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HAEMATOLOGICAL PROFILES OF *CLARIAS GARIEPINUS* FINGERLINGS FED GRADED LEVELS OF BLOOD MEAL – BOVINE RUMEN DIGESTA BLEND DIETS

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ABSTRACT

An experiment was conducted to test the effect of feeding graded levels of Blood meal – Bovine rumen digesta blend diets (BMBRD) on the haematological profiles of *Clarias gariepinus* fingerlings (10.32±1.96 g). Five approximately iso-nitrogenous (35% crude protein) experimental diets were prepared with 0%, 25%, 50%, 75% and 100% BMBRD meal inclusion respectively to replace the fishmeal component of the control diets. The prepared experimental diets were fed in duplicate to 8-week old *C. gariepinus* fingerlings in each dietary group for 86 days. A commercial feed was exclusively fed as the control diet. The fish stocked in 12 glass tanks (60 cm x 30 cm x 30 cm) at the rate of 12 fish per tank were fed at the rate of 5% of their body weight per day, in two equal installments. Analysis showed that the water pH in each of the culture media significantly differ ($P < 0.05$) from each other. The haemoglobin values of the fish fed the control and 75% BMBRD diets were significantly different ($P < 0.05$) from the haemoglobin. However, the MCHC values of the fish fed experimental diets were not significantly ($P > 0.05$) from the MCHC value of the fish fed control (DBR) diet. The study concluded that blood meal – bovine rumen digesta could satisfactorily replace fishmeal in the diet of *C. gariepinus* fingerlings without affecting the haematological profiles of the fish.

Keywords: Blood meal, Rumen digesta, *Clarias gariepinus*, Haematology.

INTRODUCTION

Clarias gariepinus Burchell is one of the most cultured fish species in Nigeria because of the good adaptability to captivity condition, rapid growth rate, flesh tastiness, hardiness and disease resistance ability (Skelton, 1993; Olagunju *et al.*, 2007; Anoop *et al.*, 2009 and Vanguard, 2009). The plasticity of the catfish diet (Anoop *et al.*, 2009) and its ability to convert waste by-product feedstuffs into useful fish flesh make the species the choice fish of culture in Nigerian aquaculture (Anoop *et al.*, 2009 and Emokaro, 2010).

Fish meal which is the main ingredient in *Clarias gariepinus* diet is a primary choice protein source because it increases feed efficiency and fish growth through better feed palatability, digestion and nutrient absorption

(Mile and Chapman, 2012). Due to its expensiveness however, promising alternative plant and animal protein sources that are cheap and affordable have been tried in catfish diets to replace the fish meal component (Solomon and Sadiku, 2007; Goda *et al.*, 2007; Ingweye *et al.*, 2010 and Ferouz *et al.*, 2012). In most cases, total replacement of the fish meal component has not been achieved due to lack of some essential amino acids (Anderson *et al.*, 1995), presence of anti-nutritional factors or toxicants (Murray *et al.*, 2010 and Ferouz *et al.*, 2012) or poor feed digestibility (Murray *et al.*, 2010).

Blood meal, a common sustainable source of animal protein (Otubusin *et al.*, 2009), has not been successfully utilized as a wholesale substitute for fish meal in catfish feeds due to observed deficiencies (Otubusin *et al.*, 2009).

Documented deficiencies include poor amino acids balance with lysine being relatively high (7-8%) and isoleucine being very low (Sauvant, 2004). The relatively high lysine content in the blood meal makes it an excellent supplemental protein source to complement plant included feed ingredient such as cattle rumen digesta (McDonald *et al.*, 2002).

The heterogenous nature of cattle rumen digesta made up of dense microbial population (Czerkawski, 1986) and digested feed at different stages of degradation (McDonald *et al.*, 2002) made it a suitable candidate in various animal feed preparations (Agbabiaka *et al.*, 2012). Odunsi (2003) and Dairo *et al.* (2005) had also reported that blend of cattle rumen digesta complemented the amino acid in-balance reported in the blood meal. Rumen contents has also been shown to be suitable feed ingredient in catfish diets because it contains no anti-physiological factors (Adeniji and Balogun, 2002). Studies on blend of bovine blood meal and rumen digesta (BBRDM) as a replacement for fishmeal have been reported in the diet of *C. gariepinus* fingerlings (Adewumi, 2012; Adewole, 2014). The main objective of the present study was to examine the haematological profiles of *Clarias gariepinus* juveniles fed differently substituted Blood Meal – Bovine Rumen Digesta diets

