HAEMATOLOGICAL RESPONSES OF HYBRID OF CATFISH FED DIETARY LEVELS OF *IPOMEA BATATAS* LEAF MEAL

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ABSTRACT

The haematological effects of feeding 35% isonitrogenous dietary levels of 0%, 5%, 10%, 15% and 20% Ipomea batatas leaf meal on Hybrid of Heterobranchus bidorsalis and Clarias gariepinus post fingerlings were assessed. These were fed to the fingerlings, randomly assigned to 5 treatments – control(TCN), 5%(TI_1), 10%(TI_2), 15%(TI_3) and 20% (TI_4) IBLM in 3 replicates of 15 post fingerlings each using 15 plastic aquaria of 250 x 150cm dimension. The fish were fed at 5% body weight twice daily within the experimental period of 56 days. Samples of blood were coollected from the treatments bi-weekly and analyzed to evaluate some of the haematological profile, and data subjected to a one way analysis of variance (ANOVA). T The haematological responses of Heteroclarias (H. bidorsalis x C. gariepinus) fed varied dietary levels of Ipomea batatas leaf meal are shown in table 4. There were significant ($p<0.05$) differences in the haematological responses of the fisf across treatments, except for the Haemoglobin, packed cell volume and MCHC that were not significant ($p>0.05$) for the various dietary inclusion levels of Ipomea batatas. The red blood cell values of 3.0 x $10^6/mm^3$ for TCN and 2.73x $10^6/mm^3$ for TI_4 were not different ($p>0.05$) from TI_2 (2.56 x $10^6/mm^3$) and TI_3 (2.58 x $10^6/mm^3$), but significantly ($p<0.05$) more than TI_1 (2.26 x $10^6/mm^3$).The white blood cells on the other hand for TCN (4.75 x $10^4/mm^3$), TI_1 (4.78 x $10^4/mm^3$) were significantly ($p<0.05$) higher than TI_4 (4.45 x 10^4mm^3). However, the MCV value of 106.2mm^3 for TI_1 was significantly ($p<0.05$) higher than the rest of the treatments. This was followed by TI_2 (94.45mm^3) and TI_3 (94.96mm^3), which were similar ($p>0.05$), TI_4 (87.28mm^3) and then treatment TCN (79.2mm^3). Similarly, the MCH value of 26.34pg for TI_1 was significantly ($p<0.05$) higher than the rest of the treatments. This was followed by TI_2 (23.79pg) and TI_3 (24.03pg), which were similar ($p>0.05$), TI_4 (22.0pg), and then TCN (20.82pg).

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INTRODUCTION

Many fish species appear to be able to utilize simple carbohydrates more effectively than complex starches. Catfishes and carps are known to utilize high levels of dietary carbohydrates (Okoye, 2003). *Ipomea batatas* leaves have been very useful in feeding rabbits, and most researchers like Oyenuga, (1978) and Anyanwu, (2008) found it useful as feedstuff for poultry and other livestock, due to its measurable high level of crude protein (24.65 – 26.70%) and other essential food nutrients

Assessment of nutrient utilization and biological values of feeds and feedstuffs may seem inconclusive without adequate consideration on their implications on the haematological profile of the fish. Haematological parameters of different species of fish and screening test provide a bank of useful information from which valuable and informative conclusions could be drawn (Nlewadim and Alum, 1999). The haematological and biochemical indices of farm fish as with other farm animals namely - haemoglobin, red blood cells, white blood cells, packed cell volume, plasma protein, blood glucose, specific gravity of blood plasma and whole blood, coagulation time etc. have been analyzed and variously reported as useful tools in assessing the performance, viability and health status of farm fish and animals (Blakhall and Daisley, 1973; Bhaskar and Rao, 1990; Musa and Omoregie, 2001; Harikrishnan *et al,* 2003; Anyanwu *et al,* 2003; Hemre *et al*, 2007; Ochang *et al*, 2007; Anyanwu *et al*, 2011).

The objective of this study therefore was to determine the effect of IBLM on the haematology of hybrid catfish (*H. bidorsalis* × *Clarias gariepinus*) post fingerlings.

