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Utilization of sunnhemp (*Crotalaria juncea.* L) seed as a protein supplement in fish feed

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Abstract: A static indoor experiment consisting of nine glass aquaria was conducted with Tilapia (GIFT) to evaluate sunnhemp seed meal as an indigenous protein source and as protein concentrate substitute. Five iso-nitrogenous diets were formulated to contain about 35% protein by replacing protein concentrate from sunnhemp seed. Protein concentrate was used as principle source of protein in control diet (diet 1). The fishes were fed twice daily at a rate of 5% of their body weight. One replicate for diet 5 treatment and two replicates for the rest treatments were used. Each replicate contained 16 fishes. Water in aquaria was changed daily during removal of uneaten food or feces. The evaluation of the experimental diet was done based on the growth parameters; mean weight gain, weight gain (%), mean length, survival rate (SR) (%), whereas the evaluation of food utilization was done by food conservation ratio (FCR) and protein efficiency ratio (PER). Diet 5 however showed the best growth performance (compared to control) but exhibited the lowest FCR than the others. Diet 2 demonstrated the lowest growth performance among the diets.
Key words: Sunnhemp, approximate composition, protein supplement, Tilapia fish

Introduction

From nutritional standpoint, animal protein is the best for human being. However, per capita consumption of animal protein in our country is below the standard requirement due to low production of animals. Recently there has been increasing awareness among public for the need of animal proteins. Here farmers are becoming interested in fish and poultry farming as a profitable agricultural practice. This not only will increase their income but also will help to overcome much of the protein demand of our country. At present a lot of fish meal or animal protein concentrate (APC) have mainly been used as a dietary protein source due to their high nutritive value and palatability in artificial feed of fish and poultry worldwide. Moreover, calorie requirement is filled by corn, maize, wheat etc. All these ingredients are costly and mostly are imported from foreign countries. Sometimes our fish and poultry industries have run into trouble when shipment of these ingredients becomes uncertain. To save the industry from such a situation and to make the industry profitable, it is necessary to reduce the feed cost through the utilization of cheap and non conventional protein and carbohydrate rich ingredients. In this regard indigenous legume seeds which are rich in protein, lipid and carbohydrate should be considered as an economic and valuable ingredient for animal feed. Sunnhemp *(Crotalaria juncea)* is a leguminous plant can fix atmospheric nitrogen by rhizobial symbiosis, which is a popular fibre yielding crop in Bangladesh and India, and ranks next to jute (Narayan and Yagna, 1982). In Bangladesh, however, its agricultural use has been confined to green manuring for the improvement of the soil fertility as well as to fodder (Alom *et al.,* 1989). Moreover, its seeds are rich in protein and carbohydrate and also contains fair amount of oil (Javed *et al.,* 1999). Therefore, this plant have enough scope not only to be used as fodder for livestock but also its seed may also be used as an important indigenous feed ingredient for fish and poultry as there is no complain about its toxic effect like grasspea, soybean (Van der Ingh *et al.,* 1991). Some workers (Lewis *et al.,* 1974) attempted to replace the costly feed ingredient like APC, corn, wheat etc. with a variety of pulse crops; lentil, gram, chick pea, black gram, mustard, sesame, ground nut, sunflower but most of them are directly used as human food except APC. Therefore, it is necessary to find out an alternative source of proteins and calories for those farmed animals that are indigenous, non-toxic, locally available and not consumed directly by human being. Based on the above consideration, this work was designed to study the nutrient composition of sunnhemp seed as well as to evaluate the nutritive value of sunnhemp seed meal as protein supplement in formulated fish feeds.

Materials and Methods

The experiment was conducted in a static indoor system in the Department of Biochemistry, Bangladesh Agricultural University, Mymensingh.

Source of fry and acclimatization: Fry of *Tilapia nilotica* (GIFT) were collected from Freshwater Station of Bangladesh Fisheries Research Institute, Mymensingh and were acclimatized to the experimental system for 24 days. During acclimatization the fish were fed with formulated feed (1% of body weight) containing 35% protein.

Experimental system and procedure: Nine glass aquaria of 91 × 47 × 30 cm^3 were used as experimental tank. Each of the aquaria was filled with about 30 L of filtered water.

Preparation of experimental diets

Selection of ingredient: Ingredients selected to prepare experimental diets were: i) Protein concentrate: Protein concentrate was obtained from Jasoport, Joyson Agrovert Ltd and its percent composition is; Crude Protein - 60.00, Moisture - 7.00, Crude Fat - 10.0, Fibre - 4.0, Ash - 21.0 and ME - 3230 kcal/kg, ii) Soybean meal, iii) Maize starch (Korean), iv) Sunnhemp seed meal, v) Pure fish oil (China), vii) Soybean oil, viii) Vitamin premix

All ingredients, except sunnhemp seed meal, were purchased from local market and sunnhemp seed meal was prepared in the laboratory. Composition of vitamin premix used in different experimental diets has been shown in Table 1.