MATERIALS AND METHODS

Experimental Design

One hundred and forty four (144) laboratory acclimatized 8-weeks old *C. gariepinus* juveniles (10.32 ± 1.96 g) were fed graded levels of 35% crude protein iso-nitrogenous Blood Meal – Bovine Rumen Digesta based diets. The BMBRD meal was made from mixture of fresh bovine blood and rumen digesta in the ratio of 1:1 (weight/weight), cooked for 60 minutes with stirring until it formed a paste which was sundried on a clean drying slab for 4 days (Adewole, 2014). The dried BMBRD blend was later ground into a meal and analyzed for the proximate composition (A.O.A.C., 1990). Experimental diets were then formulated using fish meal, wheat offal and yellow maize meals (purchased from Segsco Farm Feeds, Ile-Ife) mixed with graded levels of BMBRD meal. In the experimental diets, the fish meal component

of the diet was replaced at 0, 25, 50, 75 and 100% respectively by the BMBRD meal. The ingredients analysed for their proximate composition (Table 1) were weighed according to the formulations (Table 2), moistened, thoroughly mixed and pelletized using 2 mm die in a Horbart pelleting machine. The moist pellets tagged 0% BMBRD, 25% BMBRD, 50% BMBRD, 75% BMBRD and 100% BMBRD diets respectively were sundried for 5 days until crispy and stored at room temperature for subsequent experimental feeding. The experimental diets were analyzed for the proximate composition (AOAC, 1990) to confirm the formulations (Table 1).

Experimental Feeding

The fish were stocked in duplicates for each dietary treatment and the control at the rate of 12 juvenile per tank (60 cm x 30 cm x 30 cm). The entire test fish were starved for 24 hr prior to the commencement of the experimental feeding to allow for digestion of already eaten food and also prepare the fish for the test diets. The test diets were fed twice daily, at the rate of 5% of the body weight, shared between 0800hr to 0900hr and 1700hr to 1800hr daily for a period of 86 days. The tanks were cleaned every 3 days and replenished with water from a holding reservoir

Water Quality Determination

The water quality parameters analysed in duplicates in the culture media were temperature, pH and dissolved oxygen which were determined *in situ* daily using mercury in glass bulb thermometer, Jenway 3020 pH meter and Milwaukee Dissolved Oxygen Meter (MW 600) respectively. The levels of calcium, magnesium, and nitrate ion were determined weekly according to the methods of Ademoroti (1996).

Haematological Techniques

Blood samples were taken by caudal vein puncture using a 2 ml syringe on a set of four *Clarias gariepinus* juveniles from each treatment tank separately into a 2 ml heparinized tubes. Haematocrit (Hct) was determined using the methods of Snieszko (1960) while the haemoglobin level were determined using Cyanomethaemoglobin (Larsen and Snieszko, 1961). The red and white blood cell counts were determined using Improved Neubauer Counting

Chamber while the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) were obtained according to the method described by Dacie and Lewis (2001).

Statistical analysis

The data collected were reported as means (\pm S.D) and subjected to analysis of variance (ANOVA) and Duncan's New Multiple Range Test ($P < 0.05$) using the statistical software package SPSS 16.0

RESULTS

Water Quality

The mean temperature of water in the culture tanks which varied between $25.64^{\circ}\text{C} \pm 0.06$ (75% BMBRD treatment) and $26.25^{\circ}\text{C} \pm 0.35$ (control treatment) were not significantly different ($P > 0.05$) from each other (Table 3). Irrespective of the feed offered, the water in all the culture tanks was slightly acidic varying between 6.11 ± 0.03 (25% BMBRD) and 6.66 ± 0.01 (75% BMBRD). Analysis also showed that the water pH in each treatment tank were significantly different ($P < 0.05$) from each other. Introduction of the experimental feed into the culture tanks decreased the dissolved oxygen content significantly. Analyses showed a significant ($P < 0.05$) variation in DO content between the treatments with the dissolved oxygen levels varying between 2.36 ± 0.74 mg/L (50% BMBRD treatment) and 7.76 ± 0.79 mg/L (control treatment).

The NO_3 levels in the culture water were not significantly affected by the introduction of the experimental and the control feeds (Table 3). However, the Ca^{2+} concentrations in the culture tanks which varied slightly with treatments were not significantly different ($P > 0.05$) but was found to be significantly lower ($P < 0.05$) than the Ca^{2+} level recorded when the fish was fed the control diet. The Mg^{2+} concentrations which varied between 7.01 ± 0.15 mg/L (25% BMBRD treatment) and 7.83 ± 0.20 mg/L (0% BMBRD treatment) were not significantly different ($P > 0.05$) in the experimental and the control culture tanks.

