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Growth Response and Nutrient Utilization of *Heterobranchus Longifilis* (Valenciennes,1840) Fingerlings Fed Raw and Boiled *Mucuna Cochinchinensis* Seed Meal

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Abstract: Feeding trial was conducted in static water to assess the growth and nutrient utilization of *Heterobranchus longifilis* fingerlings fed Mucuna Seed Meal (MSM). Raw and boiled MSM were used at 10%, 20, 30 and 40% inclusion levels to formulate isonitrogenous (CP 40%) and isocaloric (2.8 Kcal g^{-1}) diets similar to a fishmeal-based control diet. It was observed that after 8 weeks of feeding at 5% body weight daily, diets 2 (10% raw MSM), 6 (10% boiled MSM) and 7 (20% boiled MSM) performed well with SGR, AFCR and APER that were similar ($p<0.05$) to the control diet (1) which was significantly different from the other diets. While the fish carcass fat showed similar trend as the SGR, AFCR and APER for the MSM containing diets, the carcass protein for all fish fed diets containing boiled MSM had similar values as the control diet. The significantly lower performance of fish fed diets containing higher levels of MSM might be due to the increasing levels of antinutritional factors present in particularly raw MSM and the lower quality of boiled MSM arising from both heat treatment and thermostable antinutritional factors. Other methods of processing MSM to improve its nutritive value should be investigated.

Key words: Fishmeal substitute, *heterobranchus longifilis mucuna cochinchinesis*, MSM, antinutrional factors

INTRODCUTION

Nigeria aquaculture produced over 20,000 tonnes of various fish species in the year 2000, which involved the input of supplementary and complete feeds that accounted for between 40-60% of production costs. Since diets generally represent the largest single cost item of most fish farm operations, it follows that the selection of feed ingredients for use within diets will play a major role in dictating the ultimate nutritional and economic success for farmed fish.

Fishmeal which serves as the main protein sources for fish feed because of its high quality protein content , is not only very expensive but also usually unavailable (Tacon and Barg, 1998) particularly in developing countries. Efforts to replace fishmeal with vegetable protein from more sustainable sources have been embarked upon by various workers (Fagbenro, 1999; Fagbenro and Davies, 2001; Ogunji and Wirth, 2001; Osuigwe *et al*., 2002; Fagbenro and Davies, 2003; Ogunji *et al*., 2003).

Mucuna, a tropical legume is widely cultivated as a cover crop and the seeds which have relatively high protein content is hardly consumed by man. This study is an effort to evaluate the nutritional potential of *Mucuna cochinchinensis* seed meal as an alternative to fishmeal in diets for *H. longifilis* a commonly cultured catfish.

MATERIALS AND METHODS

Dry mature seeds of *M. cochinchinensis* obtained from Micheal Okpara University of Agriculture Research Farm were hammer milled and divided into two lots. One lot was subjected to wet boiling for 60mins. by introducing the moistened meal wrapped in polythene sheets into water brought to boil (100-150°C) in plastic buckets using electric boilers. The boiled meals were thereafter dried in an oven at 60°C.

Samples of the meals were assayed for proximate composition using the Association of Official Analytical Chemists (A.O.A.C., 1990) procedures. Micro Kjeldahl method was employed for Ether Extract (EE). The gross energy was determined using adiabatic oxygen bomb colorimetry techniques. The mineral content was determined by atomic absorption spectrometry after wet digestion with perchloric and nitric acids by the Johnson and Ulrich (1959) method. Phophorus content was determined following the development of colour with ammonium molybdate on Spectronic 20 spectrophotometer (Table 1).

The alkaline titration method of A.O.A.C was used to determine the hydrocyanic acid in MSM. Heamagglutinin extract was prepared by a modified Liener (1955) method. The method of Hoff and Singleton (1977) was employed to determine tannin while the chemical method described by Kakade *et al*. (1994) was used for assessment of trypin inhibitor (Table 1).

