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Research

Dietary effects of Sun-hemp (*Crotalaria juncea* Linn.) in the diet of African catfish, *Clarias gariepinus* juveniles

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Abstract

With an increasing interest in new feedstuff sources, the seeds of wild plants including the tribal pulses are receiving more attention, because of their high resistance to diseases and pests and in addition, they exhibit good nutritional qualities. The seed of Crotalaria juncea was used to replace soybean meal at 5%, 10%, 15%, 20%, 25%, 30% inclusion levels respectively and the control diet was set up without the test ingredient. C. gariepinus juveniles used for the experiment were fed to satiation thrice daily and their water was changed every other day. The weekly feed supplies and weight gains were recorded and used to compute the growth, nutrient utilization and the economic parameters. There were significant differences ($P<0.05$) in all the growth and nutrient utilization parameters examined. The highest average weight gain (AWG) was recorded in the control treatment (107.69±2.41g) while the least value (66.09±0.48g) was recorded in fish fed with D6 (30%). Similar patterns of results were observed in the other growth parameters as well as nutrient utilization parameters. The best values for Pin, ICA and ECR were recorded in diet 2 after the CTR compared with other dietary treatments. The packed cell volume, white blood cell and haemoglobin were significantly ($P<0.05$) higher in the control than other tests. Similarly, the control recorded significant ($P<0.05$) values in the proximate composition of carcass . Sun-hemp seeds do not have any adverse effect on haematological parameters, and carcass proximate composition of the fish. Furthermore, the cost analysis showed that more profit awaits the farmer if diet 2 (10% sun-hemp seed and 20% soy bean inclusion level) is adopted in addition to the medicinal values of the test ingredient.

Introduction

The optimum aim of every livestock investor is to make profit at the end of the culture season and this is also applicable to aquaculture. However, the cost of feed production in aquaculture has become a burden limiting the profit potential. Feed is the single largest operating cost item in intensive fish culture and in the desire to obliterate this effect, a variety of feed ingredients of both plant and animal origins have been used in the preparation of commercial artificial diet in intensive aquaculture (Muthukumarappan *et al.* 2009).

Hence, research geared towards utilization of locally available feedstuffs from plant source becomes crucial for sustainable aquaculture.

Plant seeds are important sources of proteins and oils because of their nutritional, industrial and pharmaceutical applications. However, among plant species, grain legumes are considered as the major source of dietary proteins because of their rich protein composition, energy, mineral content and wide distribution in the tropics (Ogunji *et al*. 2005). With an increasing interest in new feed sources, the seeds of wild plants including the tribal pulses are receiving more attention, because they are highly resistant to diseases and pests and exhibit good nutritional qualities. The legumes from wild sources have tremendous potential for commercial exploitation and offer good scope to meet the ever increasing demand for vegetable protein (Janardhanan *et al*., 2003). Sun-hemp (*Crotalaria juncea*) an example of tribal pulses, widely distributed in the tropics and subtropics is appreciated for its food, fibre and medicinal values.

Sun-hemp seed is preferred to other legumes because of its rich amino acid, starch, fibre, phosphorus, ether extract and calories. Its lysine content is better than many other legumes, including soybean. (Dhan *et al*. 1987). However, anti-nutritional substances including Trypsin inhibitor and alkaloid, Retusamine-N-oxide (Chaudhury *et al.* 2010) that are present in the seed could limit its application.

Considering factors such as quality, palatability and functional properties of feed ingredients on fish status; necessitate the investigation of effects of replacing soybean meal with sun-hemp seed meal on the growth performance, nutrient utilization, carcass composition, haematological parameters and economic performance in *Clarias gariepinus*.

Materials and Methods

Experimental Location: The experiment was conducted at the Nutrition Unit of the Department of Marine Sciences, Faculty of Science, University of Lagos, Akoka, Nigeria for a period of 10 weeks.

Experimental Fish: The *Clarias gariepinus* juveniles were purchased from Agbede farm at Egbeda, Lagos State. The fish were acclimatized for two weeks in twenty one plastic tanks (52.5 X 33.5 X 21.0 cm). During the period of acclimatization, they were fed with Coppens commercial feed of 2mm size.

Fish weight was recorded after acclimatization with a digital scale (Camry EH 5055), sorted and stocked in experimental tanks at 10 fish per tank with average weight of 12.00 ± 0.00g per fish. The tanks were covered with nylon net of mesh size 2mm to prevent the fish from jumping out. The whole experiment was run in triplicate.

