Haematological responses of hybrid of *Heterobranchus bidorsalis* and *Clarias gariepinus* fed dietary levels of *Carica papaya* leaf meal.

ANYANWU[a], D.C., UDEDIBIE[b], A.B.I. AND OSUIGWE[b], D.I., Ogwo[a], V. O.

[a]Department of Agric. Science, Alvan Ikoku Federal College of Education, Owerri.
[b] School of Agriculture and Technology, Federal University of Technology, Owerri.
ahamefula_dan@yahoo.com

ABSTRACT: The haematological effects of feeding 35% isonitrogenous dietary levels of 0%, 5%, 10%, 15% and 20% *Carica papaya* 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% (TC_1), 10% (TC_2), 15% (TC_3) and 20% (TC_4) CPLM 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. The haemoglobin and mean cell haemoglobin concentration levels for TC_4 were significantly (P>0.05) lower than the rest of the treatments. TC_1 was significantly the least in red blood cell level, while the highest for the packed cell volume. The white blood cell value was highest for TC_1, followed by TC_4 and TCN, while TC_2 and TC_3 were the least. The mean cell volume of TC_1 was significantly (P<0.05) higher than the rest of the treatments, followed by TC_3, TC_4, TC_2 and then TCN. The mean cell haemoglobin value for TC_I was significantly (P<0.05) higher than the rest of the treatments, followed by TC_2, TC_3, TC_4 and then TCN, which was the least. TC_2 and TC_3 were not significantly (P>0.05) different from each other.

[Anyanwu, Udedibie, Osuigwe, Ogwo. **Haematological responses of hybrid of *Heterobranchus bidorsalis* and *Clarias gariepinus* fed dietary levels of *Carica papaya* leaf meal.** World Rural Observations 2011;3(1):9-12]; ISSN: 1944-6543 (Print); ISSN: 1944-6551 (Online). http://www.sciencepub.net/rural.

Keywords: Haematological; hybrid; *Heterobranchus bidorsalis; Clarias gariepinus*; *Carica papaya*

INTRODUCTION

Assessment of nutrient utilization and biological values of feeds and feedstuffs may seem inconclusive without adequate consideration on their implications on the physiological and health status of the animal for example 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). Regular monitoring of haematological parameters of farmed fish according to Bhaskar and Rao (1990) can be used to prevent damage to farming activities. The use of haematological parameters in diagnosing the health of fish is gaining ground as a tool in the management of fish farms. It has been realized that changes in haematological parameters due to unfavourable exogenous factors like adverse water quality, overstocking, starvation etc. are indices of the ill health of cultivated fish. 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 analysed and variously reported as useful tools in assessing the performance, viability and health status of farm fish and animals(Blakhall and Daissssley, 1973; bhaskar and Rao, 1990; Musa and Omoregie, 2001; Harikrishnan *et al,* 2003; Anyanwu *et al,* 2003; Hemre *et al,* 2007). The objective of this study however, was to determine the effect of CPLM 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 *Carica papaya* 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 TC_1, TC_2, TC_3, and TC_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.

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 *Carica papaya* 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), TC_1, TC_2, TC_3 and TC_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 (1990) methods and Kekeocha (2001). 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 7.5).

RESULTS

The haematological responses of hybrid of *Heterobranchus bidorsalis and Clarias geriepinus* fed varied dietary levels of *Carica papaya* leaf meal are shown in table 4. Most of the responses in the study however showed significant differences. The haemogloblin values of fish on TCN (5.9g/100ml), TC_1 (6.25g/100ml), TC_2 (6.0g/100ml) and TC_3 (5.9g/100ml) were significantly ($p<0.05$) higher than TC_4 (5.48g/100ml). The RBC value of 3.0 x10^6/mm^3 for TCN was significantly ($p<0.05$) higher than TC_1 (2.35 x 10^6/mm^3). This was however not different ($p>0.05$) from TC_2 (2.55 x 10^6/mm^3), TC_3 (2.41 x 10^6/mm^3) and TC_4 (2.45 x 10^6/mm^3), while in the same vein these were not significantly ($p>0.05$) different from TC_1.

The white blood cell values of 5.03 x 10^4/mm^3 and 4.8 x 10^4/mm^3 for TC_1 and TC_4 respectively were not significantly ($p>0.05$) different, but significantly ($p<0.05$) different from the rest of the treatments. Similarly TCN (4.75 x 10^4/mm^3) was not significantly ($p>0.05$) different from TC_3 (4.53 x 10^4/mm^3), but was significantly ($p<0.05$) higher than TC_2 (4.46 x 10^4/mm^3), which was the least value. The PCV value of 24.75% for TC_1 was significantly ($p<0.05$) higher than the rest of the treatments. In the same vein, the MCV of TC_1 (105.32mm^3) was significantly ($p<0.05$) higher than the rest of the treatments, and followed by TC_3 (97.51mm^3), TC_4 (95.30mm^3), TC_2 (93.14mm^3) and then TCN (79.2mm^3). Similarly, the MCH value of 26.60pg for TC_1 was significantly ($p<0.05$) higher than the rest of the treatments, and this was followed by TC_2 (23.53pg), TC_3 (24.48pg), TC_4 (22.21pg) and then TCN (20.82 pg), which was the least. TC_1 and TC_2 were not significantly ($p>0.05$) different from each other.