Table 1: Proximate composition and some antinutritional factors in raw and 60 min. boiled mucuna seed meal (% on dry matter basis)

Component	Raw Mucuna	Boiled Mucuna
Dry matter	89.44	86.96
Crude protein	30.19	32.18
Crude fibre	9.33	7.48
Crude lipid	4.28	4.30
Ash	3.58	2.97
Nitrogen-free extract	52.62	53.34
Gross energy (Kcal g^{-1})	4.63	4.65
Calcium	0.08	0.07
Phosphorus	1.06	0.89
Magnesium	0.16	0.15
Sodium	0.94	0.75
Trypsin inhibitor (mg g^{-1})	8.03	4.39
Tannin (Mg g^{-1})	5.52	4.25
Haemagglutinin (Hu g^{-1})	4264.0	2130.0
Cyanide (Mg Kg^{-1})	40.0	25.0

Hu-Haemagglutinating unit, Kg-Kilogramme, G-Gramme, Kcal-kilocalorie, Mg-Milligramme

Nine isonitrogenous (CP 40%) and isocaloric (Kcal g^{-1} 2.8) diets were formulated using raw and 60 minutes boiled MSM (Table 2). Diet 1 without MSM served as the control while diets 2,3,4 and 5 had the fishmeal replace progressively by raw MSM at 10, 20, 30 and 40%, respectively.

Diets, 6,7,8 and 9 had the fishmeal replaced by 60 mm boiled MSM at 10, 20, 30 and 40%, respectively.

The fingerlings of *Heterobranchus longifilis* used for the experiment were collected from Green Lake Farm near Owerri and transported to Michael Okpara University of Agricultrue, Umudike. Individuals of fairly uniform size of initial average body weight 1.46 g that had been acclimated in 20 litre square plastic aquaria for seven days were used.

The test diets were assigned randomly to triplicate groups of ten fingerlings in the aquaria. The experimental fish were fed the assigned diets twice daily for 56 days at 5% body weight per day. At the commencement of the trial, fish were batch-weighed with Acculab 33 to the nearest gram and subsequently every two weeks. The quantity of feed to be dispensed was adjusted from the fortnightly weight records to reflect the new weight. During this period, water was replaced from each aquarium every 3 days by siphoning. The water temperature, pH and dissolved oxygen were also monitored daily.

For fish carcass crude protein and crude lipid, four fish collected at the commencement of the study and two fish from each aquarium at the end were sacrificed, stored in freezer and subsequently analysed. From the data collected the following parameters were calculated from each treatment. Specific Growth Rate (SGR), Apparent Feed Conversion Ration (AFCR), Apparent Protein Efficiency Ratio (APER), fish carcass crude protein and crude lipid.

The indices were calculated using the following formulae.

$$*\text{Specific growth rate} = \frac{\text{LogeW2} - \text{LogeW1} \times 100}{T}$$

W2 = Final body weight; W1 = Initial body weight; T = Duration of study in days.

$$*\text{Apparent Feed Conversion Ratio AFCR} = \frac{\text{Weight of dry feed disped}}{\text{Live weight gain}}$$

$$*\text{Apparent Protein Efficiency APER} = \frac{\text{Weight gain (g)}}{\text{Apparent protein intake}}$$

Statistical analysis: One way Analysis of Variance (ANOVA) was used in determining any statistical variation among the treatment groups: The Duncan's multiple Range test was used in separating means.

RESULTS

The proximate composition and some antinutrational factors in raw and boiled MSM are presented in Table 1. The crude protein and nitrogen free extracts of the boiled MSM were slightly higher than those of the raw MSM while its ash and crude fibre content were lower than that of raw MSM (Table 1). Boiling also reduced the concentration of heat labile antinutritional factors such as trypsin inhibitor activity, haemagglutinin and cyanide more than tannins.

The mean values of some physicochemical parameters recorded were water temperature 29±1.2°C, PH 6.5±0.3 and Dissolved Oxygen (DO) 6.2±0. 5 mg L^{-1}. These values are considered suitable for fish production in the tropics.