Experimental Feed Preparation and Formulation: Sun-hemp seeds were collected from Weed Department of International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria, while other feed ingredients; indomie nodule waste, fishmeal, groundnut cake, soy bean meal, palm oil, fish premix, salt, dicalcium phosphate were obtained from Soleace feed mill industry, Oko-Oba market, Agege, Lagos State, Nigeria.

The proximate composition of sun-hemp seeds (Table 1) was determined at the Department of Animal Science, Faculty of Agriculture, University of Ibadan, Oyo State, Nigeria. The sun-hemp seed (*Crotalaria juncea*) along with other feed ingredients were separately grinded at a feed mill.

The sun-hemp seeds were sieved to reduce the chaff so as to allow for smooth and even mixing with other feed ingredients. The chaff and the fine parts of the seed were measured and recorded at ratio 5:1.

Seven experimental diets were formulated; the control diet (CTR) was without the test ingredient, while the test ingredient was used to replace soybean meal in the other six diets (D1, D2, D3, D4, D5 and D6) at 5%, 10%, 15%, 20%, 25% and 30% inclusion levels respectively (Table 2). The feed ingredients were thoroughly mixed with hot water at a temperature of 100^{o}C into a homogenized paste. The paste was pelletized (2mm) with a locally fabricated hand pelletizing machine.

Experimental Procedures: The juvenile fish were fed to satiation thrice daily (9.00am, 12.00pm and 6.00pm) with the formulated experimental diets. Good water quality was maintained by changing the water every other day, and the fish were bulk-weighed at the end of every week to determine the mean weight gain of fish.

Laboratory Procedures:

Haematological Analysis Blood sample of fish taken at random from each tank were collected in both syringe and heparinized bottles for haematological assay and taken to Bioassay Diagnostic Laboratory Cele-Egbe, Ikotun-Lagos. The blood samples were analysed for haemoglobin (Hb), white blood cells (WBC), packed cell volume (PCV), mean corpuscular haemoglobin concentration (MCHC), neutrophils and lymphocytes according to methods in Brown (1980).

Proximate Analysis of Carcass: The proximate composition analysis of carcass included the determination of dry matter, ash, crude protein and ether extract. The proximate analysis was done by standard methods (AOAC 2000) at the Department of Animal Science, Faculty of Agriculture, University of Ibadan.

Growth, Nutrient Utilization and Economic Parameters: The weekly weight gains and feed supplies previously recorded were used to compute the growth and nutrient utilization parameters following the methods of Burel *et al.* (2000) while the economic evaluation of the diets were calculated from the methods of New (1989) and Mazid *et al.* (1997).

i) AVERAGE WEIGHT GAIN (AWG)

Average Weight Gained (g) = Average Final Weight (AFW)(g) - Average Initial Weight (AIW)(g)

ii) SPECIFIC GROWTH RATE (SGR)

$$\text{Specific growth rate} = \frac{\ln W_2 - \ln W_1}{T_2 - T_1 \text{ (day)}} \times 100$$

T_2-T_1 = Experimental period

W_1 = Initial weight gained

W_2 = Final weight gained

In=Natural logarithms

iii) PROTEIN INTAKE (PI)

$$\frac{\text{Total Feed Intake}}{\text{Protein Content of Feed}}$$

iv) FEED CONVERSION RATIO (FCR)

$$\text{Feed Conversion Ratio} = \frac{\text{Weight of Feed Intake (g)}}{\text{Weight Gained (g)}}$$

v) AVERAGE FEED INTAKE (AFI)

$$\text{Average Feed Intake} = \frac{\text{Feed Intake During Experimental Period}}{\text{Number of Days}}$$

vi) INCIDENCE OF COST ANALYSIS (ICA)

$$\text{ICA} = \frac{\text{Cost of Feed}}{\text{Mean Weight Gained}}$$

vii) PROFIT INDEX (PIn)

$$\text{PIn} = \frac{\text{Cost of Fish (CFF)}}{\text{Cost of Feed (CFD)}}$$

viii) Economic Conversion Ratio (ECR)

ECR = Cost of feed × Feed Conversion Ratio

Statistical Analysis: All data collected were subjected to analysis of variance (ANOVA). Comparisons among diets means were carried out using Duncan Multiple Range test (Duncan 1955) at significant level of 0.05. All computations were performed using Statistical package SPSS15.0 (SPSS Inc., Chicago, IL, USA).