Diet formulation: Prior to the formulation of diet all the ingredients were subjected to proximate analysis and the results are presented in Table 2. Thus 5 iso-nitrogenous and isocaloric diets containing 35% protein were formulated (Wee and Ngamnsae, 1987; Jauncey and Ross 1982). Attempts were also made to formulate diets by progressively replacing protein concentrate by the proteins from sunnhemp meal, soybean meal (Table 3). Protein concentrates of diet 1 served as the principal source of protein. It was treated as control diet. Maize starch was

mainly used as carbohydrate source in all the experimental diets. Diets were subjected to proximate analysis and results are presented in Table 4.

Table 1. Composition of the 'vitamin premix used in the experimental diets

Ingredient	Amount per kg premix
Vitamin A	4850000 IU
Vitamin D_3	850000 IU
Vitamin E	8000 mg
Vitamin K_3	800 mg
Vitamin B_1	400 mg
Vitamin B_2	1600 mg
Vitamin B_6	200 mg
Vitamin B 12	4 mg
Nicotinic acid	10000 mg
Pantothenic acid	4800 mg
Folic acid	200 mg
Biotin	20 mg
Cobalt	160 mg
Copper	3200 mg
Iron	12800 mg
Iodine	320 mg
Manganese	25600 mg
Zinc	16000 mg
Selenium	64 mg
DL-Methionine	25000 mg
L-Lysine	15000 mg
Choline Chloride	11000 mg

Source: KIM Agrovert, Bangladesh Ltd.

Diet preparation: All the dietary ingredients were finely ground by mortar and sieved through 0.5 mm mesh and were mixed homogeneously with vitamin and mineral premix. Sufficient amount of water was added to make dough, which was passed through l mm diameter pelleting machine (Hobart Mixture Machine Model A200). The resultant pellets were sun dried for two days and stored in airtight plastic box until use.

Feeding rates: The fishes were fed the formulated diets twice daily; at 09:00 AM and at 16:00 PM at the rate of 5% of their body weight. Data about the feed fed were kept for subsequent calculation of food conversion ratio and protein efficiency ratio.

Water quality: Water quality parameters; temperature and pH were monitored by using pH meter (Portable digital pH meter, OSK 1148) and a graduated celsius thermometer, respectively.

Sampling procedure: Fish were netted, using a fine mesh scoop net and excess water was then removed from fish body by gently blotting on a soft tissue paper. Fish were weighed to the nearest 0.01 g on an electronic balance (Setra, 200S-USA) and their lengths were also recorded. It was done at 7 days interval up to 42 days and compared with initial data.

Table 2. Formulation of test diets

Ingredients	% Composition (g)				
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Protein concentrate	49.18	39.34	29.50	19.67	29.50
Soybean meal	11.00	11.00	11.00	11.0	11.00
Maize starch	32.60	21.77	12.19	2.60	12.19
Sunnhemp	-	20.83	40.33	59.83	40.33
Fish oil	2.36	2.28	2.24	2.20	2.24
Soybean oil	2.36	2.28	2.24	2.20	2.24
Vitamin mix	2.50	2.50	2.50	2.50	2.50
	100 g	100 g	100 g	100 g	100 g

* Diet 1= Control, Diet 2 = 20%, Diet 3 = 40%, Diet 4 = 60%, Diet 5 = 40% replacement of protein concentrate

Table 3. Proximate composition of the protein sources used (% dry matter basis)

Protein sources	Dry matter	Protein	Fat	Ash
Protein concentrate	93.00	64.51	10.75	22.58
Soybean meal	90.16	44.32	3.87	9.89
Sunnhemp seed meal	88.56	36.67	6.30	5.40
Sunnhemp seed meal (boiled)	87.24	35.78	5.96	5.26

Table 4. Proximate composition of the experimental diets

Parameters	Diet No.				
	1	2	3	4	5
Dry matter %	91.84	86.57	85.34	93.5	94.0
Crude protein %	33.85	34.8	34.72	34.72	34.28
Ash %	10.98	9.86	8.58	8.46	9.57
Oil %	10.44	10.52	10.62	10.70	10.32.

