APPARENT PROTEIN DIGESTIBILITY AND AMINO ACID AVAILABILITIES OF DIETARY FISH MEAL, SOYBEAN MEAL AND FULL FAT SOYBEAN IN THE NILE TILAPIA *OREOCHROMIS NILOTICUS* FINGERLINGS.

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Key words: Apparent protein digestibility, amino acid availabilities, fish meal, soybean meal, full fat soybean Nile tilapia.

ABSTRACT

This study was conducted to evaluate the apparent protein digestibility and amino acid availabilities of fish meal replaced with soybean meal and full fat soybean at two levels of 50% and 75% in the Nile tilapia fingerlings.

The carcass protein, lipid and gross energy gain were improved when dietary fish meal was replaced with soybean meal or full fat soybean at the levels of 50% than 75% .

Diets containing either soybean meal (50%) or full fat soybean (50%) had the highest significant ($P \geq 0.01$) value for protein digestibility coefficient than the other treatments.

The over all pattern of apparent amino acids, availability corresponds to that of protein digestibility .The availability of threonine from fish meal and that of methionine of soybean meal at 75% replacement level of fish meal were the lowest in the respective protein sources. Cystine availabilities of fish meal and full fat soybean at 75% replacement level were low compared with other amino acids.

This study demonstrates that soybean meal or full fat soybean may used as a best source of protein in combination with fish meal in the diet for Nile tilapia at the level up to 50% of total dietary protein, in order to ensure a favorable essential amino acid profile which closely meets the requirements.

INTRODUCTION

In formulating practical fish feeds, information on the availability of nutrients from feed ingredients is necessary to improve the quality of the feeds (Yamamoto *et al.,* 1998). Despite the fact that fish meal is the single most expensive major ingredient in aquaculture feeds (Tacon, 1993), it is widely used as the main source of dietary protein for most commercially formed fish species. In the mean time, the shortage in world production of fish meal, coupled with increased demand and competition with terrestrial domestic animals has further increased fish meal prices (EL– Sayed, 1998). In the long – run, many developing countries may not be able to depend on fish meal as a protein source for aqua-feeds, therefore, it is necessary to test and find other fish meal replaces. In this regard, many attempts have been made to partially or totally replacement of fish meal with less – expensive protein sources in fish feeds (Pongmaneerat, 1994).

Soybean meal is the best plant protein source in terms of protein contents and favorable essential amino acid profile that closely meets the requirements of fish. However, it is limited in sulfur containing amino acids (Met. , Lys. , Cys.) .Many studies have considered soybean meal and other soybean products as a partial or total fish meal alternative for tilapia , with varying results (EL – Sayed , 1999; Elangovan & Shim, 2000) .

The nutritional value of a protein depends highly on its digestibility and its amino acid composition. However, since alternative protein sources for fish meal have different amino acid compositions, the availability of amino acids from these protein sources should be assessed to ensure an effective substitution of fish meal.

The present study was conducted to evaluate the apparent protein digestibility and amino acid availabilities of fish meal, replaced with soybean meal and full fat soybean at two levels of 50% and 75% for Nile tilapia fingerlings.

MATERIALS AND METHODS

Experimental diets

The proximate and amino acid composition of the protein sources tested are shown in (Table,1) . Five isocaloric (14.88 + 0.16 Mj/ME/Kg) ,isonitrogenous (30.38 ± 0.25 cp /Kg) diets were formulated as presented in Tables (2 and 3) . The control diet was formulated with fish meal as the sole protein source , two diets containing soybean meal to replace fish meal at levels of 50 % and 75% and another two diets were formulated to contain replaced at the same levels with full fat soybean as replaces of fish meal .

Feedstuffs were mixed using mixer and combined with 400 ml water per Kg dry ingredients then pelleted using a mincing machine. The diets were then cut into approximately 5 mm pellet length then stored at – 20 ^{0}C untill being fed to the fish.

Experimental procedure

Nile tilapia fingerlings with an average initial weight of 18.70 ± 1.86 g were allocated in five groups of 30 fish each in 180-l tanks and were Kept for acclimatization for 14 days. Fish were fed on the experimental diets twice daily (8.30 and 16.00 h) to apparent satiation. After being fed the respective diets for 7 days, fish were starved 1 day to evacuate previous feed. On the next day, they were given feed only in the morning, two hours after the feeding, five fish were randomy removed from each tank and tronsferred to a faeces collection tank. Faeces were taken every 2h for the first 6h and every 4h for the subsequent 8h to minimize leaching loss. For the replicate determination ,different fish were reallocated from the same stock and fed in the same manner as describeted above.

