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Evaluation of Alfalfa Leaf-Protein-Concentrate as Feed for *Clarias lazera* (Pisces: Clariidae)

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Abstract

Alfalfa *(Medicago satival)* leaf-protein-concentrate (LPC) was chemically and biologically evaluated as a feed for *Clarias lazera*. Based on proximate composition values alone, it could be concluded that LPC diet used was suitable for a high protein demanding monogastric such as *C. lazera*. However, the growth performance of *C. lazera* during the feeding trial presented PLC interior to the control which is a fishmeal based diet. Toxic principles identified included saponin, alkaloids, tannins and phytate. A supplementation of the LPC diet with lysine and methionine improved the growth performance of the LPC by 419%. Organ index showed that while the liver and the gills were not affected by the LPC diets, kidney, heart and spleen were slightly enlarged. The experiment demonstrate that LPC is inferior to fishmeal based diets. However, LPC may be an alternative to fishmeal for herbivorous fishes or even *C.lazera* in the face of a dwindling purchasing power of the developing world.

Introduction

The problem of malnutrition is still very much with the third world. One of the ways of ameliorating this problem is by converting leaf proteins to animal proteins. It is generally known that fish is an efficient converter of plants to animal protein. Since, in most of the third world, fish farm production is still underexploited (FAO 1966) it will be beneficial to encourage massive fish farming. However, one of the forseeable problems is the inavailability of good quality fish feed (Afolabi *et al* 1984) Fishmeal, which is the traditional protein base for fish feeds, is still an import item and it is highly demanded by other animal feeds. In the face of a dwindling purchasing power, the high cost of fish feed could constitute a problem for the development of fish farming industry. This project was therefore designed to investigate the possibility of utilising LPC as a fish feed. Alfalfa LPC was used as the feed while *C. lazera* was the test animal.

Materials and Methods

Experimental diet

Alfalfa leaf protein concentrate (LPC) used was imported from Brazil under an agreement between our research team and a Brazilian research laboratory. Method of LPC preparation was as described by Pirie (1971). Two experiments were performed. The first experiment was a preliminary one which was designed to evaluate the performance of LPC

as a fishmeal replacer in fish feeds. In the second experiment the effect of tannin and amino acid supplementation on the growth performance of *Clarias lazera* was investigated. Table 1 shows the experimental diets used during the two feeding trials. Diet components were purchased and used as such. The diets were formulated in experiment 1 such that the fishmeal (FM) content of control diet (i.e. that containing 68% fishmeal; designated FM diet) was progressively replaced by LPC at 20%, 40% and 60% respectively. Diets containing 100% LPC and the energy diet were included. In experiment 2, LPC was supplemented by methionine and lysine. In addition, multiple levels of the quantity of tannin found in 100% LPC diet were added to FM diet at the following levels: 0.24, 0.48, and 0.96% of the total diet. Feed formulation was designed such that the protein level in the various diet types is about 50% which represent the amount of protein contained in 100% LPC. Diets were prepared by mixing the various diet components using "Ken-wood Chef" K-blade set at 40 rev/min and adding sodium alginate at 2% to serve as the binder.

Feeding trial:

Clarias lazera used in this study was obtained from a private commercial farm in Oyo State, Nigeria. Eight fish specimens were used per diet. Average weight of each fish was 11.69g. The fish specimens were acclimatized for two weeks in six glass aquaria, measuring 0.9m XO.32m XO33m each, while placed on a commercial fish feed. Before the commencement of the feeding trial, the fish specimens were starved for twenty four hours in order to sharpen the appetite of the fish. During the feeding trial, water temperature *ranged between* 28-29°C, and pH was maintained at 7.0 using 'Hart 3' pH test kit for aquaria. 50% of the water was changed every fourth night while taking the weights of the animals. The fish specimens were fed at 3% of their body weight (Igbinosun *et al* 983). At the end of the experiment, the fish specimens were weighed and then sacrificed, the organs removed and weighed. Physico-chemical conditions prevailing during the experiment is included in Table 6. This was obtained by adjusting all these parameters to equal levels.

Proximate Comporsition of Experimental Diet:

Moisture, crude protein, crude fibre, fat and ash contents were determined as described by AOAC (1980).

Phytochemical Screening:

Tests for alkaloids, saponins, tannins, phlobatannins, anthraquinones, were carried out as described by Odebiyi and Sofowora (1978). Tannin content was estimated by the modified vanillin-hydrochloric acid method of Price and Butler (1977), phytate content was determined by the anion exchange method of Harland and Oberleas (1986).