Growth parameters of the experimental fish: xperimental data collected during the growth trials and those obtained during subsequent analysis of diets, body weight, body length, survivability and fed food were used to determine the following nutritional parameters:

a) Body weight gain: It was calculated as: Body weight gain (g) = Mean final weight (g) - Mean initial weight (g).

b) % Weight gain: It was calculated as:

$$\% \text{ Weight gain} = \frac{\text{Mean final weight (g) - Mean initial weight (g)}}{\text{Mean initial weight (g)}} \times 100$$

c) % Survival rate: It was as calculated as:

$$\% \text{ Survival rate} = \frac{\text{Final number of fish}}{\text{Initial number of fish}} \times 100$$

d) Protein efficiency ratio (PER): Protein efficiency ratio is defined as the gain in weight per gram of crude protein fed and was calculated as:

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Weight gain (g)}}{\text{Protein intake (g)}} \times 100$$

e) Feed conversion ratio (FCR): It was calculated as:

$$\text{Food conversion ratio (FCR)} = \frac{\text{Food fed (g)}}{\text{Weight gain (g)}} \times 100$$

Results and Discussion

Nutrient composition of the sunnhemp seed meal (% dry matter basis): The proximate composition of sunnhemp seed meal was determined under two conditions: boiled and unboiled (Table 3). At both condition seed contained prestigious amount (36.67%) of protein which will be comparable to soybean meal (44.32%). Thus it was revealed that soybean protein can easily be substituted by sunnhemp protein in fish feed.

Table 5. Rearing and feeding conditions of the experimental Tilapia (GIFT) fry

Items	Condition
Experimental period	42 days
Tank size	91× 47 × 30cm^3
Water content	30 L/aquarium
Water temperature	17-18°C
pH of water	7.5-8.18
Water change	Once daily
Feeding frequency	Two times daily (9.00 A.M. & 4.00 P.M.)
Type of diet	Pellet
Size of diet	2 mm

Proximate composition of experimental diets: Proximate composition of each experimental diet is presented in table 4. There were only minor differences of protein contents among the various experimental diets (Table 3). Ash content varied from 8.46 to 10.98. Protein concentrate was used as the sole source of protein in diet 1 (Control).

Water quality parameters: The water quality parameters; water temperature and pH were monitored and are shown in table 5 along with other experimental conditioning. The temperature ranged from 17°C to 18°C and pH from 7.50 to 8.18.

Acceptability of diets: The acceptability of individual experimental diet was judged by observing the feeding responses. In all treatments, the fish became habituated to the experimental diets within first three days of feeding but the degree of acceptance of all diets was not similar. The fish were observed to intake actively diet 1 followed by 5, 3, 4 and 2. Detailed results are presented under the following headings:

A. **Survival rate of the experimental fishes:** During feeding trial a number of fish fries were died in each diet group. The lowest survival rate of the fish fry was observed in diet 2 (37.5%), which was highest in diet 5 (81.25%) (Table 6). Jackson *et al.* (1982) reported that at the oilseed meal diets containing 25% plant protein supported comparable good growth to fish. Inclusion of sunflower seed meal and lupin *(Lupinus albus)* seed meal protein up to 22% and 30% respectively in rainbow trout diet have also been reported to increase its life span without any adverse effect on growth (Higuera *et al.*, 1988).

B. Growth rate of the experimental fishes: The growth response in terms of increase in average length, percent length, body weight and percent body weight of fish was done (Table 6).

Table 6. Effect of protein replacement with Sunnhemp on the growth and survival of Tilapia (GIFT)

Parameters		Diet group*				
		No. 1	No. 2	No. 3	No. 4	No. 5
No. of fry**	Initial	16+16	16+16	16+16	16+16	16
	Final	7+10	6+6	12+11	9+13	13
Percent survival rate		53.12	37.5	71.87	68.75	81.25
Average length (cm)	Initial	3.750	3.700	3.900	3.700	3.810
	Final	4.035	3.800	4.420	3.960	4.130
Percent increase in length		7.6	2.7	13.3	7.0	8.4
Average body weight (g)	Initial	0.907	0.858	1.050	0.910	0.927
	Final	1.105	0.88	1.171	0.985	1.080
Weight gain (g)		0.197	0.030	0.121	0.075	0.153
Percent weight gain		21.76	3.40	11.52	8.24	16.50
PER		0.043	0.008	0.028	0.019	0.037
FCR		2.80	14.81	3.75	5.53	0.97

* No. l, control; No. 2, 20% replacement; No. 3, 40% replacement; No. 4, 60% replacement; No. 5, 40% replacement with heat treated (sunnhemp meal). **Experiment was done in duplicate, except diet group No. 5.

