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HAEMATOLOGICAL RESPONSES OF HETEROCLARIAS FED DIETARY LEVELS OF *MICRODESMIS PUBERULA* LEAF MEAL

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Abstract: The haematological responses of feeding 35% isonitrogenous dietary levels of 0%, 5%, 10%, 15% and 20% *Microdesmis puberula* leaf meal on Heteroclarias (*Heterobranchus bidorsalis* x *Clarias gariepinus)* post fingerlings were assessed. These were fed to the fingerlings, randomly assigned to 5 treatments: 0%-control(TCN), 5%(TM_1), 10%(TM_2), 15%(TM_3) and 20% (TM_4) MPLM 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 collected 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). There were significant ($p>0.05$) differences among the treatments, except for the packed cell volume. The *haemoglobin* value for TM_4 was significantly ($p>0.05$) higher than all the other treatments. TCN (control) was different ($p<0.05$) from TM_1 and TM_3. Besides, there was no significant ($p>0.05$) difference between TM_1 and TM_3 and between TCN and TM_2 ($p>0.05$). The red blood cell values for TCN and Tm_4 respectively were significantly ($p>0.05$) higher than the other treatments. These were followed by TM_2 and TM_3, which were in the same vein significantly ($p>0.05$) different from TM_1. The white blood cell of the fish of TM_4 was significantly ($p>0.05$) higher than the other treatments. TM_3 on the other hand was significantly ($p<0.05$) more than TM_1 and TM_2. There was, however, no significant difference between TM_1 and TM_2, and between TM_3 and TCN (control) ($P>0.05$). TM_1 had the highest MCV value of 100.47mm^3. This was followed by 92.53mm^3, 91.30mm^3, and 83.02mm^3 for TM_3, TM_2 and TM_4, respectively, while TCN (79.20mm^3) was the least. The MCH values of 23.80pg, 22.68pg and 23.86pg for TM_1, TM_2 and TM_4, respectively were significantly ($p>0.05$) higher than those of TCN (20.82pg) and TM_3 (21.25pg).The mean cell Haemoglobin concentration value of 28.73% for TM_4 however was significantly ($p>0.05$) higher than the rest of the treatments. This was followed by TCN (25.02%) and TM_2 (24.84%) which were significantly ($p>0.05$) higher than TM_3 (22.94%). TM_2 (23.70%) was similar to the values for TCN and TM_2.

Key Words: Haematological; Heteroclarias; Microdesmis puberula leaf meal.

INTRODUCTION

Alternative protein and energy sources using tropical browse plant leaf meals like *Microdesmis puberula* and other terrestrial or fresh water aquatic plant leaf meals due to their easy availability, potentials for nutrients provision and low procurement cost, would seem to provide acceptable feeding regimes for species of fish like *Clarias gariepinus*, *Heterobranchus bidorsalis, hybrids,* other catfishes and farm animals generally (Udedibie and Opara, 1998; Reyes and Fermin, 2003; Ochang *et al* , 2007 Anyanwu *et al.,* 2011). Besides, they grow luxuriously in the humid tropics, and much of these are underexploited.

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. however have been analysed 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; Anyanwu *et al.,* 2011). Nutrient utilization and biological values of feeds and feedstuffs evaluation may seem inconclusive without adequate consideration on their implications on the physiological and health status of the animal for example fish. It is against this background that this study was designed to determine the effect of MPLM on the haematology of Heteroclarias (*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 *Microdesmis puberula* 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 coloration. 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 TM_1, TM_2, TM_3, and TM_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.

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.

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Table 1: Experimental diets using *Microdesmis puberula* leaf meal (MPLM)

Ingredients	Dietary levels of MPLM				
	0%	5%	10%	15%	20%
Maize	30.6	26.8	23.1	19.3	15.5
Fish meal	19	19	19	19	19
Soyabean meal	45	43.8	42.5	41.3	40.1
MPLM	0	5	10	15	20
Cassava starch	2	2	2	2	2
Palm oil	1	1	1	1	1
Bone meal	1	1	1	1	1
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	100	100	100	100

