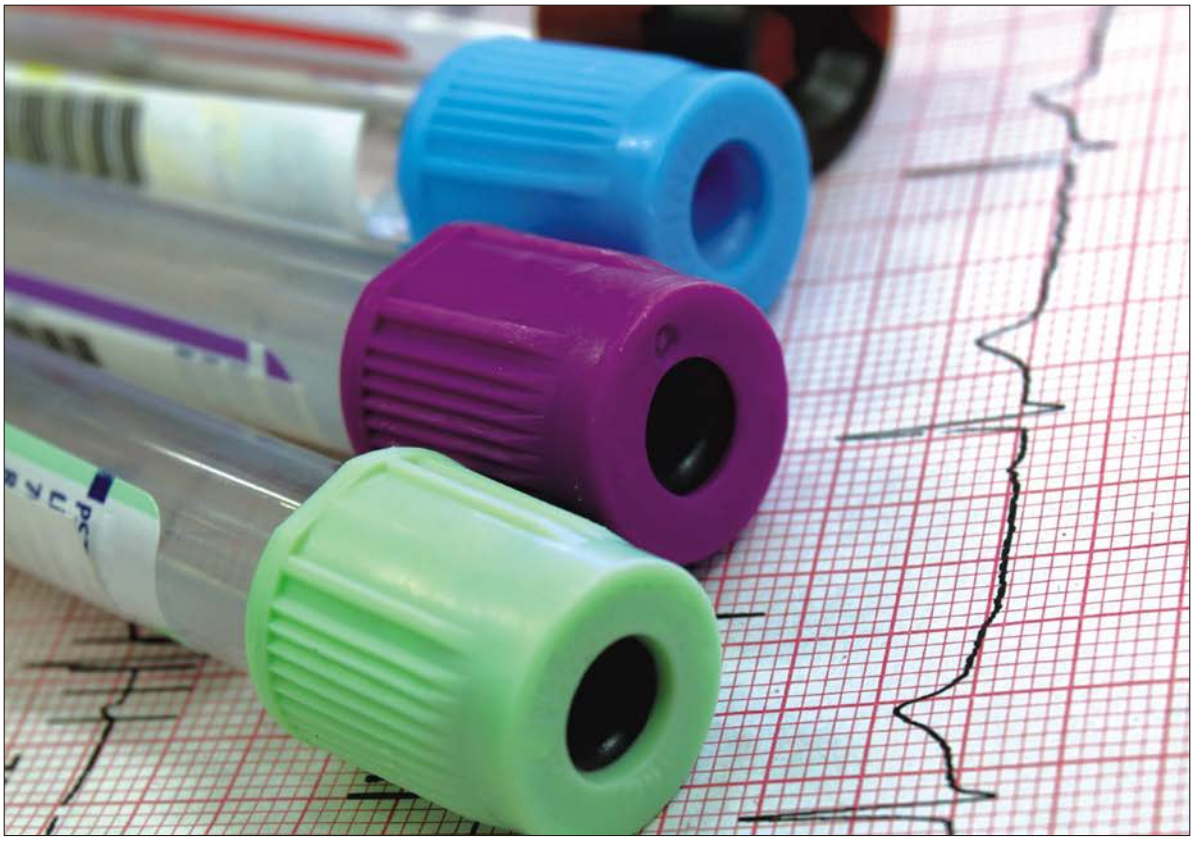

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Research Article
Haematological and Immunological Changes in the Blood of African Catfish (*Clarias gariepinus*, Burchell 1822) Reared Under Different Sex Combinations

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Abstract
The effect of sex combinations on growth and survival of African catfish was evaluated using haematological indices. This study was conducted at the Wet Laboratory of Aquaculture and Fisheries Management Department, University of Ibadan. The experimental set-up involved four treatments labelled as NP (Natural population), MM (Only Male population), FF (Only Female population) and MF (Male and Female population). Juvenile *C. gariepinus* (66.56±2.25 g) were randomly stocked in twelve plastic aquaria (2.9 m^3) at a density of 10 fish/tank and fed twice daily in split-rations with commercial diet of 42% crude protein at 3% b.wt. for 84 days. The differences in blood and plasma concentrations in fish before and after experiment were noted. The Pack Cell Volume (PCV) of the treatment groups (32.00±0.10 g L^{-1}) increased significantly relative to initial value (24.00±0.01), while their platelets counts showed significant variations compared to that of initial value. Significantly, Red Blood Cell (RBC) in treatments decrease (2.62±0.01 to 3.81±0.00 mg dL^{-1}) compared to initial value (11.77±0.00 mg dL^{-1}). However, there was no significant difference ($p>0.05$) in the mean Hemoglobin (Hb) values between the initial (8.00±0.10 g L^{-1}) and treatment groups. Also, the treatment groups showed a significant variation in serum biochemical values of total protein (6.00±0.01), albumin (2.23±0.33), cholesterol (105.00±1.00), aspartate aminotransferase (208.00±0.23) and glucose (27.00±0.28) compared to the initial values, total protein (5.27±0.01), albumin (0.75±0.01), cholesterol (117.84±0.02), aspartate aminotransferase (56.90±0.01) and glucose (64.77±0.01). There was also a significant increase in the creatinine concentrations. Hence, it can be concluded that the slight haematological changes observed between the initial value and treatment groups values cannot significantly affect the health of *C. gariepinus* reared under different sex combinations.