MATERIALS AND METHODS

The experiment was carried out in a farm's fisheries house of 8 x 6m^2 situated in Owerri, Imo State. A total of 15 plastic aquaria (250cm x 150cm), covered with mosquito mesh nylon screen to prevent fish from jumping out and possible predation were used. The *Ipomea batatas* leaves were harvested from bushes at the outskirt of the Owerri capital territory, along Owerri/Onitsha Road, Imo State. These were spread under the sun and dried for three days until they became crispy while still retaining the green colouration. The dry leaves were milled, using a hammer mill to produce to leaf meal.

The leaf meal was used to make 4 35%CP Isonitrogenous diets at inclusion levels of 5%, 10%, 15%, and 20% for TI_1, TI_2, TI_3, and TI_4 respectively. Maize was used as the major source of energy in the diets, while soyabean meal and fish meal as major sources of protein (Table 1), besides, the use of lysine and methionine at 0.2% levels of inclusion. 1% bone meal was used, with Vitamin/mineral premix and common salt at 0.5% levels of inclusion as main sources of vitamins and minerals. Cassava starch was used at 2% level of inclusion as a binding material.

Table 1: Experimental Diets using *Ipomea batatas* leaf meal (IBLM)

	Dietary levels of IBLM				
Ingredients	**0%**	**5%**	**10%**	**15%**	**20%**
Maize	30.6	27.8	25.0	22.2	19.5
Fish meal	19.0	19.0	19.0	19.0	19.0
Soyabean meal	45.0	42.8	40.6	38.4	36.1
Ipomea leaf meal	0.0	5.0	10.0	15.0	20.0
Cassava starch	2.0	2.0	2.0	2.0	2.0
Palm oil	1.0	1.0	1.0	1.0	1.0
Bone meal	1.0	1.0	1.0	1.0	1.0
Lysine	0.2	0.2	0.2	0.2	0.2
Methionine	0.2	0.2	0.2	0.2	0.2
Vit./min premix	0.5	0.5	0.5	0.5	0.5
Common salt	0.5	0.5	0.5	0.5	0.5
	100.00	100.00	100.00	100.00	100.00

The feedstuffs were finely ground and mixed up into a dough form in a plastic bowl using hot water. The mixture was then pelleted by passing through a mincer of 2mm die to produce 2mm diameter size of the pellets. The pellets were then sundried to about 10% moisture content, packed in polythene bags and kept safely dry for use.

Two hundred and twenty-five post fingerlings of *Heteroclarias* collected from the African Regional Aquaculture Centre (ARAC) fish farm, Port Harcourt were stocked in an experimental tank for acclimatization. The fish were acclimatized for 7 days during which they were fed with the control diet containing 35% crude protein and of zero *Ipomea batatas* leaf meal twice daily, 08.00 – 09.00h and 17.00 – 18.00h. At the end of the acclimatization period, the 225 post fingerlings were completely randomized in 3 replicates of 15 post fingerlings per replicate for the 5 treatments – TCN (Control), TI_1, TI_2, TI_3 and TI_4. The initial weight of fish in each aquarium was taken and recorded. Feeding commenced an hour after weighing exercise and the fish fed at 5% of their body weight twice daily, morning (08.00 – 09.00h) and evening (17.00 – 18.00h). The water in the aquaria was regularly monitored for the physico-chemical properties, and was renewed completely every other day within the experimental period that lasted 56 days of culture. Temperature was determined using mercury in glass thermometer calibrated from 0-100^{o}c; immersed 5cm deep on the water surface. The pH and dissolved oxygen readings were taken using pH and oxygen meters respectively. Biweekly blood collection and sampling of the fish were carried out in line with Nlewadim and Alum (1999). The fish was anaesthesized in benzocain solution, using 0.4g dissolved in 1ml of 98% alcohol, and then added unto 1 litre of water. The fish was placed on its back in a trough, and blood collected from the posterior end of the abdomen, towards the tail, using a 2cm3 sterile plastic syringes and no 21 needle. The blood was emptied into EDTA (Ethylene Diamine Tetra Acetic Acid) treated bottle from Chemisciences Nig. Ltd. Owerri. Red blood cell and white blood cell counts were determined in line with Conroy and Herman (1970). Haemoglobin concentration and haematocrit (packed cell volume) estimates were determined with the procedure described by Wedemeyer and Yasutake (1977) and Blakhall and Daisley (1973) respectively. Mean cell volume, mean cell haemoglobin and mean cell haemoglobin concentration, expressed in fento litres, Picogram and grams per 100ml respectively, were also calculated as reported by Anyanwu (2008).