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 *Microdesmis puberula* 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), TM_1, TM_2, TM_3 and TM_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°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 anaesthetized 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 corpuscular volume, mean corpuscular haemoglobin and mean corpuscular 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 fiber 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 *Microdesmis puberula* leaf meal were 19.32%, 8.00%, 4.60%, 17.35%, 40.03% and 10.70% for crude protein, ash, lipids, crude fibre, nitrogen free extract and dry matter respectively. Water quality condition in the experimental aquaria of mean values 26.05 ± 0.01°C, 6.60 ± 0.03 and 4.91 ± 0.06mg/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%)	Tm_1 (5%)	Tm_2 (10%)	Tm_3 (15%)	Tm_4 (20%)	Mean
Temperature (°C)	26.05	26.02	26.04	26.06	26.1	26.05±0.01
pH	6.5	6.75	6.6	6.58	6.55	6.60±0.03
Dissolved Oxygen (mg/l)	5.15	4.85	5.01	4.8	4.78	4.91±0.06

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 MPLM				
	0%	5%	10%	15%	20%
Crude protein(%)	34.98	35.02	35.01	35	34.99
Crude fibre (%)	2.93	3.98	5.08	6.03	7.12
Ether extract (%)	7.35	7.9	8.38	9.09	10.1
Ash (%)	13.7	13.44	13.28	12.46	12.34
ME (Kcal/kg)	3244.74	3119.84	2994.74	2869.49	12.34

The haematological responses *Heteroclarias (H. bidorsalis* ♀ *C. gapinusrie)* fed varied dietary levels of *Microdesmis puberula* leaf meal are shown in table 4. There were significant ($p>0.05$) differences among the treatments, except for the packed cell volume. The haemoglobin value for TM_4 (6.56g/100ml) was significantly ($p>0.05$) higher than all the other treatments. TCN (5.90g/100ml) was different ($p<0.05$) from TM_1 (5.50g/100ml) and TM_3 (5.20g/100ml). Besides, there was no significant ($p>0.05$) difference between TM_1 and TM_3 and between TCN and TM_2 ($p>0.05$). The red blood cell values of 3 x 10^6 /mm^3 and 2.75 x 10^6/mm^3 for TCN and Tm_4 respectively were significantly ($p>0.05$) higher than the other treatments. These were followed by TM_2 (2.46 x 10^6/mm^3) and TM_3 (2.45 x 10^6/mm^3), which were in the same vein significantly ($p>0.05$) different from TM_1 (2.31 x 106/mm3).

The white blood cell of the fish of TM_4 (5.05 X10^4/mm^3) was significantly ($p>0.05$) more than the other

treatments. TM_3 (4.85 x 104/mm3) on the other hand was significantly ($p<0.05$) more than TM_1 (4.58 x 104/mm3) and TM_2 (4.60 x 104\mm3). There was, however, no significant difference between TM_1 and TM_2, and between TM_3 and TCN (4.75 x 104/mm3) ($P>0.05$). TM_1 had the highest MCV value of 100.47mm^3. This was followed by 92.53mm^3, 91.30mm^3, and 83.02mm^3 for TM_3, TM_2 and TM_4, respectively, while TCN (79.20mm^3) was the least.

The MCH values of 23.80pg, 22.68pg and 23.86pg for TM_1, TM_2 and TM_4, respectively were significantly ($p>0.05$) higher than those of TCN (20.82pg) and TM_3 (21.25pg). The mean corpuscular haemoglobin concentration value of 28.73% for TM_4 however was significantly ($p>0.05$) higher than the rest of the treatments. This was followed by TCN (25.02%) and TM_2 (24.84%) which were significantly ($p>0.05$) higher than TM_3 (22.94%). TM_2 (23.70%) was similar to the values for TCN and TM.

Table 4: Haematological responses of *Heteroclarias* (*Heterobranchus* bidor*s*alis x *Clarias gariepinus*) fingerlings fed varied levels of *M. puberula* leaf meals.