The MCHC values of 25.08%, 25.25%, 26.26% and 25.10% for TCN, TC_1, TC_2, and TC_3, respectively were significantly ($p<0.05$) higher than that of TC_4 (23.47%), which was the least.

DISCUSSION

The heamatological responses of the experimental fish fed varied dietary levels of *C. papaya* Leaf Meal as summarized in table 4 showed significant differences in the various haematological indices for the treatments (TCN, TC_1, TC_2, TC_3 and TC_4). The heamoglobin values of 5.4g/100ml, 6.25g/100ml, 6.0g/100ml and 5.9/100ml for TCN, TC_1, TC_2, and TC_3 respectively were significantly ($P<0.05$) higher than TC_4 (5.48g/100ml). The red blood cell value of

3x10^6/mm^3 for TCN was similar to those of TC_2 (6.0g/100ml), TC_3(5.9g/100ml) and TC_4(5.48g/100ml), while TC_1 (2.35x10^6/mm^3) was also significantly the least. The PCV value of 24.75% for TC_1 was significantly ($P<0.05$) higher than the rest of the treatments, which were not different. The trends observed in the haemoglobin, red blood cells and packed cell volume responses for the experimental fish were indications that the dietary inclusion levels of *C. papaya* compared favorably with the control diet. The white blood cells value of 5.03X10^4/mm^2 for TC_1 however was similar to that of TC_4, and significantly ($P<0.05$) higher than the rest of the treatments, while TC_2 (4.48x10^4/mm^3) was the least. The variability in the WBC responses of the fish might be due to some exogenous and endogenous factors in the experiment, and in relation to the dietary inclusion levels of the leaf meal in the diets. *Okoli et al* (2003) and Bairagi (2004) reported deleterious effects of some anti-nutritional factors in unconventional feedstuffs as leaf meals. Bhasker and Rao (1990), however 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. They also reported 1.75 -9.25x10^{4}mm^3 for the white blood cells. Similar range values were also reported by Blakhall and Daisley (1973) and Ochang *et al* (2007). These were in agreement with the ranges of 5.48 - 6.25g/100ml, 2.35 - 3.0x106/mm^3, 2.335 - 24.75% and 4.48 - 5.03X10^4/mm^3 for the heamoglobin, red blood cells, packed cell volume and white blood cells values, respectively, observed for the experimental fish fed varied dietary inclusion levels of *Carica papaya* leaf meal in this study.

The mean cell volume of 105.32 fl for TC_1 was significantly ($P<0.05$), the highest while TCN (79.2fl) was the least. Similarly, for mean cell haemoglobin, TC_1 (26.60pg) was significantly ($P<0.05$) higher than the rest of the treatments, while that of TCN (20.82pg) was the least. The mean cell haemoglobin concentration values of 25.02%, 25.25%, 25.26% and 25.10% for TCN, TC_1, TC_2 andTC_3 were significantly ($P<0.05$) higher than that of TC_4(23.47%) which was the least. The trends in MCV, MCH and MCHC values of this study seemed to be suggestive 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 - 47.20pg and 10.90 – 38.10% respectively in their studies in relation to stocking and feeding conditions. Ochang *et al* (2007) observed similar range values, although with higher MCHC values. These seemed to be in agreement with the observations in this study. The result of this trial tended to show that *Carica papaya* leaf meal has no strong haematological attribute as feedstuff in the diet of catfish hybrid (*Heterobranchus bidorsalis* × *Clarias gariepinus).*

Table 1: Experimental Diets using *Carica papaya* leaf meal (CPLM), Dietary levels of CPLM

Ingredients	0%	5%	10%	15%	20%
Maize	30.6	28.8	26.9	25.1	23.4
Fish meal	19.0	19.0	19.0	19.0	19.0
Soyabean meal	45.0	41.8	38.7	35.5	32.2
CPLM	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

Table 2: Chemical composition

Crude protein (%)	34.98	35.04	35.05	35.05	35.06
Crude fibre (%)	2.93	3.54	4.34	4.84	5.50
Ether Extract (%)	7.35	7.18	7.87	8.42	8.75
Ash (%)	13.70	13.33	13.82	12.93	12.72
ME (Kcal/kg)	3244.74	3187.89	3130.94	3074.09	3017.34

Table 3: Limnological characteristics of the experimental ecosystem.

Parameters	TCN	TC_1	TC_2	TC_3	TC_4
Temperature(^{0}C)	26.05	26.05	26.08	26.07	26.o8
Ph	6.50	6.70	6.68	6.72	6.45
Dissolved oxygen(mg/l)	5.15	5.05	5.12	5.02	4.80

Table 4: Haematological responses of hybrid of *Heterobranchus bidorsalis* and *Clarias gariepinus* fingerlings fed varied levels of *C. papaya* leaf meal.