Ten faecal samples were collected in 10 day experimental period for each experimental diets, the samples were collected using siphon technique to determine the apparent digestibility of protein and avalilabilitis of amino acid using Cr_2o_3 as external marker according to Riche and Brown (1996) and Masumoto *et al.*, (1996).

Experimental analysis

Feedstuffs, diets and faecal matter were analyzed for proximate composition in duplicates according to A.O.A.C. (1990) methods. Protein was determined using Micro – Kyeldahl technique, lipid by solvent extraction and ash by ashing in a muffle furnace for 12h at 550°C. Alpha – plus Amino Acid Analyzer (LKB Bichrom Ltd., Cambridge, England) was used for amino acid determination.

Energy values were calculated using multiplier factors of 9.45, 5.65 and 4.2 Kcal g^{-1} for lipid, protein and carbohydrate, respectively (Hepher *et at.*, 1983). Cr_2o_3 was estimated by the method of Furukawa and Tsukahara (1966). Apparent digestibility coefficients (ADC) and apparent amino acid availability (AAAA) of the diets were determined according to Jauncay and Ross (1982); Carter and Hauler, (2000):

$$ADC\% = 100 - [100 (\%I_{diet} / \%I_{faeces}) \times (\%N_{faeces} / \%N_{diet})] .$$

Where:
I : is the inert marker
N: is the nutrient.

$AAAA(\%)=100-[100\ (\%I_{diet}/\ \%I_{faeces})\ x\ (\%A_{faeces}\ /\ \%A_{diet})]$.
Where:
I : is the inert marker
A: is amino acid.

Statistical analysis:

Statistical significance (P ≥0.01)of differences between protein digestibility and individual amino acid availability values within the same test protein source was computed for ANOVA test one way classification . For comparing a control to all other means Duncan's multiple range test was done (Duncan , 1955) .

RESULTS AND DISCUSSION

A proximate analysis of the test protein sources showed that crude protein contents were highest in fish meal (70.10) and lowest in full fat soybean meal (38.00) wheat brean (16.40) in decreasing order (Table, 1). Crude fat was highest in full fat soybean meal (18.00 %) followed by fish meal (9.08) . Plant protein sources , had higher total carbohydrate than the fish meal . The ash contents of fish meal was higher than that of the other ingredients . Fish meal was high in lysine and methionine, however , soybean meal and full fat soybean were low in methionine and had essentially the same amino acids profule . Wheat bran content had lowest values of all amino acids t han requreid for Nile tilapia according to NRC (1993) .

The tabulated results (Tables 4 and 5) illustrate the protein , lipid and gross energy retention values derived from either dietary intake feed and carcass gain . The carcass protein , lipid and gross energy gain were improvrd when dietary fish meal replaced with soybean meal or full fat soybean at the level of 50% then 75% . No significant differences in protein retention were observed .The protein intake also insignificantly varied with the diets (P ≥0.05) . The opposite trend were observed for lipid retenition and gross energy retention .Reigh and Ellis (1992) reported that growth has often been reduced in fish fed diets with soybean meal replacing all the fish meal.

One possible reason may be the reduced activity of protease due to trypsin inhibitors in crude or inadequated heated soybean meal (Wilson and Poe, 1985) .Webster *et at.* (1992 a,b) reported that the activity of protease may not be of practical importance since commercially availa`ble soybean meal usually has little trypsin inhibitor activity if adequately processed . A second possible reason may be for suboptimal amino acid balance which represents a possible reason for suboptimal amino acid balance of soybean meal (Dabrowski *et al.*, 1989) .A third possible explanation may be that the energy content of soybean meal is lower than that of fish meal in diets for fish (Hilton and Slinger , 1986) . Finally , soybcan may have reduced digestibilities of minerals , especially phosphorus , compard with fish meal (Goda *et al.* , 2002) .

The reduced carcass fat content with increased soybean meal in diet was also consistent with ealier findings of Mohsen and Lovell (1990) for catfish , Reigh & Ellis (1992) for red drum , Olli *et al.* (1995) for Atlantic salmon. Olli & Krogdahl (1995) demonstrated that alcohol – soluble component of soybean meal comprises antinutrients , which negatively affect fat digestibility particularly the long chained , saturated and mono saturated fatty acids in Atlantic salmon . This may be one of the reasons for reduced carcass fat retention and gross energy retention in fish fed diets either soybean meal or full fat soybean meal at replacemat level of 75 % than 50 % from fish meal (Table 5) .