Results

The average weight gain (AWG) showed significant ($P<0.05$) reduction with increased inclusion of the test ingredient (Table 3). The fish fed control diet recorded the highest AWG (95.69±2.41), while the least value was recorded by fish on diet 6 (54.09±0.48), though no significant difference ($P>0.05$) was observed between fish fed diet 5 and 6. Similar result pattern was observed for the specific growth rate (SGR) of the fish, with the control group recording the highest SGR (3.48±0.35 %day^{-1}) followed by diet 1 group (3.33±0.01%day^{-1}), while the least was recorded by diet 6 group (2.71±0.01%day^{-1}). The best feed conversion ratio (FCR) (0.89±0.01) was recorded for the control diet, while diets 1 to 5 recorded significantly related values; however, the poorest value was recorded with diet 6. Similarly, the same pattern was recorded in the result of the protein intake (PI).

Cost analysis of *C. gariepinus* fed diets containing varying replacement levels of soybeans with sun-hemp seed was shown in Table 3. There was no significant difference (P>0.05) in profit index across diets with the exceptions of control diet and Diet 2 however, the highest profit index was recorded in dietary treatment D 2 (3.13±0.06), followed by control diet (3.10±0.45). The result of ICA for the CTR and diet 2 differed significantly (P <0.05) from other diets while diet 2 recorded the least value for ICA after the control diet. Significant difference was recorded in the ECR across diets though, D 2 had the highest value (180.06±5.4) after the control while diet 6 had the least value (122.34±9.96).

The Packed cell volume (PCV), the haemoglobin (Hb), the white blood cell (WBC) and the mean corpuscular haemoglobin concentration (MCHC) of CTR differed significantly (P<0.05) compared with other treatments however, with increased inclusion of the test ingredient the PVC and Hb values were elevated from diets 1 to 6. There was reduction in the value of WBC from diets 2 to 6 with increased inclusion of the test ingredient. The neutrophil and lymphocyte also showed significant differences (P<0.05) across the treatments (Table 4).

There were significant differences (P<0.05) in the recorded carcass proximate composition of fish carcass fed varying levels of test diets (Table 4). The highest values of dry mater (27.77±0.12), crude lipid (0.08±0.07), and ash (7.00±0.00) were recorded in the control diet and were significantly different (P<0.05) from other test diets. Conversely, the highest crude protein (69.98±3.24) and ether extract (23.91±0.09) were recorded in the fish fed with 30% inclusion level of sun-hemp seed (D6).

Discussion

There was decrease in average final weight gained (AFW), average weight gained (AWG), and specific growth rate (SGR), as the inclusion level of Sun-hemp seed increased across diets. This may be due to high fibre content in the test diets which reduced the palatability of the diets consequently reduced the feed intake (Bureau *et al.* 1999, Refstie *et al.* 1998, Arndt *et al.* 1999). Similarly, Keembiychetty & De Silva (1993) reported decrease in weight gain at high fibre level inclusion when they fed cowpea and blank grain to Nile tilapia. According to Aderolu & Oyedokun (2009), high fibre level accumulates into increased cell wall materials and non polysaccharide which invariably limit the rate of digestion and nutrient absorption.

Considering nutrient utilization, protein in fish feed is expected to translate into fish flesh, but in the present study it was evident that protein was poorly utilized in the test diets from the values reported in protein intake. This may be related to unavailability or poor protein digestibility. It has been reported that protein digestion and amino acids assimilation could set a limit on growth rate in juvenile fish (Blier *et* *al.* 1997). Accordingly, trypsin activity, a key enzyme of protein digestion, is correlated to growth rate and survival in various fish species (Sharma & Chakrabarti 1999, Lemieux *et al.* 1999). The presence of alkaloid and trypsin inhibitor anti-nutritional factors in sun-hemp seed was reported by Chaudhury *et al.* (2010). The deleterious effects of anti-nutritional factors include reduced feed intake and depressed growth (Alarcon *et al.* 1999; Arndt *et al.* 1999). Hence, growth performances are thus clearly limited by the efficiency of digestive system to provide amino acids for protein synthesis and energy distribution (Savoie *et al.*, 2011).