Key words: Hematology, *C. gariepinus*, juvenile, sex combination

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Aquaculture production has increased significantly in the last few years in Nigeria, mostly due to increase in farmed catfish (FAO., 2009). In fact, farmed catfish accounts for approximately 50% of Nigeria's domestic annual fish production as demand for fresh and smoked catfish has continued to increase. Tastes and preferences for catfish have been increasing due to availability, cost, consumer preference for white meat and perceived health benefits over other substitutes (Dixie and Ohen, 2006). Hence, catfish farming in Nigeria is becoming more and more intensive with different manipulations to boost its production and enhance its acceptability. In efforts to boost productivity, *Clarias gariepinus* has been subjected to various farming techniques which include sex composition or variability of stocked fish in culture system. This includes monosex culture, mixed sex culture etc., as reported by Omitoyin (2007). However, the effect of various stocking manipulations on physiology of the fish as regard to stress has not been adequately addressed. According to Soivio and Oikari (1976) the application of haematological techniques has proved valuable in monitoring stress responses. Consequently, this study examined the haematological and immunological changes in African catfish reared under different sex combinations.

MATERIALS AND METHODS

Experimental procedure: Two hundred juveniles *C. gariepinus* (66.59±1.86 g) were purchased from a commercial farm in Ibadan, Nigeria. The fishes were transported in two open 25l plastic containers to the Aquaculture and Fisheries Management Laboratory of University of Ibadan and acclimated for 14 days in twelve plastic aquaria specification (2.9 m^3). These tanks were disinfected and filled with dechlorinated well water prior to the commencement of the experiment. The test animals were randomly distributed into the tanks at a density of 10 fish/m^3, labeled as NP (Natural population), MM (Only Male population), FF (Only Female population) and MF (Male and Female population) in replicates of 3. The fishes were fed twice daily (900 and 1800 h) in split-rations with commercial diet of 42% crude protein at 3% b.wt. for 84 days. The negative effect of unconsumed feed and feacals were controlled by exchanging water every 72 h to ensure good water quality. The tanks were covered with a net of 2 mm mesh size to protect the fish from jumping out. Fish was removed from each tank every week and batch-weighed using weighing scale (OHAUS model Cs 5000, capacity 5000×2 g) and the amount of feed was adjusted accordingly. Physico-chemical parameters (Dissolved oxygen, temperature, pH, ammonia and nitrate) were monitored throughout the duration of this experiment using LaMotte freshwater aquaculture test kit (Model AQ-2/AQ-3).

Haematological and serum biochemistry studies: Haematological studies were carried out on the fishes before and after the experiment. The fishes were taken out individually using a small hand net and placed belly upward on a table. Blood samples of about 5 mL was collected from the caudal peduncle (Stoskopf, 1993) with the aid of a 2 mL plastic syringe, 2 mL of the blood was dispensed into Ethylene Diamine Tetra-acetic Acid (EDTA) anticoagulant for haematological studies while 3 mL was transferred into a tube containing Lithium Heparin (LH) anticoagulant to obtain plasma for biochemical analysis. The plasma obtained by centrifugation from the lithium heparinised samples was stored at 20°C until analyzed. The blood samples were analyzed at the Physiology laboratory of Animal Science Department, University of Ibadan, Ibadan, Nigeria within 2 h of collection.

The Packed Cell Volume (PCV) and Hemoglobin (Hb) were determined using the micro haematocrit method and cyanmethehemoglobin method respectively as described by Mitruka and Rawnsley (1977). Erythrocyte count (RBC) and Leukocyte count (WBC) were determined using the improved Neubauer haemocytometer after appropriately diluted (Schalam *et al*., 1975). Differential leukocyte counts were determined by scanning Giemsa's stained slides in the classic manner (Schalam *et al*., 1975).