Haematological Indices

Analyses of the haematological indices of the cultured fish (Table 4) revealed that the packed cell volume (PCV) were not significantly different ($P > 0.05$) in the fish fed the experimental diets and the control. Fish fed 0% BMBRD diet had the lowest haemoglobin (Hb) value (6.40 ± 1.56 g/dL) while the highest haemoglobin value (9.12 ± 1.68 g/dL) was recorded for the fish fed 75% BMBRD diet. However, the haemoglobin values of the fish fed the control and 75% BMBRD diets were not significantly different ($P > 0.05$) from each other, but were significantly different ($P < 0.05$) in the fish fed 0% BMBRD, 50% BMBRD and 100% BMBRD diets (Table 4).

In spite of the recorded variations in the RBC, there were no significant differences ($P > 0.05$) in the RBC of the fish fed the experimental graded BMBRD diets and the control. The lowest white blood cell count (WBC) ($1.64 \times 10^{-12}/\text{L}$) was recorded in the fish fed 50% BMBRD diet while the highest value ($2.39 \times 10^{-12}/\text{L}$) was recorded in the fish fed 0% BMBRD diet. The recorded WBC which were not significantly different ($P > 0.05$) between the fish fed the control diet and 50% BMBRD diets were significantly lower ($P < 0.05$) in the fish fed 0% BMBRD diet. Mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and the mean corpuscular volume (MCV) values of the fish fed different experimental diet were generally low in the fish fed 0% BMBRD diet. The mean MCH was significantly lower ($P < 0.05$) in the fish fed 0% BMBRD diet when compared with those fed on other experimental and control diets. The MCV in the fish fed on the control, 0% BMBRD, 50% BMBRD, and 75% BMBRD diets were found to be significantly lower ($P < 0.05$) than in the fish fed 25% BMBRD and 100% BMBRD diets

The fish fed control diet however, had the highest mean corpuscular haemoglobin concentration (MCHC) (33.23 ± 0.24 g/dL) which was significantly higher ($P < 0.05$) than the MCHC of the fish fed 0% BMBRD diet. However, the MCHC values were found not to be statistically different ($P > 0.05$) from those fed 25% BMBRD, 50% BMBRD, 75% BMBRD and 100% BMBRD diets.

DISCUSSION

Temperature of aquatic environment is important for ensuring survival and normal metabolism of fish (Forghally *et al.*, 1973). In all the treatments during the period of study, water temperature was within the recommended range of between 25⁰C and 32⁰C for optimum growth in warm water fishes (Boyd and Licktoppler, 1979). The pH, which was observed to be slightly acidic in the culture tanks, could be as result of the presence of rumen digesta in the experimental diets. Li *et al.* (2013) had earlier reported that pH of rumen digesta of cows fed by high forage diets are always between 6.0 and 7.0 which is considered to be the optimum for cellulolytic bacteria activity (Mould *et al.*, 1983). The presence of the rumen–digesta blend as an important component of the experimental diets was probably responsible for lowering the pH of culture water. However the pH recorded were still within the recommended range for freshwater fish culture (Boyd, 1979; Olatunde, 2004). The low level of dissolved oxygen in the treatment tanks could probably be attributed to depletion of dissolved oxygen during bacterial decomposition of the organic matters in the uneaten feed and fecal matter. Bacteria respiratory activities in the treatment tanks would also contribute to depletion of dissolved oxygen concentration. Veado *et al.* (2000) observed that introduction of excess organic matter results in depletion of oxygen from aquatic systems especially in warm stagnant conditions.

Haematological Profile

The haematological profile recorded in the fish showed that the experimental diets were not in any form or at any level toxic to the fish indicating that the diets supported the fish eco-physiology to a reasonable extent. Haemoglobin and PCV determinations carried out routinely to check the health status of fish (Agbede *et al.*, 1999) was lower than the values reported by Osuigwe *et al.* (2005) and Agbabiaka *et al.* (2013) for the African catfish fed a tiger nut diet. However, the result was similar to those reported by Adeyemo *et al.* (2003) on haematological response of *C. gariepinus* to temperature variations and Gabriel *et al.* (2004) on the influence of sexual variation and acclimation temperature on the haemoglobin