The apparent and true amino acids availability values are shown in Table (6) . Generally , the apparent and true amino acids availability values were determined from faeces by using syphon technique.The sedimentation followed by syphon technique was chosen due to the fact that it allows to obtain faeces from fish at less stressful conditions and with less contamination of body fluids (Spyridakis *et al.*, 1989 and Masumoto *et at.*, 1996) .

Protein digestibility of the diets differed significantly from each other ($P \geq 0.01$). The data revealed that diets containing either soybean meal (50%) or full fat soybean (50%) had the highest significant differences for protein digestibility coffeicient (Table 6). The performance of fish meal as major protein source in formulated feeds for aquaculture is easily understood by the very high digestibility of protein and the availability of amino acids (Table 6). Results of Table (6) are agreement with high digestibility cofficients recorded for Henely (1987) ; Luquet (1991) and El – sayed

& Toshima (1992) for the Nile tilapia and other species including salmonids (Sugiura *et al*.,1998), red drum (McGoogan & Reigh, 1996). However, Lovell (1990) reported that the protein of dehulled solvent extracted soybean meal was 85% digestible , equal to or higher than that of whole fish meal protein .However , somewhat lower average digestibility cofficient values have bean reported for herring meal (81.97%) , menhaden meal (7.30%) for rainbow trout (Anderson *et al* ., 1992) and menhaden meal (69.20 – 82.90%) for channel catfish (Wilson *et al.,* 1981) .The variation in the apparent digestibility coefficient values of protein reported for fish meal may be due to differences in fish meal processing methods or the quality of the raw material used . Moreover , oxidation of fish meals resulting from overheating or prolonged storage many affect protein quality and amino acid availability (Masumoto *et al.,* 1996) . Davies & Morris (1997) reported that soybean meal and soybean derived product without amino acids supplements have been shown to be adequate for the partial replacement (50%) , while higher substitution levels may warrant an addition to the diet of specific amino acids to meat the essential amino acid (EAA) requirements of the Nile tilapia and other species ,while maintaining a suitable balance of EAAS .

Table (3) relates the amino acid in the diets to the requirements data of NRC (1993) to establish the limiting amino acids. The apparent amino acid availability (AAAA) of the two soybean products at two replacement levels (50% and 75%) for fish meal showed higher value than that for fish meal (Table 6) . The overall pattern of apparent amino acids availability corresponds to that of protein digestibility . The availability of threonine from fish meal and that of methionine of soybean meal at 75% replacement level of fish meal were the lowest in the respective protein sources . Cystine availabilities of fish meal and full fat soybean at 75% replacement level of fish meal were lower compared with other amino acids. Kaushik & Luquet (1980) ,working with rainbow trout , and Jackson and Copper (1982) ,working with tilapia *Oreochromis mossambicus* showed that excess dietary methionine has a depressive effect on growth . Jackson and Copper (1982) related this to the inhibitory effect of methionine on the absorption through the intestinal wall of certain neutral amino acids . Inhibition of leucine transport by methionine, may be related to the high affinity of the methionine lipophlic side chain for the transport mechanism (Hepher,1988). However, Sadiku & Jauncey (1995 a,b) presented the availability of methionine and cysteine in combination with fish meal and soybean meal . Arginine concentrations , however, appear to run parallel with Met. levels in the plasma despite a constant dietary level . Leucine and Trp. levels were significantly reduced in fish with Met. deficiency. The same tendency was observed in the results of digestibility cofficient of arginine.Wilson *et al.* (1981) investigated the availability of amino acids in soybean for channel catfish and found that arginine was the most available , while the least available was glycine .

Tyrosine which is non- essential , showed the highest significant ($P \geq 0.01$) value of digestibility coffeicient with phenylalanine for tilapia fed soybean meal or full fat soybean at 50% replacement level of fish meal than the other treatment . The same results was obsorved for cystine (non-essntial) with methioinine . In both of these last cases the non-essential amino acid cannot completely replace the essential acid , but because of their capacities for partial replacement of the essential amino acids , they are often included with them (Hepher , 1988) .

In concolusion, this study demonstrates that soybean meal or full fat soybean may be used as a best source of protein in combination with fish meal in the diet for Nile tilapia . The resullts of this experiments suggest the possibility of partial replacement of fish meals by vegetable proteins (soybean meal or full fat soybean) up to 50% without negative effect on feed utilization or nutrient retention . The availability of amino acids in the diets containing fish meal and soybean meal or full fat soybean were higher than the diet containing fish meal only . Futher work is necessary in order to undarstand the factors goverining or affecting feed intake in Nile tilapia fed fish meal free diet . Further evaluation of different types (dehulled and solvent extracted) soybean meal and other soybean products (soyflour and soybean protein concentrates) as a sole sources of dietary protein in diets supplemented with amino acids or other plant protein sources on growth of Nile tilapia should be conducted in the future .