From table 6 average body weight gain was found to be the highest (0.197 g) in diet 1 (control) followed by diet 5 (0.153 g), diet 3 (0.121 g), diet 4 (0.075 g) and the lowest gain (0.030 g) was found with diet 2. Same trend was observed for percent weight gain. The highest (21.76%) was found in diet 1 (control) followed by diet 5 (16.50%), diet 3 (11.52%) and diet 4 (8.24%) whereas the lowest in diet 2 (3.40%). This finding is more or less similar to that of Mahata *et al.* (1994), who reported that 38% of fish meal protein in *P. gonionolus* diet can be replaced by silkworm pupae meal. However, Hossain *et al.* (1994) also reported that up to 50% of the fishmeal protein in P. *gonionotus* diet can be replaced by oilseed proteins (mustard oilcake and sesame meal) without affecting the growth performance.

Although the average initial length of the fishes under each diet group was not identical but a little increase in average length was found in all diet groups. The average percent increase in length was found to be the highest in diet 3 (13.33%) followed by diet 5 (8.39%), diet 1 (7.60%), diet 4 (7.02%) and the lowest was in diet 2 (2.70%). One of the reasons for better growth of Tilapia fry fed on diet 5 might be better nutritional balance. It has been suggested that the use of multiple protein

sources in combination are more effective than a single source in replacing fish meal in carp diet. It also can prevent a high inclusion level of any single anti-nutritional factor in the diet (Hossain and Jauncey, 1990). It is also reported that a mixture of plant and animal proteins is much more efficient than a single source of either types of protein (Meske and Pruss, 1977).

Table 7. Weekly feed consumption (g)

Dietary treatment	Week	1st	2nd	3rd	4th	5th	6th
	Tank 1	3.14	2.60	2.13	2.25	1.80	1.45
Diet 1	Tank 2	3.16	2.70	2.11	2.33	1.76	1.49
	Average	3.15	2.65	2.12	2.29	1.78	1.47
	Tank 1	2.30	1.78	1.70	1.47	1.33	1.17
Diet 2	Tank 2	2.22	1.82	1.84	1.43	1.37	1.13
	Average	2.26	1.80	1.77	1.45	1.35	1.15
	Tank 1	2.40	2.39	1.13	1.90	1.82	1.69
Diet 3	Tank 2	2.30	2.42	2.07	1.94	1.88	1.71
	Average	2.35	2.36	2.10	1.92	1.85	1.70
	Tank 1	2.50	2.36	1.75	1.66	1.56	1.47
Diet 4	Tank 2	2.30	2.34	1.83	1.67	1.61	1.43
	Average	2.40	2.35	1.79	1.65	1.58	1.45
	Tank 1	2.40	2.36	2.08	1.85	1.60	1.50
Diet 5	Tank 2	-	-	-	-	-	-
	Average	2.40	2.36	2.08	1.85	1.60	1.50

C. Food conversion ratio (FCR): Food conversion ratios (FCRs) for the various test diets are presented in table 6. The diet 2 exhibited the highest FCR (14.81) followed by diet 4 (5.53), diet 3 (3.75) and diet 1 (2.80) and diet 5 showed the lowest FCR (0.97). Chakraborty *et al.* (1973) reported that the mixture of silkworm pupae, ground nut, oilcake and wheat bran is better utilized by the fry of *Labeo rohita* and *Cirrhinus mrigala* than the mixture of mustard oilcake and rice bran.

D. Protein efficiency ratio (PER): The level of protein utilization for the different dietary protein sources fed to Tilapia fry was evaluated in terms of PER (Table 6). The highest PER value (0.043) was obtained for diet 1 followed by diet 5 (0.037), diet 3 (0.028), diet 4 (0.019) and the lowest value (0.008) was found for diet 2 (Table 6). PER is usually used for evaluating protein from different sources for animal growth, and among the different factors affecting PER, the protein quality is an important one (Steffens, 1989). In the present study the protein quality as well as PER values was observed to be different, which is the agreement of the above statement. The highest PER value for diet 5 in this study indicates its maximum utilization of protein and it might be due to the proper combination of different protein sources used in the diet.

E. Food consumption: The weekly food consumptions are shown in table 7. Fishes reared with diet 1 were found to be the highest total food consumption (13.47 g) followed by diet 3 (12.28 g), diet 5 (11.78 g), diet 4 (11.22 g) and diet 2 (9.78 g). Data revealed that fishes reared with raw sunnhemp meal did not ingest enough and showed no immediate toxic effect. On the other hand, boiled sunnhemp meal was found to be accepted easily, which indicating that boiling made the meal palatable and non-toxic or less toxic for the fish.

Although sunnhemp seed meal is not so attractive for fish but the growth parameters are not so significantly different from that of control. Considering the economical condition of our country it can be suggested that as a chief non-conventional protein sources, it can be utilized for the partial replacement of costly protein source of the formulated fish feed.

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