Results and Discussion

Proximate composition:

The proximate composition of the experimental diets is presented in Table 2. The moisture content of the feeds in both experiments ranged between 2.85-8.39%, increasing

TABLE 1: PERCENT COMPOSITION OF EXPERIMENTAL DIETS

Feed Component	Experiment 1[c]						Experiment 2[c]					
	FM Diet	20% LPC	40% LPC	60% LPC	LPC Diet	Energy Diet	FM Diet	LPC Diet	LPC Diet +AA	FM Diet +0.24% T	FM Diet +0.48% T	FM Diet +0.96% T
Palm Kernel meal	10.0	10.0	10.0	10.0	-	-	10.0	10.0	10.0	10.0	10.0	10.0
Wheat offal	18.0	15.0	13.5	10.0	-	-	18.0	4.5	3.5	17.76	17.52	17.04
Fishmeal	68.0	54.4	40.8	27.2	-	-	68.0	-	-	68.00	68.00	68.00
LPC	-	13.6	27.2	40.8	100.0	-	-	68.0	68.0	-	-	-
Vitamin/Mineral Supplement	0.5	0.5	0.5	0.5	-	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Corn oil	1.5	1.5	1.5	1.5	-	1.5	1.5	1.5	1.5	1.50	1.50	1.50
Casein	-	3.0	4.5	8.0	-	-	-	13.5	13.8	-	-	-
Sodium alginate	2.0	2.0	2.0	2.0	-	2.0	2.0	2.0	2.0	2.0	20.	2.0
Methionine	-	-	-	-	-	-	-	-	0.5	-	-	-
Lysine	-	-	-	-	-	-	-	-	0.2	-	-	-
Tannin	-	-	-	-	-	-	-	-	-	0.24	0.48	0.96
Corn Starch	-	-	-	-	-	96.0	-	-	-	-	-	-

[a] *Casein added in some cases to make diet virtually isonitrogenous*
[b] *Vitamin/mineral supplement is as previously described (Afolabi and Oke 1981)*
[c] *% Feed component in the columns refer to % replacement of fishmeal by LPC.*

TABLE 2: PROXIMATE COMPOSITION OF THE EXPERIMENTAL DIETS

	Experiment 1						Experiment 2					
	FM Diet	20% LPC	40% LPC	60% LPC	LPC Diet	Energy Diet	FM Diet	LPC Diet	LPC +AA	FM +0.24%T	FM 0.48%T	FM +0.96%T
Moisture	3.09	6.68	7.20	4.80	8.39	5.09	3.52	4.45	4.90	2.85	2.93	2.69
	±0.01	±0.13	±0.08	±0.70	±0.29	±0.05	±0.06	±0.01	±0.15	±0.03	±0.09	±0.06
Crude	52.32	50.57	49.96	49.74	49.04	0.74	52.63	49.74	50.00	52.15	51.75	51.97
Protein	±0.14	±0.14	±0.22	±0..22	±0.17	±0.09	±0.17	±0.09	±0.17	±0.14	±0.17	±0.25
Lipid	8.80	4.05	4.37	4.36	3.78	1.53	9.17	4.00	3.53	8.33	8.01	8.25
	±0.14	±0.07	—0.18	±0.20	±0.11	±0.04	±0.23	±0.00	±0.04	±0.46	±0.00	±0.36
Ash	18.48	18.81	17.33	14.15	19.96	2.94	18.94	11.55	11.83	19.63	14.63	19.91
	±0.19	±0.14	±0.15	±0.04	±0.02	±0.21	±0.37	±0.01	±0.03	±0.43	±0.70	±0.91

Values are means ± standard deviation of means.

TABLE 3: TOXICANTS IDENTIFIED IN THE EXPERIMENTAL DIETS

	Experiment 1						Experiment 2					
Toxicants	FM Diet	20% LPC	40% LPC	60% LPC	LPC Diet	Energy Diet	FM Diet	LPC Diet +AA	FM Diet	FM Diet 0.24%T	FM Diet +0.48%T	LPC Diet +0.96%T
Alkaloids	x	✔	✔	✔	✔	x	x	✔	x	x	x	✔
Saponins	x	✔	✔	✔	x	x	✔	x	x	x	✔	
Tannins	x	✔	✔	✔	✔	x	x	✔	✔	✔	✔	✔
Anthroquinones	x	x	x	x	x	x	x	x	x	x	x	x
Phlobatannins	x	x	x	x	x	x	x	x	x	x		
Tannin (mg/g)	0.50	0.81	1.32	1.66	2.44	0.0	0.41	2.47	2.33	4.20	9.94	2.19
	±0.00	±0.31	±0.14	±0.32	±0.41	±	±0.11	±0.58	±0.29	±0.58	±0.57	±0.00
Phytate (mg/g)	23.55	10.41	9.78	8.70	4.33	6.15	22.64	6.88	25.36	23.18	22.46	6.69
	±0.52	±0.38	±0.50	±1.03	±0.00	±0.51	±0.25	±0.52	±0.00	±0.42	±0.58	±0.25

