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Research Article

Growth Response of *Heterobranchus Longifilis* Fingerlings Fed Diets Supplemented with *Moringa Oleifera* Leaf Meal as Replacement of Soybean Meal

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Abstract

Moringa leaf meal (MLM) is a source of protein from a highly valued plant *Moringa oleifera.* Its protein has a profile of amino acids similar to that of soybean. A completely randomized design with three replicates was used in a 42 days study to determine digestibility and growth performance of 150 *Heterobranchus longifilis* fingerlings. Graded levels (0, 5, 10, 15 or 20) % of dried MLM was used to replace soybean meal in a 42.5% crude protein *H. longifilis* diet fed at 5% in the first 2 weeks and subsequently 3% body weight. The results showed that there were significant differences in weight gain (WG) and specific growth rate (SGR) of the fish with the significant highest observed in the group that fed on diet 2 with 5% MLM supplementation ($P<0.05$) though not significantly different from the control ($P>0.05$). The significant poorest WG and SGR were obtained in the group that fed on diet with 20% level of MLM supplementation ($P<0.05$). There was no significant difference between treatments in terms of initial weight, feed intake and survival ($P>0.05$). However there were significant differences in feed conversion ratio (FCR), SGR, total length and standard length among the groups that fed on diets with 15% and 20% supplementations ($P<0.05$). The haematocrit and hemoglobin were also significantly reduced in the group that fed on the highest level of supplementation ($P<0.05$). The in-vitro trial revealed significant reduction in digestibility with increase in MLM supplementation level ($P<0.05$) the tannin and phytic-acid levels following the same trend. However there was no pathological defect observed in the fish from all the groups. There was no significant difference in the hepathosomatic index. The feed cost analysis showed a reduction in cost per 100kg feed with increased level of supplementation with 0.00, 11.50, 23.20, 34.00 and 50.64% for diets 1, 2, 3, 4 and 5 respectively. It was concluded that 5 – 10% MLM supplementation could be used in the diets of *H. longifilis* to obtain a reduction in cost of feeding it at 11.2% and 23.5% per 100Kg feed respectively. There is need to try other processing methods if the level could increase above that obtained from this study.

Keywords: *Heterobranchus longifilis;* Moringa leaf meal; Soybean meal; Digestibility and performance

Introduction

Aquaculture is the fastest growing sector of the world's animal production with an annual increase of about 10% [1]. To sustain such high rates of increase in production, a matching increase in fish feed production is imperative. Because fish meal is a limited primary source and plants are widely available and reasonably priced, the use of plant protein sources in aqua feeds should be considered [2]. Therefore, in order to attain more economically sustainable, environmentally friendly and viable production, research interest has been directed towards the evaluation and use of non-conventional sources of plant and animal protein. Soybean meal is one of the most nutritious of all plant protein sources because of its high protein content, high digestibility, and relatively well-balanced ami no acid profile [3]. Owing to its reasonable price and steady supply, soybean meal is widely used as a cost-effective feed ingredient for most aquaculture species [4]. It is currently the most commonly used plant protein source in fish feeds [5]. However, soybean meal use in animal feeds competes with human food use, and hence there is a need to identify other protein-rich plant resources that could be used in fish diets. Another potential alternative plant protein source for fish feeds is moringa (*Moringa oleifera*). This plant is receiving much attention because its leaves, flowers and seeds can all be used as food [6]. Moringa leaf contains crude protein (CP) with about 260g/kg of leaf, of which about 87% is true protein [7]. Essential amino acids found in moringa leaf are methionine, cyste-

ine, typtophan and lysine [6]. Analyses of the leaf composition have revealed them to have significant quantities of vitamins A, B and C, calcium, iron and protein. According to Optima of Africa, Ltd., a group that has been working with the tree in Tanzania, "25 grams daily of Moringa Leaf Powder (MLP) will give a child" the following recommended daily allowances: Protein 42%, Calcium 125%, Magnesium 61%, Potassium 41%, Iron 71%, Vitamin A 272%, and Vitamin C 22%. A comparison between the amino acid composition of raw moringa leaf and that of soybean revealed an almost identical pattern of the essential amino acids [8]. There is an abundant total amount of these essential amino acids plenty in the leaf that can be used as animal feed [9]. The objective of this present experiment was to investigate the effect of different levels of dietary moringa leaf meal as a partial replacement of full fat soybean meal in the diet of *Heterobranchus longifilis*, (giant African catfish) fingerlings on the growth performance, nutrient digestibility, hematology and liver histopathology.