Plasma Glucose (PG), alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and cholesterol were determined by spectrophototometric method. Urea was determined by Urease method and creatinine by Folin-Wu filtrate methods as described by Connors *et al*. (1950). Total serum protein was determined using the biuret method as described by Stoskopf (1993) while albumin was determined using the BGG (Bromocresol green) method as described by Peters *et al*. (1982).

Statistical analysis: All the results were subjected to analysis of variance (ANOVA) using 17.0 version. Duncan multiple range test (Duncan, 1955) was further used to evaluate the mean differences at 0.05 significant levels.

RESULTS AND DISCUSSION

Haematological and plasma parameters: The haematology parameters (Hb, PCV, RBC, WBC, lymphocytes and monocytes) investigated before (initial) and after the experiment as presented in Table 1. These results showed that the mean Hb value ranged from 8.00±0.10 to 11.00±0.10 g L^{-1}. The highest hemoglobin concentration was obtained in only female population (11.00±0.10 g L^{-1}). The Hb values recorded among treatment groups were comparable with the initial value as there were no significant ($p>0.05$) differences in the mean values obtained among the treatment groups. A measurable increase in the hemoglobin concentration as observed in this study was corroborated by Fagbenro *et al.* (2010) who fed sunflower and sesame meal based diet to *Clarias gariepinus*. Similarly, this result is consistent with the findings of Adeyemo *et al.* (2008), who observed that healthy African catfish had mean hemoglobin value of 8.89 g L^{-1}. An increase in the concentration of hemoglobin in blood is usually an indication of suitability of the environment.

The PCV level varied between 24.00±0.10 and 32.00±0.10%. The maximum PCV value (32.00±0.10%) was discerned in only female population whereas the initial had the lowest value (24.00±0.10%). These differences found in PCV were significant ($p<0.05$) among treatments. Packed Cell Volume (PCV) showed marked increase between various sex combinations which is an indication of conduciveness of the experimental conditions on *C. gariepinus*. The mean PCV values were within the range observed by Gabriel *et al.* (2004) who attempted to determine if significant variations do exist and whether this could be attributed to some internal or external factors. Initial RBC value was relatively higher compared to other treatment groups, but these values fell within the range (2.3-2.9×10^6 mm^{-3}) described by Gabriel *et al.* (2004) and Adeyemo *et al.* (2008). For WBC, the initial value recorded was 15,950.00±15.27 10^6 μL^{-1} whereas, treatment NP, MM, FF and MF had 16,950.00±28.86 10^6 μL^{-1}, 12,800.00±35.11 10^6 μL^{-1}, 18,600±0.00 10^6 μL^{-1} and 15,500±32.14 10^6 μL^{-1}, respectively. There were significant difference ($p>0.05$) for the values obtained for RBC among treatments as indicated in Table 1. The initial and final serum biochemistry of experimental fish under different sex combinations are summarized in Table 2.

Total Protein (TP) was found within the range of 4.00±0.01 mg dL^{-1} and 6.00±0.01 mg dL^{-1}. This was significantly ($p<0.05$) higher in the MF treatment (6.00±0.01 mg dL^{-1}) than other treatments and initial value. Meanwhile, the lowest TP value (4.00±0.01 mg dL^{-1}) was observed in the initial and only female treatment. From this study, total plasma protein level was observed to increase among treatment groups except for a slight fall in treatments MM and FM. The result was similar to that reported by Tavares-Dias (2000). However, these values were significantly

Table 1: Haematological changes in *C. gariepinus* of different sex combinations

	Parameters					
Treatments	Hb (g L^{-1})	PCV (%)	RBC 10^6 μL^{-1}	WBC ×10^3 μL^{-1}	Lymphocytes	Monocytes
Initial	8.00±0.10^a	24.00±0.10^a	11.77±0.01^e	15,950.00±15.27^b	76.00±0.43^e	3.00±0.10^c
NP	8.00±0.15^a	25.00±0.15^b	03.74±0.01^c	16,950.00±28.86^c	66.00±0.43^a	3.00±0.10^c
MM	9.00±0.05^a	28.00±0.05^d	02.62±0.01^a	12,800.00±35.11^a	74.00±0.43^d	2.00±0.10^b
FF	11.00±0.10^a	32.00±0.10^e	03.58±0.00^b	18,600.00±0.00^d	68.00±0.43^b	1.00±0.10^a
MF	9.00±0.05^a	27.00±0.05^c	03.81±0.00^d	15,500.00±32.14^b	71.00±0.43^c	3.00±0.10^c

Means with different superscript in the column are significantly different at ($p<0.05$), NP: Natural population, MM: Only male population, FF: Only female population, MF: Male and female population, PVC: Pack cell volume, WBC: White blood cells, RBC: Red blood cells