The profit index (PIn), of the fish fed with 10% sun-hemp seed and 20% soy bean inclusion level, D2 was recorded to be the highest and it was not significantly different (P>0.05) from control diet. This could be as a result of; lower price of sun-hemp seed compared to soy bean. This agreed with the work of Dhan *et al.* (1987) that reported the promising of sun-hemp seed (*Crotalaria juncea*) as a source of low cost protein for possible use as feed to meet the gap of protein deficiency.

Haematological characteristics help fish biologists to interpret physiological responses by fish and deviation from normal response may indicate a disturbance in the physiological process (Rainza-paiva *et al.* 2000). The crashed in PCV and Hb values in the test diets especially in diets 1 and 2 compared to control diet in the present study may be connected to the presence of anti-nutritional factors in the diets. Tacon (1993) reported that nutritionally deficient diets decrease haemoglobin concentration; reduce haematocrit (PCV) and red blood cell volume. Dick *et al.* (1976) found that nutritional toxicity is associated with anaemia in fish. Nworgu *et al.* (2008) in their work reported the haemolytic activity of alkaloid when they tested *Landolphia owariensis* leaf invitro extract on the liver function profile and haemoglobin concentration of albino rats. Osuigwe *et al.* (2007) corroborated these observations by attributing the low values of WBC, PCV and Hb concentrations of *C. gariepinus* to the presence of anti-nutritional factors inherent in plant ingredient when they fed raw Jackbean seed to *C. gariepinus*. The drop observed in the WBC of the test diets compared with the control, may be related to the medicinal properties of test ingredient. It purifies blood and its seed is used in psoriasis (Chaudhury *et al.* 2010).

The inclusion of the test ingredient in the fish diet did not have any out of ordinary effect on the fish carcass composition. The range of the various proximate parameters tested for did not show any negative side effect of sun-hemp inclusion in catfish diet.

In conclusion, control treatment has the highest performance in growth and nutrient utilization; however, the cost analysis showed that more monetary profit awaits the farmer if diet with 10% sun-hemp seed and 20% soy bean inclusion level, D2 is adopted in addition to the medicinal values of the test ingredient.

TABLE 1: Result of proximate analysis of sun-hemp seed.

Composition	%
Crude protein	39.99
Ether extract	7.50
Ash	5.00
Crude fibre	15.00
Dry matter	87.97
Moisture content	12.03

TABLE 2: Experimental feed formulation.

Ingredient (Kg)	Control	D 1	D2	D3	D4	D5	D6
Indomie	20	20	20	20	20	20	20
Fish meal	22	22	23	23	24	24	25
GNC	25	25	24	24	23	23	22
Soy beans	30	25	20	15	10	5	-
Sunnhemp seed	-	5	10	15	20	25	30
Palm oil	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Premix	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Dicalcium Phosphate	1.00	1.00	1.00	1.00	1.00	1.00	1.00
% Crude protein	43.40	42.84	42.88	42.68	42.72	42.52	42.56
% Crude fibre	4.21	4.40	4.76	5.16	5.52	5.92	6.28
% Crude lipid	7.97	8.17	8.37	8.57	8.77	8.97	9.17

TABLE 3: Growth performance, nutrient utilization and cost analysis of *C. gariepinus* juveniles fed different experimental diets.