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Table (1): Proximate and amino acid compositions of the ingredients.

Ingredients	**Fish Meal**	**Soybean meal**	**Full fat soybean**	**Wheat bran**
Proximate composition (%)				
Crude protein	70.10	44.10	38.00	16.40
Crude fat	9.08	1.86	18.00	4.00
Total carbohydrate	0.32	47.82	39.50	74.30
Crude ash	20.50	6.22	4.50	5.30
Gross energy (*Mj* / Kg)	20.68	18.22	23.03	18.51
Amino acid composition: (g/100g protein)				
Arginine	4.59	3.32	3.08	0.76
Histidine	1.64	1.25	1.05	0.39
Isoleucine	3.45	2.00	1.95	0.51
Leucine	5.38	3.32	3.08	0.90
Lysine	5.45	2.78	2.72	0.48
Methionine	2.01	0.63	0.56	0.20
Cystine	0.51	0.68	0.41	0.26
Phenylalanine	2.57	2.22	2.10	0.59
Tyrosine	1.98	1.66	1.52	0.40
Threonine	3.08	1.71	1.72	0.42
Tryptophan	0.75	0.64	0.63	0.21
Valine	4.46	2.03	2.46	0.64
Alanine	4.60	1.90	1.64	1.05
Aspartic acid	7.62	4.70	3.05	2.36
Glutamic acid	12.60	8.96	6.72	4.05
Glycine	2.99	1.89	1.61	0.90
Proline	3.88	2.21	1.90	1.17
Serine	2.54	2.20	1.80	1.11

Table (2): Proximate analysis compositions and ingredients content of test diets.

	FM	SBM		FSBM	
		50%	75%	50%	75%
Ingredient (%)					
Fish meal	27.00	15.50	10.00	18.50	15.50
Soybean meal	-	23.00	33.00	-	-
Full fat soybean	-	-	-	22.0	30.50
Wheat bran	67.50	56.00	51.00	57.00	51.50
Mineral mix.(1)	1.00	1.00	1.00	1.00	1.00
Vitamin mix.(2)	1.00	1.00	1.00	1.00	1.00
Chromic oxide	0.50	0.50	0.50	0.50	0.50
Oil	3.00	3.00	3.50	-	-
Nutrient composition(3):					
Crude protein	30.16	30.36	30.15	30.51	30.74
Crude fat	8.37	7.12	7.12	7.88	8.92
Total carbohydrate	51.89	54.89	55.90	53.86	53.11
Crude ash	9.58	7.63	6.83	7.75	7.23
Gross energy (MJ/ Kg)(4)	19.54	19.59	19.75	19.79	20.12
Metabolizable energy(MJ / Kg)(4)	14.98	14.69	14.81	14.84	15.09

1Mineral mixture : each 1 kg of mixture contains: 200 g. Choline., 4 g. Copper., 0.4 g. Iodine., 12 g. Iron., 22 g. Manganese., 22 g. Zinc., 0.04 g. Selenium

2Vitamin mixture: each 1 kg of mixture contains : 4.8m. I.U, Vit A., 0.8m.I.U. Vit D_3., 4.0 g. Vit E., 0.8 g. Vit K., 4.0 g. Vit B_{12}., 4.0 g. Vit B2., 0.6 g. Vit B6., 4.0 g. Vit Pantothenic acid., 8.0 g. vit Nicotinic acid., 400 mg. vit Folic acid., 20 mg. vit Biotin.

aAalyzed and calculated on dry matter basis .

4 Gross energy content was calculated using the values 5.65, 4.2 and 9.45 Kcal/ gm for protein, carbohydrate and lipid, respectively and applying the coefficient of 0.75 to convert gross energy to metabolizable energy according to Hepher *et al.*, (1983).

Table (3): Amino acid compositions of the test diets (g / 100 g /cp)