The proximate analysis of the test feedstuff and diets were carried out to determine the moisture content, ash, lipid, crude protein, crude fibre and nitrogen free extract, using the A.O.A.C (2000) methods. Experimental results were subjected to analysis of variance (ANOVA) as described by Steel and Torrie (1980). Test of significance was by Duncan multiple Range Test (DMRT) at 95% confidence level, using statistical package for social sciences (SPSS) for windows (version 15).

RESULT

The proximate composition for the *Ipomea batatas* leaf meal were 26.70%, 13.00%, 4.25%, 11.60%, 33.40% and 11.05% for crude protein, ash, lipids, crude fibre, nitrogen free extract and dry matter respectively. Water quality condition in the experimental aquaria of mean values 26.07 ± 0.01^{0}C, 6.61 ± 0.06 and 4.93 ± 0.07mg/l for temperature, pH and dissolved oxygen respectively, showed little variations throughout the experimental duration (Table 2).

Table 2: Water parameters of the experimental trials

Variable Parameters	TCN(0%)	TI_1(5%)	TI_2(10%)	TI_3(15%)	TI_4(20%)	Mean
Temperature (^{o}C)	26.05	26.05	26.10	26.12	26.07	26.07±0.01
pH	6.50	6.80	6.68	6.70	6.40	6.61±0.06
DissolvedOxygen (mg/l)	5.15	5.05	4.90	4.80	4.78	4.93±0.07

The dietary feeds chemical compositions are shown in table 3. The energy level of the diets decreased with increased levels of leaf meal.

Table 3: Percentage Proximate Composition of Experimental Diets

	Dietary levels of IBLM				
Nutrients	**0%**	**5%**	**10%**	**15%**	**20%**
Crude protein (%)	34.98	35.00	35.07	34.97	34.95
Crude fibre (%)	2.93	3.16	3.57	3.75	4.04
Ether extract (%)	7.35	7.47	8.05	8.54	8.90
Ash (%)	13.70	13.06	13.13	12.13	11.66
ME (Kcal/kg)	3244.74	3199.95	3155.24	3110.49	3065.84

The *haematological* responses of *Heteroclarias* (*H. bidorsalis x C. gariepinus*) fed varied dietary levels of *Ipomea batatas* leaf meal are shown in table 4. There were significant ($p<0.05$) differences in the responses, except for the *Haemoglobin*, packed cell volume and MCHC that were not significant ($p>0.05$) for the various dietary inclusion levels of *Ipomea batatas*. The red blood cell values of 3.0 x $10^6/mm^3$ for TCN and 2.73x $10^6/mm^3$ for TI_4 were not different ($p>0.05$) from TI_2 (2.56 x $10^6/mm^3$) and TI_3 (2.58 x $10^6/mm^3$), but significantly ($p<0.05$) more than TI_1 (2.26 x $10^6/mm^3$).

The white blood cells on the other hand for TCN (4.75 x $10^4/mm^3$), TI_1 (4.78 x $10^4/mm^3$) were significantly ($p<0.05$) more than TI_4 (4.45 x 10^4mm^3). However, the MCV value of 106.2mm^3 for TI_1 was significantly ($p<0.05$) higher than the rest of the treatments. This was followed by TI_2 (94.45mm^3) and TI_3 (94.96mm^3), which were similar ($p>0.05$), TI_4 (87.28mm^3) and then treatment TCN (79.2mm^3). Similarly, the MCH value of 26.34pg for TI_1 was significantly ($p<0.05$) higher than the rest of the treatments. This was followed by TI_2 (23.79pg) and TI_3 (24.03pg), which were similar ($p>0.05$), TI_4 (22.0pg), and then TCN (20.82pg).