✔ = *present*
x = *absent*

TABLE 4: % AVERAGE WEIGHT GAIN IN FISH FED ON EXPERIMENTAL DIETS

Weeks after Commencement of Experiment	FM Diet	20% LPC	40% LPC	60% LPC	LPC Diet	Energy Diet	FM Diet	LPC Diet	FM Diet +0.24%T	FM Diet +0.48%T	FM Diet +0.96%T	FM Diet
2						-6.08	32.88	-2.83	-0.15	19.79	37.92	34.64
3	62.66	16.12	22.72	5.08	-5.47	-37.94						
4						-	83.55	-2.85	1.01	43.94	70.09	75.79
5	96.57	40.32	36.96	23.74	-9.04	-84.42						
6	152.57	62.58	57.38	32.89	-15.62	-132.07	167.70	0.55	1.09	121.65	127.77	111.44
7	-	-	-	-	-	-						
8	-	-	-	-	-	-	315.72	1.39	13.19	227.30	198.66	177.30
9		-	-	-	-	-	-					
10	-	-	-	-	-							
11	-	-	-	-								
12	-	-	-	-	531.62	9.97	38.72	399.27	289.72	265.28		
13	-	-	-	-	-	-						
14	-	-	-	-	-	-	847.60	15.34	76.58	620.52	375.83	289.48
15	-	-	-	-	-	1233.64	20.08	104.22	855.33	470.33	470.33	384.39

$$\% \text{ Average Weight Gain} = \frac{\text{Average Weight at Week of Interest - Average Weight at week 0}}{\text{Average Weight at Week 0}} \times 100\%$$

TABLE 5: ORGAN WEIGHT: BODY WEIGHT RATIO OF FISH ON EXPERIMENTAL DIET

	Experiment 1								Experiment 2			
Organs Index	FM Diet	20% LPC	40% LPC	0% LPC	LPC Diet	Energy Diet	FM Diet	LPC Diet	LPC Diet +AA	FM Diet +0.24%T	FM Diet +0.48%T	FM Diet +0.96%T
Liver	1.15	1.57	1.24	1.53	1.09	1.02	1.07	1.13	0.89	0.96	0.77	0.91
Kidney	0.56	075	0.52	0.79	0.81	0.52	0.52	0.62	0.68	0.58	0.35	0.62
Gill	2.93	4.20	2.63	3.53	4.28	3.52	3.99	3.82	2.68	4.69	2.06	4.17
Heart	0.13	0.15	0.10	0.14	0.19	0.12	0.14	0.08	0.15	0.07	0.22	
Spleen	0.10	0.12	0.09	0.10	0.17	0.12	0.08	0.07	0.07	0.11	0.03	0.03

TABLE 6: PREVAILING PHYSICO-CHEMICAL PARAMETERS OF THE WATER IN THE TEST AQUATIA

Parameter	Value
Oxygen (mg/l)	7.40 - 8.80
pH	7.0
Temperature °C	28 - 29
Salinity%	0
Condunctivity (uMHos)	28 - 30

with increase in percentage LPC. The fat content is higher than the level of oil added in the feed formulation (Table 1), probably due to other oil sources in the diets. The fishmeal appears to be contributing most of the additional oil (r=-0.68). The crude fiber content is low (0.22 - 2.23%). This low fibre content makes the feed suitable for monogastric animals such as *C. lazera*. The values of the ask content are generally high, suggesting that the feeds are rich in mineral. The protein levels in all the feeds except the energy diet, are high and virtually isonitrogenous (Table 2). Based on the proximate composition, it could be concluded that the feeds are adequate for high protein demanding fish species such as *Clarias lazera*.

Taxicants: The results of the phytochemical screening, tannin and phytate contents are presented in Table 3. In the FM diet and energy diets, the toxicants were not detectable. All the toxicants tested for, except phlobatannins and anthroquinones were present in the LPC containing diets. The values of the tannin content was as low as 0.41% in FM diet and as high as 2.47 in LPC diet. The values of tannin in LPC diet is in agreement with the result of Hanson (1972) who obtained a value between 2 and 3% of tannin in alfalfa; for the FM supplemented with various levels of tannin, values obtained confirm the accuracy of technique used for the quantitation of tannin Table 3. The phytate content was highest in the diet FM lowest in the LPC diet. The phytate content decreased with increasing LPC content (r= 0.85). The higher phytate content in the fishmeal is probably due to phytate contributed by other diet components, most especially wheat offal. This explanation agrees with the observation that the phytate content increased with increased wheat offal content (r- 0.79) (Table 3) wheeler and Ferrel (1971), Harland and Oberleas (1986), observed a generally low level of phytate in leaves.