content and PVC of *C. gariepinus*. Similar results were reported by Terry *et al.* (2000) and Adam (2004) in the Red Tilapia, Nile Tilapia, Curimbata and hybrid Tilapia. The values recorded for haemoglobin and PCV in this study were within the normal range reported for the African catfish (Erondu *et al.*, 1993; Musa and Omoregie, 1999). The red blood cell counts recorded in the fish fed the different experimental diets were higher than what was reported by Adeyemo *et al.*, (2003); Gabriel *et al.* (2004) and Agbabiaka *et al.* (2013). Similar results were however recorded for other fish by Terry *et al.* (2000); Nilza *et al.* (2003) and Adam (2004). The red blood cell count and PCV were probably affected by dietary treatment (Aletor and Egberongbe, 1998). The blood component of the BMBRD meal which is very rich in iron present in haemoglobin of the cow's blood probably enhanced erythropoiesis in the fish. Blood profiles have been reported to be influenced by water quality parameters such as temperature, dissolved oxygen, pH, ammonia (Adeyemo *et al.*, 2003). Increase in WBCs count probably occurred as a response of fish to stress resulting from unfavorable alteration in water quality or production of antibodies against the growth of bacteria in the water media. The relatively lower WBCs count recorded in the fish during the study when compared to values reported by Adam and Agab (2006) and Agbabiaka *et al.* (2013) probably showed that the fish fed the different experimental diets were less susceptible to dietary stress, alteration of water quality, and bacteria infestation when compared with previous studies. The range in the mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) obtained during the study were similar to those reported by Terry *et al.*(2000), Nilza *et al.* (2003) and Gabriel *et al.* (2004). Factors that may have induced the observed variations apart from the dietary treatment include environmental conditions, sex and age of fish. Banergee *et al.* (2002) however reported that under normal conditions, the blood profile should be reasonably constant for any species with changes falling with narrow limit as observed in this study.

Table 1: Proximate composition of the feedstuffs and the experimental diets

	Crude protein (%)	Crude fibre (%)	Lipid (%)	Ash (%)	Moisture (%)	NFE (%)
Ingredients						
Fish meal	68.50	0.40	10.40	17.40	3.00	0.30
BMBRD	45.50	9.48	1.15	10.93	9.82	23.12
Wheat offal	16.34	12.34	1.69	6.58	11.35	51.70
Yellow maize	10.80	3.50	3.60	8.40	11.81	61.89
Diets						
DBR**	45.00	2.60	14.00	7.50	11.00	19.9
0%BMBRD	35.90 ^a	2.14 ^a	3.40 ^a	8.17 ^a	9.99 ^a	40.14
	±0.11	± 0.16	± 0.02	± 0.58	±0.40	
25%BMBRD	35.99 ^a	2.91 ^a	3.50 ^a	7.85 ^a	11.54 ^a	38.21
	±0.01	± 0.19	± 0.02	± 0.34	±1.12	
50%BMBRD	35.98 ^a	3.85 ^b	3.10 ^a	8.40 ^a	10.78 ^a	37.89
	±0.02	± 0.09	± 0.02	± 0.23	±1.46	
75%BMBRD	35.59 ^a	3.87 ^b	4.00 ^a	7.82 ^a	10.49 ^a	38.23
	±0.05	± 0.16	± 0.02	± 1.15	±1.19	
100%BMBRD	35.48 ^a	4.78 ^c	3.90 ^a	8.32 ^a	9.66 ^a	37.94
	±0.02	± 0.07	± 0.02	± 0.24	±0.55	

Means within column with different superscripts are significantly different ($p < 0.05$).

**Manufacturer's nutrient specification

NFE = Nitrogen-free Extract = $100 - (\text{Crude protein} + \text{Crude fibre} + \text{Lipid content} + \text{Moisture content} + \text{Ash})$ (A.O.A.C, 1990).

Table 2: Ingredient compositions in the formulated experimental diet (g/100g)

Ingredient	0% BMBRD	25% BMBRD	50% BMBRD	75% BMBRD	100% BMBRD
Fish meal	52.0	39.0	26.0	13.0	-
BMBRD	-	1.3	2.6	39.0	52
Wheat offal	23.0	23.0	23.0	23.0	23.0
Yellow maize	23.0	23.0	23.0	23.0	23.0
Vit/Min Premix *	1.0	1.0	1.0	1.0	1.0
Vegetable oil	0.5	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100

*Biomix from Bio-organics production were provided per kg of diet: Vitamin A, 12,500 IU; Vitamin D, 2,500 IU; Vitamin E, 40 mg; Vitamin K, 2 mg; Vitamin B1, 3 mg; Vitamin B2, 5.5 mg; Niacin 55 mg; Calcium pantothenate, 11.5 mg; Vitamin B 6, 5mg; Vitamin B 12, 0.025 mg; Choline chloride, 500 mg; Folic acid, 1 mg; Biotin, 0.08 mg; Manganese, 120 mg; Iron, 100 mg; Zinc, 80 mg; Copper, 8.5 mg; Iodine, 1.5 mg; Cobalt, 0.3 mg; Selenium, 0.12 mg; Anti-oxidant, 120 mg.

