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Effect of Replacement of Soybean Meal with Afzilia Seed *(Afzilia africana)* Meal on the Growth and Intestinal Mucosa of African Catfish *(Clarias gariepinus)* Fingerlings.

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Abstract

Three hundred (300) Clarias gariepinus juveniles were fed five iso-nitrogenous (CP ≈ 40%) and iso-caloric diets containing varying levels of Afzilia africana (AAM), to determine their effects on the intestinal mucosa of the fish. At the end of the 56 days feeding trials, histological investigation of the intestinal mucosa of C. gariepinus revealed that the status of the intestinal mucosa of the fish was altered. Whereas the intestinal mucosa of the fish fed the control diet (0% AAM) were found to be normal, progressive damages to the epithelial mucosa of C. gariepinus intestines were observed with increasing dietary level of AAM. While fish fed diets with 10% AAM substituting fishmeal showed minor degeneration of the intestinal mucosa, those fed with higher inclusion level (60% AAM substituting fishmeal) showed severe damages. These damages to the intestinal mucosa may be attributed to the presence of anti-nutritional factors present in Afzilia africana seed. These damages may not be unconnected to the relatively poor growth performance of the fish fed diets that had varying levels of AAM, since intestinal mucosa are reputed in regulating the digestion and absorption of digested nutrients into the blood stream. Therefore, as a way of increasing the utilization of AAM in the diets of fish, other methods of processing the seeds like boiling, soaking etc. maybe tested. This development will impact positively on fish production by reducing the cost of fish feed

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given the comparatively lower cost of AAM when compared with soybean meal.

Keywords: Soybean meal, replacement, intestinal mucosa, African catfish

Introduction

The Food and Agriculture Organization of the United Nations (FAO, 2006) stated that Nigeria is a protein deficient country. The deficiency in the diets can be primarily remedied through the consumption of either protein- rich plants or animals sources. Animal protein is in short supply in Nigeria due to the rapid increase in human population annually as well as the decrease in livestock population which is as a result of several factors (Obidinma,2009).These factors combined have raised the cost of animal protein to a level that is almost beyond the reach of the ordinary citizen. This situation has therefore given rise to a considerable increase in the demand for fish to supplement the needed animal protein intake. Fish offers a relatively cheap and abundant way of providing protein to the populace but there are not enough fish in the wild to do the job, and so aquaculture or fish farming is seen as important answer to feeding a hungry world (Leong, 1999).

Aquaculture industry has remained the fastest growing food industry in recent times (FAO, 2012). However, just like every other food production industries, it has its own challenges. Thorarinsdottir *et al.* (2011) observed that the main cost factor in aquaculture is the cost of feed. Tacon and Metian (2008) reported that fishmeal has been adjudged the most important and expensive protein ingredient used in aquafeeds. Therefore, to increase the profitability of aquaculture industry, there is the need to reduce the reliance of the industry on fish meal as the primary deitary protein sources. A lot of cheap plant protein sources have been tested with variable successes (Hua *et al.*, 2015). One of the major local feed stuffs that meet the protein needs of most species is soybean meal (Ogunji, 2004).Soybean still adjudged the best source of plant protein till date has other competing uses in the animal feed industry as well as for human nutrition. There is therefore, the need to research into other alternative plant protein sources to reduce the pressure on soybean.

Afzilia africana, a leguminous plant, reported to be abundant in the savannah regions, has been shown to have good nutritional qualities that qualifies it as a good protein source in animal feed (Ogungbenle, 2014). Igweny *et. al.* (2013) reported the presence of the anti-nutients – oxalates, trypsin inhibitors and hemagluttinin in *Afzilia africana* and found their concentrations to be 1.08±0.02, 2.35±0.07 4.49±0.32 mg/100g., respectively.