Variable parameters	*Carica papaya* Leaf meal					SEM
	TCN(0%)	TC_1(5%)	TC_2(10%)	TC_3(15%)	TC_4(20%)	
haemoglobin (g/100ml)	5.9^a	6.25^a	6.0^a	5.9^a	5.48^b	0.19
Red blood cells ($10^6/mm^3$)	3.0^a	2.35^b	2.55^{ab}	2.41^{ab}	2.45^{ab}	0.07
Packed cell volume (%)	23.58^b	24.75^a	23.75^b	23.5^b	23.35^b	0.46
White blood cells ($10^4/mm^3$)	4.75^{bc}	5.03^a	4.48^d	4.53^{cd}	4.8^{ab}	0.11
Mean cell volume (mcv)(mm^3)	79.20^e	105.32^a	93.14^d	97.51^b	95.30^c	0.26
Mean cell haemoglobin (MCH) (pg)	20.82^d	26.60^a	23.53^b	24.48^b	22.21^c	0.45
Mean cell haemoglobin concentration (MCHC) (%)	25.02^a	25.25^a	25.26^a	25.10^a	23.47^b	0.22

[a, b, c, d] Means within a row with different superscripts are significantly different ($p < 0.05$)

References

1. Anyanwu, D. C., Umesiobi, D. O. and Orji, B. O. (2003). Diurnal Climate pressures on haematology and blood Biochemistry of West African Dwarf sheep. *The Nigerian Agric. Journal, 34:103 - 109.*
2. Anyanwu, D. C. (2008). *Effects of some tropical plants leaf meals on the performance of Heteroclarias (H. bidorsalis x C. gariepinus)*. PhD Thesis, Federal University of Technology, Owerri, Imo State, Nigeria, 203pp + xvii.
3. O. A. C. (1990). Association of Official Analytical chemist. Official methods of analysis 15th ed., Vinginia, U.S.A.
4. Bairagi, A., Sarka, G. K., Sen, S. K. and Ray, A. K. (2004). Evaluation of the nutritive value of *Leucaena leucoephala* leaf meal, innoculated with fish intestinal bacteria *Bacillus subtilis* and *Bacillus circulans* in formulated diets for rohu, *labeo rohita* (Hamitton) fingerlings. *Aquaculture Research, 35 (5):436 - 446.*
5. Bhaskar, B. R. and Rao, S. K. (1990). Use of haematological parameters as diagnostic tools in determining the health of milkfish (*Chanos chanos* (forskall), in Brackish water culture. *Aquaculture and fisheries mgt., 21:125 – 129.*
6. Blakhall, P. C. and Daisley, K. W. (1973). Routime haematological methods for use with fish blood. *Jorn. Fish boil, 5:771 – 781.*
7. Conroy, D. A. and Herman, R. C. (1970). *Textbook of fish diseases*. T. E. H. Publication Inc., Jersey City, 302pp.
8. Harikrishnan, R., Nisha, R. M. and Balasundaram, C. (2003). Hematological and Biochemical parameters in common carp, *Cyprinus carpio*, following herbal treatment for *Aeromonus hydrophilia* infection. *Aquaculture, 221;41 – 50.*
9. Hamre, G. I., Sagstd, A., Bakke – Mekellep, A., Acierno, M., Maffia, M., Osiash, M., Ystad, A. and Krogelahi, A. (2007) Nutritional, Physiological and histological responses in Allantic Salmon, *Salmo salar I.* fed diets with genetically modified maize. *Aquaculture Nutrition, 13: 186 – 199.*
10. Kekeocha, C. C. (2001). *Animal Nutrition in the tropics.* Onii Publishing House, Owerri, 152pp.
11. Musa, S.O. and Omoregie, E. (1999). Haematological changes in mudfish, *Clarias gariepinus* (Burchel) exposed to malachite green. *Journal of Aquatic Sciences, 14:37 – 42.*
12. Nlewadim, A. A. and Alum, O. U. (1999). Haematological studies of some culturable fish species in Umuahia area of Abia State. Proceeding of the 4th Annual Conference of Animal Science Association of Nigeria, September 14 – 16, 1999, Ibadan, pp 143 – 145.
13. Ochang, S. N., Fagbenro, A. O. and Adebayo, T.O. (2007). Growth performance, body composition, haematology and product quality of the African catfish (*Clarias gariepinus*) fed diets with palm oil. *Pakistan Journal of Nutrition 6(5) : 452 – 459.*
14. Ochang, S. N., Fagbaro, A. O. and Adebayo, T. O. (2007) Influence of dietery palm oil on growth response, carcass composition, haematology and organoleptic properties of Juvenile nile tilapia, Oreochromis niloticus. *Pakistan Journal of Nutrition 615:424 – 429.*
15. Steel, G. D. and Torrie, J. H. (1980). *Principles and procedures of statistics,* McGraw – Hill Book Co. Inc. New York, 633pp.
16. Wedemeyer, G. A. and Yasutake, W. T. (1977). *Clinical Methods for the assessment of environmental trees on fish health.* Technical paper, 89 Us Department of interior, fish and wildlife services, Washington D. C.pp 1 – 18.

12/21/2010