Table 2: Changes in the biochemical blood parameters levels of *C. gariepinus* held at different sex combinations

	Treatments				
Parameters	Initial	NP	MM	FF	MF
Total protein (g L^{-1})	5.27±0.01^c	4.00±0.01^a	5.00±0.01^b	4.00±0.01^a	6.00±0.01^d
Urea (mg dL^{-1})	1.25±0.01^a	1.75±0.01^b	2.00±0.10^c	2.05±0.01^c	1.95±0.01^c
Creatinine (mg dL^{-1})	0.95±0.01^a	1.00±0.10^a	0.95±0.01^a	1.15±0.01^a	1.00±0.10^a
ALP (U.l/l)	9.42±0.01^c	2.00±0.10^a	3.00±0.10^b	2.00±0.10^a	3.00±0.10^b
Cholesterol (mg dL^{-1})	117.84±0.02^d	105.00±1.00^b	101.00±0.28^a	111.00±0.28^c	104.00±0.28^b
Albumin (g dL^{-1})	0.75±0.01^a	0.80±0.00^a	0.70±0.00^a	1.00±0.10^a	2.23±0.33^b
AST (U.l/l)	56.90±0.01^a	149.00±0.17^b	177.00±0.28^c	208.00±0.23^e	187.00±0.28^d
ALT (U.l/l)	58.60±0.02^c	49.90±0.02^a	57.30±0.05^b	69.80±0.05^e	64.10±0.00^d
Glucose (mg dL^{-1})	64.77±0.01^e	37.90±0.05^d	36.40±0.05^c	27.00±0.28^a	29.00±0.33^b

Data is expressed as Mean±SD. Means with different superscript in the row are significantly different at ($p<0.05$)

Table 3: Water quality parameters of *C. gariepinus* reared under different sex combination (Values are Mean±SE)

	Water parameters			
Treatments	Dissolved oxygen (mg L^{-1})	pH	Ammonia (mg L^{-1})	Nitrate (mg L^{-1})
NP	4.21±0.16	7.42±0.02	0.21±0.01	0.12±0.01
MM	3.96±0.04	7.28±0.02	0.19±0.00	0.11±0.00
FF	3.85±0.08	7.30±0.00	0.35±0.11	0.20±0.06
MF	3.93±0.12	7.29±0.06	0.25±0.07	0.16±0.03

NP: Natural population, MM: Only male population, FF: Only female population, MF: Male and female population

higher than the values reported by O'Neal and Weirich (2001) in *Ictalus metas*. The increase observed in total protein might be an indication of efficient immune responses and body physiological reaction to various gender combinations.

The range of value of creatinine recorded was between 0.95±0.01 mg dL^{-1} and 1.15±0.01 mg dL^{-1}. Treatments FF had the highest creatinine value (1.15±0.01 mg dL^{-1}), while lowest value was observed in Initial (0.95±0.01 mg dL^{-1}). However, there was no significant difference ($p>0.05$) between creatinine values among treatments. The creatinine value was relatively stable in all treatment groups. This is an indication that the fish kidneys are functioning properly. This viewpoint is corroborated by Joshi (2011) who attributed rise in creatinine value to an indication of renal tubular damage due to zinc-induced nephrotoxicity. The observed stability in creatinine level in all treatment groups is suggestive of a normal physiological function. For alanine aminotransferase (ALT), the maximum value was obtained in Treatment FF (69.80±0.05 U.I/I), while Treatment NP gave lowest value of 49.90±0.02 U.I/I. Nonetheless, there are significant difference ($p<0.05$) for the values obtained for ALT among treatments as indicated in Table 2. The significant decrease between treatment groups in glucose concentration ($p<0.05$) may be considered to be the manifestation of little or no stress. However, increase in serum glucose levels in fish under stress was reported by Cicik and Engin (2005). The results of water quality parameters such as temperature, pH, dissolved oxygen, ammonia and nitrate were presented in Table 3.

The mean water temperature, pH, dissolved oxygen, ammonia and nitrate levels in this study were not affected by treatments during the twelve weeks feeding trial. The recorded mean values of all these parameters were within the acceptable limits for catfish growth and health (Omitoyin, 2007; Viveen *et al*., 1986). The observed water quality parameters were due to constant water change throughout the duration of the experiment. Similarly, the close range in the average temperature recorded during the experimental period was due to the fact that all the treatments were indoors.

CONCLUSION

This study provides information on the influence of various sex combinations on haematological and immunological changes in the blood of African catfish, *C. gariepinus*. The slight haematological changes observed between the initial value and treatment groups values cannot significantly affect the health of *C. gariepinus* reared under different sex combinations.

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