The gastrointestinal tract (GIT) plays a primary role in regulating processes necessary to obtain energy from ingested nutrients. In vertebrates, the entire length of the GIT contains four distinct layers: mucosa, sub-mucosa, muscularis, and adventitia or serosa. The mucosa regulates digestion, absorption, osmoregulation, and host defense. Giving the importance of fish intestine as a critical tissue that can influence both the health and nutrition of fish (Yuan *et al.,* 2015), ascertaining the influence of a feedstuff on the status of the intestine of a fish may be a criteria for choosing ingredients for fish feed formulation. The present study therefore, was designed to investigate the effect of replacing soybean meal with *Afzilia africana* seed meal on the intestinal mucosa of *C. gariepinus.*

Materials and Methods

Source and processing of *Afzilia africana*

Matured dried seeds of *Afzilia africana* were purchased from the farmers at Abakaliki Ebonyi state, Nigeria. The seeds were inspected and the defective ones discarded. The seeds were processed according to Ayanwale *et al.* (2007). The milled *Afzilia africana* seed meal (AAM) was defatted using N-hexane as recommended by Enujiugha and Akanbi (2005).

Experimental diets

Five iso-nitrogenous (40% c.p) experimental diets were formulated such that *Afzilia africana* meal (AAM) replaced soybean meal (SBM) at 10%, 20%, 40%, and 60% dietary levels respectively (Table 1). Diet with 0% *Afzilia africana* seed meal served as control. Other conventional feed ingredients such as Maize, fish meal, blood meal, vegetable oil, vitamin/mineral premix, and binder were procured from Meat market, Abakaliki, Ebonyi State. A sample of *Afzilia africana* meal and experimental diets were subjected to proximate

analysis (Tables 2 and 3) using standard procedures according to AOAC (1999).

Table 1: Ingredients composition of formulated diets (% Dry weight)

Ingredient (g/100g)	T1	T2	T3	T4	T5
Blood meal (82 % cp)	10.0	10.0	10.0	10.0	10.0
Fish meal (62% cp)	24.0	24.0	24.0	24.0	24.0
Soybean Meal (43% cp)	36.0	32.4	28.8	21.6	14.4
Afzilia africana seed meal (26% cp)	-	6.0	12.0	24.0	36.0
Maize (9% cp)	20.0	17.6	15.2	10.4	5.6
Vegetable oil	6.0	6.0	6.0	6.0	6.0
Vit/Min premix	2.0	2.0	2.0	2.0	2.0
Binder	2.0	2.0	2.0	2.0	2.0
Total	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**
Calc. Crude Protein level	40.60	40.39	40.19	39.60	39.37

Experimental design and feeding trials

Three hundred (300) African catfish juveniles (*C. gariepinus*) of average weight 24.29 ± 0.91 g were purchased from Oluchika fish farm, Abakaliki, Ebonyi State, Nigeria and transported to the department of Fisheries and Aquaculture, Abakaliki. The fish were acclimated for 7 days and were fed on commercial diet. Prior to the commencement of the experiment; all fish were starved for 24 hours. This practice was to eliminate variation in weight due to residue food in the gut and also to prepare the gastrointestinal tract for the experimental diets, while at the same time to increase the appetite of the fish. Fifteen plastic tanks of 70l capacity were randomly allocated to the five treatment diets (T1, T2, T3, T4 and T5) in triplicate and fish were randomly distributed into the tanks at a stocking density of twenty fingerlings per tank. Fish were fed 5% of their body throughout the period of the experiment, twice daily, (8.00 hrs-9.00 hrs) and (17.00 hrs-18.00 hrs). The water in culture tanks were changed twice weekly. During each change, the culture tanks were washed thoroughly to reduce the risk of infection and check fungal and algae growth. Subsequently, growth data were taken fortnightly and quantity of feed fed adjusted in accordance to the fish weight gain.

At the end of the experiment, all fish w weighed and data obtained from triplicate tanks were used to calculate weight gains, specific growth rate (SGR), feed conversion ratio (FCR) and feed intake.