	FM	SBM		FSBM		NRC
	100%	50%	75%	50%	75%	
Arginine	1.75	1.89	1.94	1.95	2.04	1.32
Histidine	0.71	0.76	0.77	0.75	0.77	0.77
Isoleucine	1.28	1.28	1.26	1.35	1.39	1.21
Leucine	2.06	2.09	2.08	2.18	2.23	2.41
Lysine	1.80	1.75	1.70	1.88	1.92	2.12
Methionine	0.68	0.57	0.51	0.61	0.58	0.75
Cystine	0.31	0.38	0.41	0.39	0.34	0.11
Phenylalanine	1.09	1.23	1.43	1.27	1.34	1.40
Tyrosine	0.81	0.91	0.95	0.93	0.97	1.46
Threonine	0.28	1.10	1.08	1.18	1.21	1.11
Tryptophan	0.23	0.38	0.39	0.40	0.42	0.32
Valine	1.63	1.51	1.44	1.73	1.77	1.52
Alanine	1.95	1.73	1.61	1.80	1.74	1.36
Aspartic acid	3.65	3.56	3.49	3.40	3.30	2.04
Glutamic acid	6.14	6.24	6.24	6.08	5.85	5.32
Glycine	1.41	1.39	1.37	1.41	1.41	2.12
Proline	1.84	1.75	1.70	1.70	1.77	3.75
Serine	1.43	1.51	1.54	1.40	1.50	1.64
Other	1.11	0.33	0.24	0.10	0.21	
Total essential A.A.	11.51	12.56	12.60	13.30	13.67	12.93
Total non-essential A.A.	18.65	17.80	17.55	17.21	17.07	17.80
EAAI*	88.63	92.7	91.93	94.58	95.42	-

* EAAI : Essential amino acids index.

Table (4): Daily dietary protein, lipid and gross energy intake and carcass protein, lipid and gross energy contents*.

	FM	SBM		FSBM	
	100%	50%	75%	50%	75%
Dietary protein intake (g/fish/day)	0.1114	0.1121	0.1113	0.1127	0.1135
Dietary lipid intake (g/fish/day)	0.0304	0.0259	0.0259	0.2860	0.03238
Dietary gross energy intake (Kcal /fish/day)	0.9165	0.8776	0.8731	0.9071	0.9472
Carcass protein gain (g/fish/day)	0.0315	0.0329	0.0228	0.0307	0.0200
Carcass fat gain (g/fish/day)	0.0093	0.0098	0.0072	0.0088	0.0059
Carcass energy gain (Kcal /fish/day)	0.2659	0.2785	0.1969	0.2566	0.1688

- The mean value calculated from the experimental periods (21 days)

Table (5): Digestibility coefficients of protein, indigestible protein, lipid and gross energy retention.

	FM	SBM		FSBM	
	100%	50%	75%	50%	75%
Digestibility coefficient of protein (%)	89.32^{c}	93.93^{a}	91.97^{b}	92.69^{a}	89.21^{c}
Indigestible protein (g/fish/day)	0.0995	0.1053	0.1022	0.1045	0.1013
Protein retention (%)	18.27	17.72	17.61	17.66	17.36
Lipid retention (%)	29.95^{b}	35.98^{a}	28.63^{b}	31.46^{a}	19.46^{c}
Energy relation (%)	29.00^{a}	31.72^{a}	22.52^{b}	28.23^{a}	17.86^{c}

Table (6): Apparent protein digestibility and amino acid availability of test diets (%) for Nile tilapia at $28^{0}C$.

	FM	SBM		FSBM	
	100%	50%	75%	50%	75%
Digestibility coefficient of protein (%)	89.32^{c}	93.93^{a}	91.79^{b}	92.69^{a}	89.21^{c}
Essential and semi-essential A.A.					
Arginine	89.18	95.40	92.68	93.19	91.95
Histidine	89.80	95.41	92.43	92.92	91.47
Isolencine	96.42	94.78	91.90	93.12	91.73
Leucine	88.64	93.37	90.72	91.93	90.24
Lysine	91.02	94.19	92.50	93.82	92.51
Methionine	90.17	91.34	89.28	92.39	90.09
Cystine	73.04	89.30	87.56	88.09	83.09
Phenylalanine	85.69	93.15	90.83	90.60	88.97
Tyrosine	83.49	92.02	87.34	88.94	86.88
Threomine	50.27	91.81	89.54	91.85	90.16
Tryptophan	N.D*	N.D	N.D	N.D	N.D
Valine	89.06	93.84	90.64	92.71	91.18
Non – essential amino acids					
Alanine	89.14	93.12	90.72	92.44	90.33
Aspartic	91.91	94.78	93.95	94.24	92.66
Glutamic acid	93.10	96.04	94.57	95.03	93.61
Glycine	81.43	88.72	86.44	88.47	85.73
Proline	90.92	94.69	92.50	93.17	91.88
Serine	90.26	94.04	92.67	92.89	91.79
Mean value**					
Essential	83.71	93.42	90.49	91.78	89.84
Non – essential	89.71	93.57	91.81	92.71	91.00

*N.D : Not deterimined

** Means of the essential and semi – essential amino acid availabilities , non- essential amino acid availabilities and total amino acid availabilities,respectively.