Parameter	Control Diet	D1	D2	D3	D4	D5	D6
AIW	12.00±0.00	12.00±0.00	12.00±0.00	11.93±0.00	12.00±0.00	12.13±0.00	12.00±0.00
AFW	107.69± 2.41^{a}	98. 04±0.80^{b}	82.97±0.71^{c}	77.85±1.03^{d}	72.08±0.80^{e}	67.27±0.90^{f}	66.09±0.48^{f}
AWG	95.69±2.41^{a}	86.04±0.08^{b}	70.97±0.71^{c}	65.92±1.03^{d}	60.08±1.38^{e}	55.13±1.53^{f}	54.09±0.48^{f}
SGR	3.48±0.35^{a}	3.33±0.01^{b}	3.07±0.01^{c}	2.98±0.23^{d}	2.84±0.02^{e}	2.72±0.02^{f}	2.71±0.01^{f}
FCR	0.89±0.01^{d}	1.09±0.02^{b}	1.02±0.02^{c}	1.06±0.02^{bc}	1.03±0.02^{c}	1.07±0.02b^{c}	1.24±0.02^{a}
PI	46.13±0.69^{a}	34.08±0.55^{b}	29.69±0.03^{c}	26.69±0.03^{d}	25.17±0.24^{e}	22.18±0.08^{f}	18.84±0.40^{g}
CFD	175.37±0.00	170.87±0.00	167.62±0.00	165.87±0.00	164.45±0.00	162.92±0.00	160.07±0.00
CFF	15.65± 2.28^{a}	13. 08±0.86^{b}	11.58±0.58^{bc}	11.95±2.19^{bc}	10.94±0.30^{cd}	9.65±0.71^{cd}	8.99±1.16^{d}
PIn	3.10±0.45^{a}	2.81±0.48^{ab}	3.13±0.06^{a}	2.53±0.28^{ab}	2.70±0.17^{ab}	2.73±0.16^{ab}	2.57±0.13^{ab}
ICA	0.15±0.02^{c}	0.18±0.04^{ab}	0.16±0.00^{c}	0.20±0.03^{a}	0.19±0.01^{ab}	0.19±0.02^{ab}	0.21±0.02^{a}
AFI	89.27±13.01^{a}	76.53±5.02^{ab}	69.10±3.49^{bc}d	72.07±13.23^{bc}	66.53±1.81^{cd}	59.23±4.36^{cd}	56.17±7.22^{d}
ECR	203.34±35.8^{a}	168±34.47^{abc}	180.06±5.4^{ab}	138.01±17.2^{cd}	144.58±10.2^{bcd}	139.26±12.3^{c}	122.34±9.96^{d}

All values on the same row with different superscripts are significantly different ($P<0.05$)

TABLE 4: Haematological indices and Carcass proximate composition *of C. gariepinus* fed experimental diets.

Parameter	Control	D1	D2	D3	D4	D5	D6
PCV (%)	42.50±2.50^{a}	26.00±3.00^{c}	28.50±2.50^{bc}	30.50±5.50^{abc}	35.50±1.50^{abc}	37.50±2.50^{abc}	38.50±4.50^{ab}
WBC (x10^{9}cells)	51550.00± 2650^{a}	24100.00±550^{b}	27100.00±700^{b}	25000.00±1600^{b}	24450.00±4050^{b}	23150.00±4250^{b}	21500.00±2100^{b}
Hb (g/dl)	13.75±0.75^{a}	8.65±0.85^{b}	9.35±0.65^{b}	10.00±1.70^{ab}	11.50±0.50^{ab}	12.00±1.00^{ab}	12.50±1.50^{ab}
MCHC (%)	32.32±0.16^{b}	33.14±0.66^{a}	32.49±0.12^{ab}	33.06±0.69^{ab}	32.40±0.05^{ab}	32.97±14.43^{ab}	32.40±0.09^{ab}
Neutrophil (%)	47.00±1.00^{ab}	53.50±3.50^{ab}	47.50±1.50^{ab}	55.00±1.00^{a}	44.00±2.00^{b}	48.00±6.00^{ab}	49.00±1.27^{ab}
Lymphocyte (%)	53.00±1.00^{ab}	40.50±2.50^{e}	52.00±2.00^{abc}	45.00±1.00^{de}	54.50±1.50^{a}	47.00±1.00^{cd}	49.00±1.00^{bcd}
Dry Matter (%)	27.77±0.12^{a}	27.17±1.10^{ab}	26.54±0.39^{abc}	25.83±0.28^{bcd}	25.19±1.41^{cd}	24.50±1.63^{cd}	25.01±0.99^{d}
Crude protein (%)	68.33± 5.66^{a}	67.01±3.24^{ab}	66.53±0.52^{ab}	66.33±1.21^{ab}	66.97±0.61^{ab}	66.04±1.49^{ab}	63.98±0.70^{b}
Ether Extract (%)	22.98±0.15^{bc}	23.99±0.23^{a}	23.00±0.09^{bc}	23.31±0.39^{b}	22.89±0.20^{c}	22.83±0.29^{c}	21.91±0.09^{d}
Crude Lipid (%)	0.08±0.01^{a}	0.07±0.01^{b}	0.07±0.00^{b}	0.06+0.01^{c}	0.05±0.00^{d}	0.06±0.00^{c}	0.03±0.00^{f}
Ash (%)	7.00±0.00^{a}	6.67±0.58^{ab}	6.67±0.29^{ab}	6.71±0.58^{ab}	6.73±0.46^{ab}	6.13±0.12^{b}	6.67±0.29^{ab}

All values on the same row with different superscripts are significantly different ($P<0.05$)

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