Weight gain = final weight – initial weight, SGR = (LnW2 -

LnW1)/(T2-T1)100 where W1 and W2 = initial and final weight of fish and T1 and T2 = time in days. FCR = feed fed/live weight gain.

Table 2: Proximate Composition of Soybean Meal (SBM) and *Afzilia africana* meal (AAM) used in the experiment.

Composition	Soybean meal	*Afzilia africana* meal
Crude protein	43.71[a]	26.34[b]
Crude fat	3.68[a]	2.47[b]
Crude fibre	4.40[a]	3.13[a]
Ash	5.93[a]	4.91[b]
Moisture	7.23[a]	7.23[a]
Nitrogen free extract	35.05[a]	34.92[a]

Table 3: Proximate Composition of Treatment Diets

EED AMPLE	%CP	% Cfat	% CFibre	% Ash	% M	% NFE
1	41.74±0.06	5.45±0.02	2.48±0.02	9.81±0.14	5.82±0.01	34.72±0.07
2	42.32±0.08	5.79±0.12	2.43±0.02	9.79±0.02	5.92±0.03	33.77±0.23
3	42.84±0.05	6.05±0.01	2.52±0.01	9.71±0.02	5.67±0.02	33.22±0.08
4	42.93±0.05	5.60±0.01	2.46±0.02	9.73±0.02	6.01±0.04	33.29±0.09
5	43.21±0.06	6.13±0.02	2.50±0.02	9.84±0.02	5.64±0.02	32.70±0.09

Histological examination

At commencement and end of the experiment, 2 fish were dissected the intestine removed and preserved in 10%buffer solution to retain the structural integrity of the cells and tissue. The dealcoholized tissues were impregnated in molten paraffin wax for 3 hours. After the impregnation, tissues were embedded using a deposable embedding mold and allowed to cool. Before sectioning the embedded tissues were placed on ice for proper sectioning. The tissues were then sectioned using Lecia rotary micotome and then transferred to the flattening table (hot plate) from the water bath to dry. Finally, the sections were stained using A & E staining procedure prescribed by Baker *et al.* (1989).

Statistical analysis

All growth and data were subjected to one way analysis of variance (ANOVA).The significant difference between means were determined by Duncan's Multiple Range test ($p<0.05$) using SPSS for windows (Version 20). Values were expressed as means ±SE.

Results

Table 4 shows the growth response of *C.gariepinus* fed varying levels of *Afzilia africana* meal. The feeding trials revealed that *C. gariepinus* responded to all the diets since increase in weight was recorded among the fish receiving all the test diets as shown in Table 4. The relative (268.72±21.2) and specific (3.32±0.81) growth rates were highest in the fishes fed the control diets and were lowest, 168.91±64.3 (RGR) and 1.87±0.31 (SGR) in the fishes fed 0% AAM based diet. The RGR and SGR of the control diets differed ($p<0.05$) significantly with the test diets but were comparable to the fishes fed 20% AAM based diet. Mean weight gain decreases with increase in the inclusion levels of AAM in the diets and were significantly ($p<0.05$) different from the control. The highest FCR, 2.33±1.03was recorded in 60% AAM diet while the least, 1.22±0.23 was obtained in 0% AAM inclusion. FCR of the control is significantly ($p<0.05$) different from the test diets, but is statistically comparable with fish fed 10% AAM.

Table 4: Growth Response of *C. gariepius* Fed Diets Containing *Afzilia africana* Seed Meal.

