabass *Dicentrarchus labrax*.

The results of sensory evaluation showed that the more fat aspect was associated to the fish fed SD. This could be due to the increase of fish fat content in this group. It is this fat increase in fish which influences the organoleptic properties of the fish.

Substitution of vegetable protein and oil by soya products in fish diets may modifies the body fatty acid profile as observed by Serot et al. (2001) in turbot *Psetta maxima*. Qualitative and quantitative variation of body lipids in response to dietary vegetable oils may significantly affect fish flesh quality (Thomassen and Rosjo, 1989; Arzel et al., 1994) and sensory characteristics (Aleman and Izquierdo, 1998). This could explain the variations observed in this fish texture and flavour.

In conclusion, this study shows that partial or total replacements of fish protein with soya protein in diet (35% crude protein) reduce the fish growth. However, lower ash and higher fat contents of fish carcass were associated with soya protein diets. In addition, partial substitution improves the organoleptic quality of *O. niloticus*.

ACKNOWLEDGMENTS

The authors thank the staff of Biochemistry and Foods Sciences Laboratory (LaBSA) of Cocody University (Ivory Coast) for their assistance in chemical analyses. Furthermore, the authors would like to express their gratitude to the 14 panellists for the aids in conducting organoleptic characteristics studies. This study is a part of the Oceanology Research Center project and was financed by Ivorian government.

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