DISCUSSION

The haematological responses of the experimental fish fed varied dietary levels of *I. batatas* leaf meal as summarized in table 4 revealed significant differences in the various heamatological indices of the treatments (TCN, TI_1, TI_2, TI_3 and TI_4) except for the haemoglobin, packed cell volume and mean cell heamoglobin concentration that showed no significant differences ($P>0.05$). The red blood cell value of 3.0x10^6mm^3 for the control treatment (TCN) was similar to the rest of the treatments except treatment TI_1 (2.26x$10^6/mm^3$) which was significantly ($P<0.05$) low. On the other hand, the white blood cells values of 4.75x$10^4/mm^3$, 4.78x$10^4/mm^3$, 4.68x10^4mm^3 and 4.8X$10^4/mm^3$ for TCN, TI_1, TI_2 and TI_3 respectively were significantly ($P<0.05$) higher than treatment T_4 (4.45x10^4mm^3). These observations seemed to suggest that the haemoglobin, red blood cells and packed cell volume responses to the increasing dietary levels of Ipomea batatas up to 20% compared well with the control. The values of 5.9 - 6.2g/100ml, 2.26 - 3.0x10^6/mm^3, 23.58 - 24.50% and 4.45 - 4.78x10^4/mm^3 for the heamoglobin, red blood cells, packed cell volume and white blood cells observed in this study were in consonance with the values of 5.0 - 15g/100ml, 1.70 - 4.0x10^6/mm^3**,** 22 - 48% and 1.75 -9.25x10^4/mm^3 for the haemoglobin, red blood cells, packed cell volume and white blood cells reported by Bhasker and Rao (1990). These also were similar to the ranges observed by Blakhal and Daisely (1973) and Ochang *et al* (2007). The mean cell volume and mean cell haemoglobin of 106.2fl and 26.34pg, respectively for TI_1 (5% *Ipoma batatas* dietary inclusion levels) were significantly ($P<0.05$) greater than the other dietary inclusion levels of the leaf meal (10%, 15% and 20% levels) which were significantly low. The control treatment (TCN) values of 79.2 fl and 20.82pg respectively were however the least ($P<0.05$). MCHC was not affected by the treatments ($P>0.05$). The MCV, MCH and MCHC values observed in this study seemed to be indications of the deleterious effects of the leaf meal on the haematological profile and performance of the experimental fish. Bhasker and Rao (1990) reported MCV, MCH and MCHC values of 132.8 - 308.4fl, 20.90 - 47.2pg, and 10.90 - 38.10% respectively in their studies in relation to stocking and feeding conditions. These values seem to be in agreement with the observations in this study. These observations were also in line with the 83.49 – 102.46fl, 28.50 – 31.50pg and 30.17 - 34.73% for MCV, MCH and MCHC values respectively reported by Ochang, *et al* (2007) in their study with palm oil. The observations of this study therefore seemed to suggest that the haemoglobin, red blood cells and packed cell volume responses to the increasing dietary levels of Ipomea batatas up to 20% compared well with the control. The observed MCV, MCH and MCHC values however seemed to be indications of the deleterious effects of the leaf meal on the haematological profile and performance of the experimental fish.

Table 4 Haematological responses of *Heteroclarias* (*Heterobranchus* bidorsalis x *Clarias gariepinus*) fingerlings fed varied levels of I. *batatas* leaf meals

Variable parameters	Dietary levels of *I. batatas* leaf meal					SEM
	TCN(0%)	TI_1(5%)	TI_2(10%)	TI_3(15%)	TI_4(20%)	
Haemoglobin (g/100ml)	5.90^{NS}	5.95^{NS}	6.08^{NS}	6.20^{NS}	6.00^{NS}	0.17
Red blood cells (10^6/mm^3)	3.00^{a}	2.26^{b}	2.56^{ab}	2.58^{ab}	2.73^{a}	0.07
Packed cell volume (%)	23.58^{NS}	24.00^{NS}	24.18^{NS}	24.50^{NS}	23.83^{NS}	0.54
White blood cells (10^4/mm^3)	4.75^{a}	4.78^{a}	4.68^{a}	4.80^{a}	4.45^{b}	0.08
Mean corpuscular volume (mcv)(mm^3)	79.20^{d}	106.20^{a}	94.45^{b}	94.96^{b}	87.28^{c}	1.40
Mean corpuscular haemoglobin (MCH) (pg)	20.82^{D}	26.34^{a}	23.75^{b}	24.03^{b}	22.00^{c}	0.42
MCHC (%)	25.03^{NS}	24.79^{NS}	25.14^{NS}	25.30^{NS}	25.17^{NS}	0.32

$^{a, b, c, d}$ Means within a row with different superscripts are significantly different ($p< 0.05$)
RBC-red blood cell, WBC-white blood cell, HB-haemoglobin, PCV-packed cell volume, MCH-mean corpuscular volume, MCH-mean corpuscular haemoglobin MCHC- mean corpuscular haemoglobin concentration

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