	Diets				
Parameters	**T1 (0%)**	**T2 (10%)**	**T3 (20%)**	**T4 (40%)**	**T5 (60%)**
Initial mean weight (g)	24.45±0.37^{a}	23.86±1.20^{a}	24.61± 0.98^{a}	24.22 ± 0.86^{a}	25.37±1.07^{a}
Final mean weight (g)	51.39± 1.56^{a}	40.20±0.55^{b}	36.34± 1.23^{bc}	39.57 ± 0.71^{b}	32.98 ±2.56^{c}
Mean weight gain (g)	26.94±1.44^{a}	16.34±0.70^{b}	11.73±1.52^{bc}	15.35±1.31^{b}	7.61±1.85^{c}
Food conversion ratio	1.22±0.23^{c}	1.44±0.81^{c}	0..48±0.77^{a}	0.63±0.47^{b}	2.33±1.03^{d}
Relative growth rate	268.72±21.2^{a}	228.42±32.1^{ab}	210.9±37.2^{b}	194.77±28.5^{bc}	168.91±64.3^{c}
Specific growth rate	3.32±0.81^{a}	2.61±0.33^{a}	2.20±0.36^{ab}	2.26±0.75^{ab}	1.87±0.31^{b}

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

The results of the histological examination of the intestines of the fish fed varying levels of *Afzilia africana* meal based diets are shown in Plates 2 to 6. Plate 1 shows the status of the intestinal mucosa of the fish at the commencement of the experiment with normal mucosal epithelium, lamina propria, muscularis and serous membrane. At the end of the eight weeks feeding trials, the status of the intestinal mucosa of the fish fed with the control diet (D1 0% AAM) remained normal (Plate 2). However, there were progressive damages of the

intestine of fish fed AAM based diets as shown in figures 3 to 6. The extent of damage was dependent on the inclusion levels of AAM in the test diets. As observed, fish fed diet 2 (10% soybean meal substituted by AAM) showed less severe degeneration of the intestinal mucosa (Plate 3) than those fed diets 3 (20% soybean meal substituted by AAM), 4 (40% soybean meal substituted by AAM) and 6 (60% soybean meal substituted by AAM) as shown in Plates 4, 5 and 6 respectively.

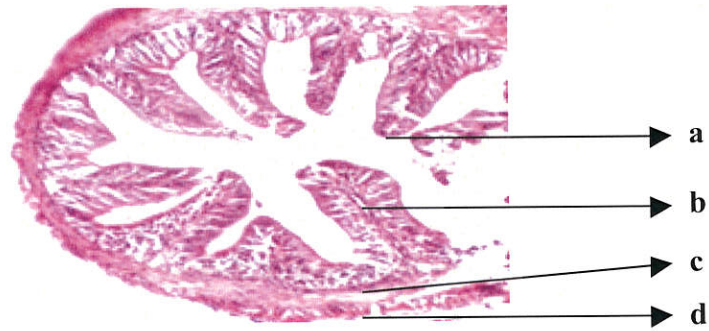


Plate 1. Normal intestinal mucosa at the commencement of the study. a. Mucosal epithelium, b. Lamina propria; c. Muscularis; d. Serous membrane.

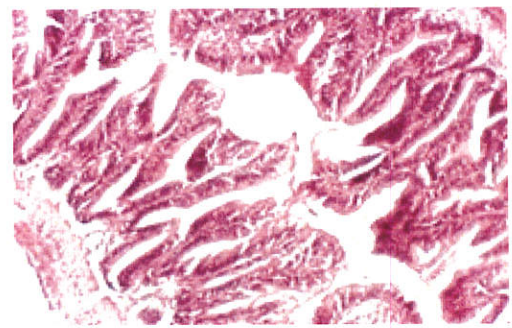


Plate 2. The status of the intestinal mucosa remained normal after eight weeks of feeding with control diet (0% AAM).