Variable parameters	Dietary levels of *Microdemis puberula* leaf meal					SEM
	TCN (0%)	Tm_1 (5%)	Tm_2 (10%)	Tm_3 (15%)	Tm_4 (20%)	+ -
Haemoglobin (g)	5.90^{b}	5.50^{cd}	5.58^{bc}	5.20^{d}	6.56^{a}	0.17
Red blood cells (10^{-6}/mm^3)	3.00^{a}	2.31^{c}	2.46^{b}	2.45^{b}	2.75^{a}	0.07
Packed cell volume (%)	23.58^{NS}	23.21^{NS}	22.46^{NS}	22.67^{NS}	22.85^{NS}	0.63
White blood cells (10^4/mm3)	4.75^{bc}	4.58^{c}	4.60^{c}	4.85^{b}	5.05^{a}	0.08
Mean corpuscular volume (mcv)(mm^3)	79.20^{c}	100.47^{a}	91.30^{c}	92.53^{b}	83.02^{d}	0.29
MCH (pg)	20.82^{b}	23.80^{a}	22.68^{a}	21.25^{b}	23.86^{a}	0.58
MCHC (%)	25.02^{b}	23.70^{bc}	24.48^{b}	22.94^{c}	28.73^{a}	0.59

[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

DISCUSSION

The haematological responses of the experimental fish fed varied dietary levels of *M. puberula* as summarized in table 4 showed significant differences in the various heamatological indices of the treatments, (TCN, TM_1, TM_2, TM_3 and TM_4) except the packed cell volume that showed no significant differences ($p>0.05$). The haemoglobin level of the fish on TM_4 (6.56g\100ml) was significantly ($P>0.05$) higher than the rest of the treatments. This was followed by TCN (5.9g/100ml), TM_2 (5.58g/100ml), TM_1 (5.5g/100ml) and then TM_3 (5.2g/100ml) which was the least. In the same vein, the red blood cells value of 3x 10^6 /mm^3 and 2.75 x10^6/mm^3 for TCN and TM_4 respectively, were significantly ($P>0.05$) higher than the rest of the treatments. These were followed by TM_2 (2.46x10^6 /mml^3) and TM_3 (2.45x10^6/mm^3) which were also significantly ($P>0.05$) higher than TM_1 (2.31x10_6 /mm^3). The packed cell volume of the experimental fish ranged from 22.46% to 23.58%, and showed no significant differences ($P>0.05$) among the treatments. These haematological responses might be an indication of effective tolerance and utilization of the leaf meal in supporting measurable haematological profile of the fish, and better still, at 20% dietary inclusion level of the leaf meal. In their haematological studies in relation to stocking and feeding conditions, Bhasker and Rao (1990) reported values of between 5.0g/100ml and 15g/100ml, 1.70x10^6/mm^3 and 4.0x10^6/MM^3 and 22% and 48% for heamoglobin, red blood cells and packed cell volume respectively. Blakhall and Daisley (1973) and Harikrishnam *et al.,* (2003) reported similar ranges in their various studies. These were in agreement with the observations of this study. The white blood cells observed in this study however ranged from 4.5 - 5.05x104mm^3, increasing at higher dietary inclusion level of the leaf meal up to 20% level. The values were also in agreement with the range of 1.75 - 9.25x10^4/mm^3 reported by Bhasker and Rao (1990).

The trend in the erythrocyte indices of the experimental fish (Table 4) indicated positive responses of the fish to the dietary inclusion levels of the leaf meal up to 20% level; indicative of the nutritive potential of the leaf meal. The values of 442.9 - 564.57fl, 145.7 -187.85pg and 32.9 - 33.26% for MCV, MCH and MCHC respectively were reported by Nlewadim and Alum (1999) in their studies. On the other hand, Bhasker and Rao (1990) observed values of 132.8 - 308.4fl, 20.90 - 47.20pg and 10.90 - 38.10% for the MCV, MCH and MCHC respectively in their studies. These values as well as the 83.49 – 102.46fl, 28.50 – 31.50pg and 3.017-34.73% for MCV, MCH and MCHC values respectively reported by Ochang *et al* (2007) were in agreement with the ranges of 79.20 to 100.47fl, 20.82 and 23.86pg to 22.94 and 2502% for the MCV, MCH to MCHC observed in this study. The trend in the erythrocyte indices of the experimental fish therefore revealed positive responses of the fish to the dietary inclusion levels of the leaf meal up to 20% level; indicative of the nutritive potential of the leaf meal.

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