Growth pattern: The growth pattern of the fish placed on experimental diet is presented in Table 4. In the first experiment, the FM diet had the greatest average weight gain, at the end of the sixth week. The weight gain decreased progressively with increasing LPC content (r=-93). Actually a depressed growth was observed in LPC diet with lysine and methionine increased the weight gain by 41% compared to the unsupplemented LPC diet. Tannic acid also caused depressed growth in the diets in which they were added. (r=-0.86). This clearly demonstrates that lysine and methionine will enhance growth performance of LPC on one hand, and on the other, tannins constitute one of the growth depressing factors in alfalfa LPC. (Bolton 1962, Hanson 1972, Ribereau-Crayon 1972). Other principles that might affect growth performance include phytate, saponins, alkaloids etc. Although, a quantitative experiment to investigate the effect of saponins and alkaloids in this study was not done, other workers (Fieser 1937, Cheeke 1983) have shown that saponins have growth depressing effects on non-ruminants.

Organ Weight Index:

In order to investigate probable organs affected by the LPC diets, the organ weight: body weight ratio was determined; and these values are presented in Table 5. In the first experiment, liver and gill did not show any directional changes; i.e. they were probably not affected by the feeds. There appeared to be some enlargement in the kidney, the heart and the spleen (r=0.64, r=.63r= 0.78 respectively) when the LPC containing diets are compared to the FM diet. In the second experiment, there was fluctuation in the weights of the organs. Therefore, the result obtained in the first experiment is probably not due to the tannin alone.

In conclusion, it is clear from the experiment carried out that LPC is inferior to FM as a fish feed. This is due large largely to the quantity of the LPC protein and the presence

Results

The estimated production functions by methods of ordinary least squares for the different groups of farmers are presented below with t-ratios in parentheses. The coefficients of all the estimated.

1. ***Cooperative Farmers***

$$\begin{aligned} LnQ = {} & 3.6989 + 0.5524\,LnX_1 + 0.5755\,LnX_2 + 0.0644\,LnX_3 \quad (2) \\ & \quad\ (2.437)^{**} \quad\ (2.754)^{***} \quad\ (0.537) \\ & + 0.1707\,LnX_4 \\ & \quad\ (2.254)^{**} \end{aligned}$$

$R^2 = 0.2108$, $F = 8.948^{***}$, $n=139$

(2) Non cooperative farmers

$$\begin{aligned} LnQ = {} & 3.5090 + 0.3246\,LnX_1 + 0.416\,LnX_2 + 0.2787\,LnX3 + 0.2263LnX_4 \\ & \quad\ (1.918)^{*} \quad\ (2.449)^{**} \quad\ (3.361)^{***} \quad\ (4.531)^{***} \quad (3) \end{aligned}$$

$R^2 = 0.4692$, $F = 32.487^{***}$ $n = 151$

(3) *Pooled Data*

$$\begin{aligned} LnQ = {} & 4.769 + 0.5289\,LnX_1 + 0.2878\,LnX_2 \\ & \quad\ (3.482)^{***} \quad\ (2.158)^{**} \\ & + 0.012_4\,LnX3 + 0.3016\,LnX_4 + 0.1756\,Dc \\ & \quad\ (0.811) \quad\ (7.647)^{***} \quad\ (1.326) \quad (4) \end{aligned}$$

$R^2 = 0.2679$, $F = 20.641^{***}$ $n = 290$

Parameters are positive in the three production functions indicating direct relationship with output in each case.

For cooperative farmers, the coefficients of labour input is significant at 1 percent while those of farm size and other farm inputs are significant at 5 percent. However, the coefficient of capital input is non-significant. The R^2 value is low at 0.2108 but is significant at 1 percent.

For non-cooperative farmers, the coefficients of all the estimated parameters are significantly different from zero. Those of capital input and other farm inputs are significant at 1 percent while those of labour and farm size are significant at 5 percent and 10 percent respectively. The R^2 value is 0.4692 and is highly significant at 1 percent.

For the pooled data, the coefficients of farm size and other farm inputs are significantly different from zero at 1 percent while that of labour is significant at 5 percent. Conversely, the coefficients of capital input and the dummy variable representing farmer type are both non-significant. The R^2 value is also low at 0.2679 but is significant at 1 percent.

(a) Test of Technical Efficiency of Cooperative and Non Cooperative Farmers

The technical efficiency of the two groups of farmers was evaluated by estimating a production function eq. (4) using a dummy variable to distinguish farmer type and testing for

* Significant at 10 percent
** Significant at 5 percent
*** Significant at 1 percent