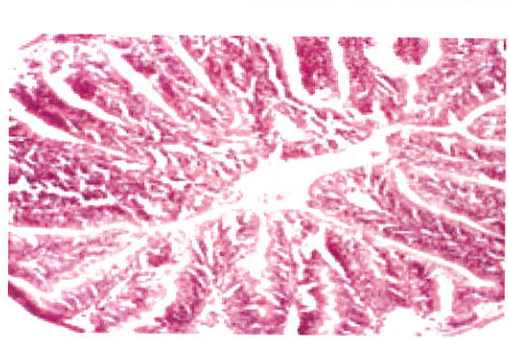
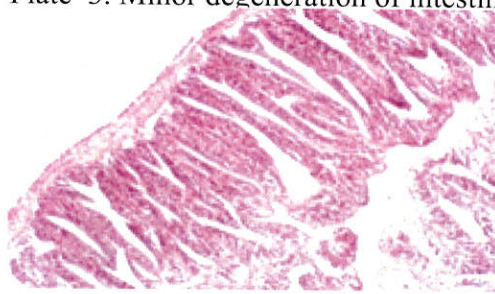
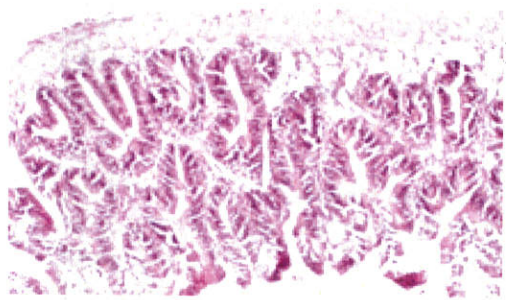


Plate 3. Minor degeneration of intestinal mucosa of fish fed diet 2





l mucosa of fish fed diet 3

Plate 5. Further degeneration of intestinal mucosa of fish fed diet

4 (40% AMM).

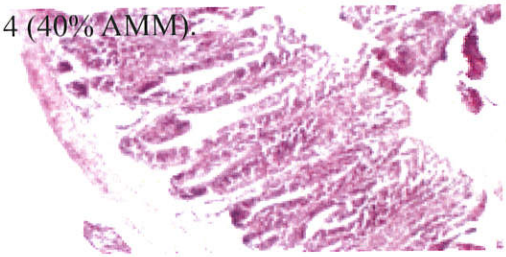


Plate 6. Pronounced degeneration of the intestinal mucosa of fish fed diet 5 (60% AMM).

Discussion

Weight gain and species growth rate are usually considered as the most important measurement of productivity of diets (Hossain *et al.*, 1995; Omitoyin and Faturoti, 2000). In the present investigation, progressively decreasing pattern of growth parameters of the fish with increase in the inclusion levels of the test diets were observed thus exhibiting an inverse relationship. This observation may be attributed to the anti-nutrional factors present in *Afzilia Africana* seed (Adesina *et al.*, 2013). However, the increase in weight gain reported in all the treatments indicated that the fish responded positively to all the diets and that the protein contents of the experimental diets adequately enhanced growth of the fish.

Extensive structural and functional disruption of the intestinal microvilli of animals fed plant protein sources with varying levels of antinutritional factors has been reported (Grant, 1991; Osuigwe *et al.*, 2006; Ogunji *et al.*,2014) amongst others. The ulcerations observed in the intestinal mucosa of *C. gariepinus* fed varying levels of AAM in this study may be attributed to oxalates, trypsin inhibitors and hemagluttinin known to be present in *Afzilia africana* seeds (Igweny *et. al., 2013).. D'Mello (1995) reported induction of intestinal abnormalities in animals fed conca* navalin A, a lectin present in jackbean seed. Bureau *et al.* (1998) observed extensive damage of intestinal mucosa

of both Chinook salmon and Rainbow trout fed Quillaja bark saponin similar to the condition of fish fed raw soybean meal diet. Osuigwe *et al.* (2006) opined that the damages observed in the intestine of *H. longifilis* fed varying dietary levels of jack bean seed meal is attributed to both lectins and saponins found in jackbean seed meal. Ogunji *et al.* (2014) obtained similar result when they fed *C. gariepinus* with raw african yam bean.

These damages observed in the intestines of fish fed varying levels of AAM based diets may be responsible for the relatively poor growth perfomances of the fish since fish intestine is not only involved in nutrient digestion but also in absorption of the digested nutrients into the blood stream through the microvilli present in the intestinal mucosa. These damages were observed to be increasing with the increased levels of AAM in the test diets.

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