Materials and Methods

Preparation of *Moringa olifera* leaf meal (MLM)

The M. *oleifera* leaves used in the diets was harvested whole in NIFFR Estate, New Bussa, Niger State and air dried under the shade. The whole leaves harvested were allowed to wilt under the shade overnight after which mere shaking it help the leaflets to drop out of the stalk before final drying. This was to avoid the stalk of the leaves mixing up with the leaflets needed and reduce fibre contribution from the MLM into the diets. The leaflets were milled to powder after they have properly dried which was used for the preparation of the experimental diets (Plate 1).

Plate 1: Moringa oleifera Leaf.

Experimentation

Three Hundred *Heterobranchus longifilis* fingerlings were acquired and acclimatized for three weeks before selection and subjection to the experiment. Completely randomized design with three replicate groups was used to study the response of 150 *Heterobranchus longifilis* fingerlings to diets supplemented with graded levels (0, 5, 10, 15 or 20)% of *Moringa* leaf meal (MLM) to replace soybean meal in a soybean and fish meal based diet together with other ingredients (Table 1). The 42 days study was carried out in an aerated aquaria using indoor at the fish nutrition laboratory in NIFFR feed mill complex. Diet 1 with a zero supplementation served as control. Chemical analysis such as phytochemicals in leaf considering tannin and phytic acid determination, proximate composition (AOAC, 2000), hematology, histopathology of feed and fish samples were carried out. Digestibility was also assessed. Fish were fed at 5% body weight at commencement of the study in the first 2 weeks but was reduced to 3% body weight when much leftover feeds were observed which could be due to *Heterobranchus* feed consumption nature which is poor. The daily rations were divided into two installments, supplied morning (8.00 - 9.00 am) and evening (6.00 – 7.00 pm). Siphoning of the bottom of the aquaria was carried out each morning and addition of freshwater to maintain water level before feeding the fish. Fortnight sampling was adopted for feed adjustments, fish observation and total cleaning of the aquaria. Water quality was monitored in the course of the study using easy test kit. Feed cost was calculated based on the prevailing market prices of the ingredients at the period of experimentation. Statistical analysis was carried out on the data obtained at the end of the study.

Table 1: Composition of 42.5% Crude Protein Experimental diet.

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Moringa leaf meal	0	5	10	15	20
Full fat soybean meal	20	15	10	5	0
Fishmeal	25	25	25	25	25
Groundnut cake	35.87	37.53	38.9	40.26	41.62
Maize bran	11.33	9.67	8.3	6.94	5.58
Starch	2	2	2	2	2
Palm oil	2	2	2	2	2
Bone meal	2	2	2	2	2
Premix*	0.5	0.5	0.5	0.5	0.5

Methionine	0.5	0.5	0.5	0.5	0.5
Lysine	0.5	0.5	0.5	0.5	0.5
Salt	0.25	0.25	0.25	0.25	0.25
B-complex	0.02	0.02	0.02	0.02	0.02
Vitamin C	0.03	0.03	0.03	0.03	0.03
Total	100	100	100	100	100

*Provides per kg diet: Vitamin A, 125000 IU, Vitamin D3 2500 IU, Vitamin E 40mg, Vitamin K 2mg; Vitamin B1 3mg; Vitamin B2 5.5mg; Choline chloride 500mg; Niacin 35mg; Vitamin B6 5mg; Vitamin B12 0.025mg; Folic acid 1mg; Biotin 0.08mg; Manganese 120mg; Iron 100mg; Zinc 80mg; Iodine 1.8 mg; Calcium pantothenate 11.5mg; Copper 8.5mg; Cobalt 0.3 mg; Selenium 0.12 mg; vitamin C 2000mg, Antioxidant 120 mg;

In-Vitro protein digestibility

The experimental diets were measured in triplicates by cleavage peptides [10]. 20 mg of each diet was added with 12 ml of 50mM phosphate buffer (pH 7.5) and incubated overnight at 30 °C. In-vitro digestion was started by adding 500µl of crude enzyme extract and incubated for 24 hours at 30 °C. After digestion, 1ml of each digested mixture was determined of cleavage peptides by measuring absorption at 750nM and converted into mg protein using a standard curve developed from the principle reported by [11].