Table 3: Mean Physico-chemical parameters of the water culture tanks before and during the feeding trials

Treatment	Water Temp. (°C)	pH	Dissolved Oxygen Conc. (mg/L)	Nitrate (mg/L)	Calcium (mg/L)	Magnesium (mg/L)
Control	26.25 ^a	6.55 ^a	7.76 ^a	1.15 ^a	27.65 ^a	7.35 ^a
± S.D	± 0.35	± 0.03	± 0.79	± 0.18	± 1.63	± 0.10
0 % BMBRD	25.83 ^a	6.63 ^b	3.84 ^b	1.05 ^a	22.59 ^b	7.01 ^a
± S.D	± 0.01	± 0.03	± 1.36	± 0.01	± 1.79	± 0.15
25 % BMBRD	25.80 ^a	6.11 ^c	2.40 ^b	2.24 ^a	21.86 ^b	7.05 ^a
± S.D	± 0.01	± 0.03	± 0.11	± 0.33	± 0.88	± 0.63
50 % BMBRD	25.80 ^a	6.54 ^d	2.36 ^b	0.78 ^a	21.01 ^b	7.80 ^a
± S.D	± 0.04	± 0.01	± 0.74	± 0.11	± 0.26	± 0.11
75 % BMBRD	25.64 ^a	6.66 ^c	2.68 ^b	1.25 ^a	21.37 ^b	7.61 ^a
± S.D	± 0.06	± 0.01	± 0.34	± 0.28	± 0.12	± 0.27
100 % BMBRD	25.66 ^a	6.60 ^b	4.58 ^{ab}	1.05 ^a	22.56 ^b	7.83 ^a
± S.D	± 0.01	± 0.01	± 1.62	± 0.01	± 1.08	± 0.20

*Means within column with different superscripts are significantly different (p<0.05).

Table 4: Heamatological parameters of the cultured fish fed the different experimental diets

Treatment	PCV (%)	Heamoglobin (Hb) g/dl	RBC x 10 ¹² L ⁻¹	WBC x 10 ¹² L ⁻¹	MCH (pg)	MCV (fl)	MCHC (g/dl)
Control ± S.D	28 ^a ± 0.70	8.89 ^a ± 0.80	3.88 ^a ± 0.20	1.75 ^a ± 0.18	26.46 ^a ± 5.60	79.66 ^a ± 17.47	33.23 ^a ± 0.24
0 % BMBRD ± S.D	26 ^a ± 2.83	6.40 ^b ± 1.56	3.45 ^a ± 0.87	2.39 ^b ± 0.29	17.26 ^b ± 0.15	73.32 ^a ± 15.30	24.09 ^b ± 5.23
25 % BMBRD ± S.D	28 ^a ± 0.01	8.47 ^{ab} ± 1.89	3.15 ^a ± 0.91	2.04 ^{ab} ± 0.85	28.98 ^{ab} ± 14.45	92.20 ^b ± 28.00	30.47 ^{ab} ± 6.41
50 % BMBRD ± S.D	27 ^a ± 0.70	7.89 ^b ± 0.94	3.94 ^a ± 0.36	1.64 ^a ± 0.50	24.71 ^a ± 0.15	84.10 ^a ± 10.95	29.85 ^{ab} ± 4.34
75 % BMBRD ± S.D	28 ^a ± 2.83	9.12 ^a ± 1.68	3.48 ^a ± 0.34	1.66 ^a ± 0.28	26.56 ^a ± 7.40	80.18 ^a ± 8.81	32.82 ^{ab} ± 5.62
100 % BMBRD ± S.D	25 ^a ± 2.12	7.11 ^b ± 1.60	2.84 ^a ± 0.48	1.71 ^{ab} ± 0.52	24.97 ^a ± 1.34	91.08 ^b ± 13.18	27.81 ^{ab} ± 5.49

Means within column with different superscripts are significantly different (p<0.05).

CONCLUSION

The study concluded that blood meal – bovine rumen digesta could satisfactorily replace fishmeal in the diet of *Clarias gariepinus* juveniles without affecting the haematological profiles of the fish.

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