No mortalities were recorded during the period of the experiment. The influence of raw and boiled MSM on growth and some nutrient utilization parameters are shown on Table 3. Generally increasing level of MSM in diet led to reduced growth. Diet 5 with the highest level of raw MSM gave the poorest performance with SGR, AFCR and APER that are significantly different from other diets. The SGR values for diets 2, 6 and 7 were however not significantly different from the control diet (1) Boiling appear to have improved the performance of diet 7 when compared to diet 3 that is of similar MSM inclusion level. Thus diet 7 had similar ($p<0.05$) values as the control diet for SGR, AFCR and APER.

Table 2:Composition of experimental diets (%)

		Raw MSM					60 min boiled MSM		
		1	2	3	4	5	6	7	8
	Control	10%	20%	30%	40%	10%	20%	30%	40
Mucuna	-	10	20	30	40	10	20	30	40
Maize	48.0	42.8	38.1	33.4	28.0	42.8	38.1	33.4	28.00
VMP	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Salt	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Oyster shell	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
SBM	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Crude protein	40.28	40.20	40.04	40.05	40.05	40.30	40.36	40.42	40.34
Gross Energy (Kcal g^{-1})	2.83	2.83	2.84	2.82	2.81	2.82	2.84	2.84	2.82

VMP=Vitamin and Mineral Premix, SBM=Soya Bean Meal

Table 3: Effect of test diets on the SGR, AFCR, APER, carcass protein and fat of heterobranchus longiflis fingerlings

		Raw MSM					Boiled MSM		
		1	2	3	4	5	6	7	8
	Control	10%	20%	30%	40%	10%	20%	30%	40
Initial mean weight (g)	14.4	15.0	14.5	14.3	14.4	14.6	14.2	15.0	14.8
Final mean weight (g)	36.3	34.9	28.5	24.3	21.8	35.8	32.4	30.6	25.7
SGR (% day)	1.64^{a}	1.51^{a}	1.15^{b}	0.95^{c}	0.74^{d}	1.60^{a}	1.47^{a}	1.27^{b}	0.98^{c}
AFCR	1.70^{d}	1.80^{cd}	2.40^{c}	3.60^{b}	4.80^{a}	1.75^{d}	1.86^{cd}	1.98^{c}	2.2^{c}
APER	1.47^{a}	1.36^{a}	0.97^{b}	0.65^{c}	0.42^{d}	1.41^{a}	$1.28a^{b}$	1.07^{b}	0.64^{c}
Protein (%)	64.4^{a}	63.9^{a}	63.2^{b}	62.6^{c}	61.5^{c}	64.8^{a}	64.6^{a}	64.4^{a}	63.9^{a}
Fat (%)	9.52^{a}	9.48^{a}	7.04^{b}	6.98^{b}	5.02^{c}	9.46^{a}	9.43^{a}	8.26^{ab}	7.34^{a}

Means on the same row with different superscripts are significantly different ($p<0.05$), SGR = Specific Growth Rate, AFCR = Apparent Feed Conversion Ratio, APER = Apparent Protein Efficiency Ratio

For fish carcass protein and fat, diet 2 had values that are not significantly different ($p<0$-05) from the controlled diet. Fish fed boiled MSM had similar carcass protein value as the control diet. However, among fish fed boiled MSM, only diet 9 had fat value that is significantly different from the control diet, while those fed raw MSM had fat values that were significantly different from the control diet except diet 2.

DISCUSSION

In this experiment, fish fed diets, 2, 6 and 7 had SGR values that are similar and not significantly different ($p<0.05$) from the control diet. Diets containing higher than 10% raw MSM and 20% boiled MSM produced significantly poorer growth and nutrient utilization indices compared to the control diet. The reduced growth performance might be attributed to the presence of various antinutritional factors present in MSM.