Results

The results of diets' proximate composition, growth performance, in-vitro digestibility, and antinutritional factors in the diets are presented in the tables 2-5 below. The growth performance results showed that there was significant difference in weight gain of the fish with the significant highest gain observed in the group that fed on diet 2 with 5% supplementation (P<0.05). The significant poorest weight gain was obtained in the group that fed on diet 5 with highest (20%) level of MLM supplementation (P<0.05). There was no significant difference between treatments in terms of initial weight, feed fed and survival (P>0.05). However there was significant difference in FCR, Specific growth rate, total length and standard length among the groups that fed on diets 4 and 5 with 15% and 20% supplementations respectively (P<0.05). The significant poorest specific growth rate (SGR) was observed with the group that fed on the highest MLM supplementation (P<0.05) while the significant highest SGR was obtained with the groups that fed on 5% MLM supplementation though it was not significantly different from the control group. The haematocrit and haemoglobin were also significantly reduced in the group that fed on the highest level of supplementation (P<0.05). The in-vitro trial revealed significant reduction in digestibility with increase in MLM supplementation level (P<0.05). The tannin and phytic-acid levels followed the same trend. However there was no pathological defect observed in the fish from all the groups. There was no significant difference in the hepathosomatic index. The cost analysis showed a reduction in cost per 100kg feed with increased level of supplementation with 0.00, 11.50, 23.20, 34.00 and 50.64 % for diets 1, 2, 3, 4 and 5 respectively. The results of the water qualities monitored have means falling within the range suitable for good growth of catfish with temperature 27.5 °C, dissolved oxygen 4.6mg/l, pH 7.51, NH_3-N 0.34mg/l and NH_2-N 0.026mg/l (Table 2 – Table 5).

Table 2: Composition of 42.5% Crude Protein Experimental diet.

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Moisture Content %	6.02	6.08	5.89	6.55	6.01
Crude Protein %	44.78	42.69	42.56	41.89	41.69
Crude Fat %	13.5	13.35	13.89	12.73	12.05
Crude Fibre%	2.02	2.41	3.54	4.98	6.02
Ash %	7.65	7.77	7.82	7.89	7.65
NFE %	26.03	27.7	27.3	25.96	26.58

Table 3: Growth performance of Heterobranchus longifilis fingerlings fed diets with graded level of MLM as replacement for toasted full fat soybean meal (0 – 6 weeks).

Parameter	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Initial Weight (g /Fish)	9.25 ±0.34a	9.14 ±0.55a	8.96 ± 0.22a	8.61± 0.16a	9.11± 0.41a
Weight Gain (g /Fish)	16.14 ±0.70ab	23.63±12.76a	16.41± 5.61ab	16.71±4.81ab	8.54±3.36c
Feed Consumed (g /Fish)	15.50±3.45a	22.45±13.31a	16.37±8.04a	17.38±16.06a	10.33±3.9a
Feed Conversion Ratio	0.96±0.14a	0.93±0.05a	1.00±0.10a	0.97±0.15a	1.2±0.02b
SGR (%/day)	2.38±0.05ab	2.90±0.94a	2.45±0.55ab	2.53±0.44ab	1.54±0.46b
Survival (%)	70.00±00a	45.83±36.08a	66.67±14.43a	58.33±28.86a	66.67±14.43a
Total Length (Cm)	9.70±0.26b	10.78±0.89a	10.55±0.26ab	9.75±0000b	10.21±0.40ab
Standard Length (Cm)	8.00±0.00c	9.10±0.81a	8.88±0.43ba	8.25±0.00c	8.77±0.25ba
Haematocrit (%)	23.76±1.16a	24.35±1.05a	24.67±1.17a	23.86±2.66a	19.02±1.23b

Haemoglobin (mg/dl)	11.20±2.32a	11.05±2.15a	11.40±2.20a	10.80±1.20a	7.02±2.34b
% Reduction in N/100Kg Feed	0	11.5	23.2	34	50.64

[a-c] Means plus standard deviation within rows with different superscript(s) significantly different (P<0.05). SGR = Specific growth rate.

Table 4: Phytochemical contents of Moringa leaf and experimental diets.

Parameters % MLM	Moringa leaf	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Total Tannin	2.82±0.69	0.82±0.06c	2.09±0.27b	2.36±0.12ab	2.51±0.28a	2.60±0.24a
Phytic acid	0.49±0.14	0.46±0.05c	1.15±0.13b	1.29±0.05ab	1.40±0.13a	1.48±0.14a

[a-c] Means plus standard deviation within rows with different superscript(s) significantly different (P<0.05).