Substantial amounts of tannins, phytic acid, saponins, protease inhibitors, lectins and 3,4-dihydroxyphenylalanine have been reported in MSM (Siddhuraju and Becker, 2001). This agrees with the detection of trypsin inhibitor, tannin, heamagglutinin and cyanide in this experiment (Table 1) monogastrics and other warm blooded animals are known to be adversely affected by the high proportion of phenolic substances present in feeds by reduction of feed intake, growth and nutrient availability and increase in endogenous losses of nitrogen through faeces (Makka and Becker, 1999). Al-Owafeir (1999) also observed significant growth reduction in fish fed diets containing low levels of tannic acid. The presence of tennins in deoiled sal *(Shorea robusta)* seed meal in the diet was shown by Mukhopadhyay and Roy (1996) to reduce growth of Indian major carp fingerlings. The level of tannin in diets 3.4, 5, 8 and 9 may have negatively affected the growth parameters of fish fed such diets in this experiment. The slight reduction of the concentration of tannin in MSM subjected to boiling may have contributed to the improved performance of diet 7 relative to diet 3.

Protease inhibitors and phytoheamagglutinin in legumes are considered as potential antinutrients and are known to decrease growth performance of animals (Liener, 1994). Many fish species have been reported to be sensitive to trypsin inhibitors (Wilson and Peo, 1985; Shu, 1992). Similarly, Abel *et al.* (1984) and Viola *et al.* (1983) found that carp are sensitive to protease inhibitors. In this study, although trypsin inhibitors and heamaglutinin were reduced by about 45 and 50% respectively by boiling MSM, the significantly reduced growth performance in *H. longifilis* fingerlings fed diets 8 and 9 implies that residual trypsin inhibitor and heamaglutinin in boiled MSM may have led to the poor performance. Related studies by some workers also reported the presence of other ant nutritional factors in mucuna such as non-protein amino acid 3,4-dehydroxyphenylanine (L-DOPA), phytates, saponins,

Non-Starch Polysaccharides (NSP) etc, Robania *et al.* (1995), Bureau *et al.* (1998), Siddhuraju and Becker (2001). These antinutritional factors which are mostly thermostable may have equally contributed to the poor growth performance experienced in fish fed diets with high inclusion levels of MSM.

In the present study, the AFCR and APER values obtained with diets, 3, 4, 5, 8 and 9 were generally significantly lower compared with the control diet and diets 2 and 6. Similarly, Osuigwe and Obiekezie (2006) observed that increasing replacement with jackbean (*Canavalia Ensiformis*) seed meal markedly reduced specific growth rate and the diet with the highest level of jackbean seed meal gave the poorest performance with SGR, AFCR and APER in *H. longifilis*. It was reported (Takashi and Kawakishi, 1997) that oxidation products of L-DOPA conjugate with SH compounds, (cysteine) of protein to form crosslinks that lead to polymerisation. The complex formation by exydised products of L- DOPA with peptide sulphur amino acide may reduce the availablility of cysteine and methionine in the diets causing reduction of growth and feed utulisation. The diets containing higher levels of raw MSM produced the significantly lowest protein and lipid. This agrees with the findings of Yurkowski *et al.* (1978) for rainbow trout with rapeseed diets. Significant reduction in lipid content of common carp fed diets containing various levels of mustard oil cake, linseed and sesame meal were also reported by Hossain and Jauncey (1989). However, in this study no significant variation was observed between the carcass protein of fish fed boiled MSM and the control diet.

The better performance of fish fed diets 2, 6 and 7 relative to other MSM containing diets might be due to the high biological value of the protein derived from the higher proportions of fish meal contained therein and relatively low levels of antinutritional factors contained particularly in boiled MSM of diet 7. The result of the study indicated that inclusion of 10 and 20% raw and boiled MSM, respectively did not affect the growth performance and feed utilization in *H. longifilis*. Other processing techniques should be investigated to identify the most appropriate method that would permit higher inclusion levels of MSM without adverse effects on growth and feed utilization.

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