Table 5: Digestibility of the Diets fed to *Heterobranchus longifilis* fingerlings (0 - 6weeks).

Parameters	Diet 1 (Control)	Diet 2	Diet 3	Diet 4	Diet 5
Digestibility coefficient (%)	83.65 ± 1.59a	79.29±0.36a	75.05±1.25b	71.45±1.78b	68.34±1.68c
Protein digestibility (%)	92.67±0.55a	91.24±0.43a	91.37±34a	88.26±0.27b	86.39±0.23c

[a-c] Means plus standard deviation within rows with different superscript significantly different (P<0.05).

Discussions

The results of this study indicated that *M. olifera* leaves possess the potential to partially replace soybean meal in any soybean and fishmeal base diet for giant African catfish (*H. longifilis*) without adversely affecting growth performance, digestibility and pathology of the fish. This is in agreement with earlier studies on *Oreochromis niloticus* by Richter et al. [12] and Abo-State et al. [13] who used MLM to replace fishmeal in the diets of the fish and found out that MLM could replace fishmeal up to 10% and 8% respectively in tilapia. *Moringa* leaf meal (MLM) could be used to reduce pressure on soybean meal. Hardy 2010 noted that soybean is currently the most commonly used plant protein sources in fish feed and there is competition with human use. Using MLM to reduce pressure on soybean will be possible because comparison between the amino acids of MLM and soybean revealed an almost identical pattern of all the essential amino acids [8]. The observation of reduced growth performance, digestibility and poorer FCR at higher inclusion level in this present study is an indication that there is a limit to the extent which MLM could be included in the diet of giant African catfish to avoid negativity in performance. This is similar to what was earlier reported on *O. niloticus* [12,13]. The poor performance at higher levels might not be unconnected to the antinutritional factors present in the raw moringa leaf. Although the MLM used in this study was air dried before use, the phytochemical analysis revealed that the phytic acids and tannin were still present in the diets though at low levels. The poor performance observed in the groups that fed on diets with higher inclusion levels of MLM might be related to the likely interaction of the antinutritional factors with utilization of other nutrients in the feeds [6]. The poor Hb and PCV levels obtained in the groups that fed on the highest MLM supplementation level might have been due to interactive effect of phytochemicals against the fish natural body functional ability to neutralize or suppress the deleteriousness of certain ingested chemicals. The appreciable digestibility of protein obtain in this study might be due to the drying process the leaf undergo before its use, the equal fishmeal levels and palm oil as a source of lipids in the diets with the fact that it is plant protein portion that was replaced. Palm oil has a natural tendency to neutralize the efficacy of poisonous substances like the one that could result from the antinutritional factors such as the tannins and phytic substances in the MLM. It had also been noted that antinutrients such as tannins and saponins inflicts bitterness in feeds and could results in poor consumption [2] due to reaction of the chemosensory receptors [14]. That might happen at high levels of inclusion which could result to high concentration of those antinutrients and subsequent effective bitterness since the intake in this study did not exhibit such criteria as there was no significant difference in feed intake. Although increased level resulted into poor feed conversion ratio which might not be unconnected to the carnivorous-ominivorous nature of the giant African catfish and tannins interaction with utilization of nutrients in feeds. The effective cost reduction also observed in the formulations with MLM is an indication that it could be used to replace some soybean in the diets of giant African catfish at the optimum level of 5-10% replacement.

In conclusion, MLM could be used to partially replace up to 5 - 10% of the full fat soybean meal in the diet of giant African catfish, *Heterobranchus longifilis* fingerlings without reduction in the growth performance of the fish. The MLM use will also lead to reduction in cost of fish feed. Using the best performing supplementation level (5%) will result to a cost reduction of 11.5% per cost of 100Kg feed base on the formulations used in this study while the 10% optimum level of supplementation will result to feed cost reduction of 23.2% per cost of 100Kg feed [15]. The cost to benefit ratio obtained in the study encourages the use of 5% level of replacement. There is need to subject moringa leaves to other processing methods for another trial to see if there could be improvement from what was obtained in this present study. There is need to evaluate the response of *Clarias* species to moringa leaf inclusion in the diet.

Acknowledgement

None.

Conflict of Interest

No conflict of interest.

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