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Comparison of Haematological Indices, Blood Group and Genotype of *Clarias gariepinus* (Burchell, 1822) and *Clarias anguillaris* (Linneaus, 1758)

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ABSTRACT

The study on haematological indices of *Clarias gariepinus* and *Clarias anguillaris* was carried out to compare and contrast their blood group, genotype and haematological profile. Little is known about the blood group and genotype of the species. The knowledge of the blood group and genotype will be useful in the genetic study of the species. Test tube techniques based on agglutination tests was used for blood grouping, while the genotype was determined by hemoglobin electrophoresis. The haematological parameters, blood group and genotype of *Clarias gariepinus* (n = 12) and *Clarias anguillaris* (n = 12) males and females, were studied. The mean value of WBC count (µL) in *C. gariepinus* males was 1621.67±10.83 µL and females 9033.33±13.63 µL, respectively. The mean RBC counts for males were 3.0833±1.23 µL and females 3.85±0.46 µL and females 3.8500±0.46 mm^3, respectively. The mean MCHC for males and females were 33.15±0.1 g dL^{-1} and 33.08±0.1 g dL^{-1}, respectively. Mean PCV for males was 51.62±6.79% and females 65.02±18.63%, respectively. Mean Hb. for males was 18.62±1.87 (g dL^{-1}), while the females had mean 22.12±5.823 (g dL^{-1}). The mean MCV for males and females were 172.17±43.03 and 169.300±31.64554 pg. The genotype of all the males and females were the same (AA). The blood group recorded 83% with O+, while 18% had AB+, respectively in the males and females. The WBC counts of males and females *C. anguillaris* were 7733.33±648.90 and 8033.33±16.47 µL, respectively. The mean RBC counts for males and females were 2.60±0.45 µL and 2.60±0.44 µL, respectively. The MCHC of both males and females were the same 33±0.00 g dL^{-1}. The mean PCV of males and females were 87.2±1.97 and 66.73±30.93%, respectively. The mean Hb for males and females were 29.77±1.20 and 22.47±10.13 g dL^{-1}, respectively. The mean MCV for males and females were 356.97±6.16 and 274±92.60 pg, respectively. The blood group, genotype and agglutination tests results were 83% Rh- O+, 18% Rh-AB+ and 100% AA, respectively and were similar to the pattern in humans.

Key words: Hematology, blood group, genotype, *Clarias gariepinus*, *Clarias anguillaris*

INTRODUCTION

Fish live in very intimate contact with their environment and are therefore very susceptible to physical and chemical changes which may be reflected in their blood components (Wilson and Taylor, 1980). In fish, exposure to chemical compounds can induce either increases or decrease in hematological levels. Blood tissues truly reflect physical and chemical changes occurring in organisms. Therefore, detailed information can be obtained on general metabolism and

physiological status of fish in different groups of age and habitat. The study of physiological and haematological characteristics of cultured fish species is an important tool in the development of aquaculture system, particularly in regard to its use in detection of healthy from diseased or stressed fish (Rainza-Paiva *et al.*, 2000; O'Neal and Weirich, 2001). Early diagnosis is also possible, when evaluating hematological data, particularly blood parameters (Luskova, 1997). The health of fish has often been reported in terms of the relationship between the weight and length increase. However, there is a need to understand the physiological concept of fish health in relation to blood and the quality of dietary protein fed. Any changes in the constituent component of blood sample, when compared to the normal values could be used to interpret the metabolic state of animal and state of health (Babatunde *et al.,* 1992). Low hematological indices are indications of anemic conditions (Haruna and Adikwu, 2001). The count of red blood cells is quite a stable index and the fish body tries to maintain this count within the limits of certain physiological standards using various physiological mechanisms of compensation. Van Vuren (1986) observed that when the water quality is affected by toxicants, many physiological changes will be reflected in values of one or more of the hematological parameters. Blood cells responses are important indicators of the changes in the internal and or external environment of animals. In fish, exposure to chemical pollutants can induce either increases or decreases in hematological levels. Their changes depend on fish species, ages, the cycle of the sexual maturity of spawners and disease (Luskova, 1997; Golovina and Trombicky, 1989). Furthermore, it should be noted that hematological indices are of different sensitivity to various environmental factors and chemicals (Lebedeva *et al.*, 1998). Previous hematological studies on the effect of nutrition (Rehulka, 2000), infectious diseases and pollutants (Rehulka, 2002) revealed that erythrocytes are the major and reliable indicators of various sources of stress (Rainza-Paiva *et al.*, 2000; O'Neal and Weirich, 2001). The use of hematological characteristics in evaluating the health status of fish as a tool for its management under captive rearing is well established and the knowledge of the hematological profile of a fish also indicates its dietary efficiency and physiological response to environmental stress. The haematological profile of few tropical African catfish species are well documented (Kori-Siakpere, 1985; Fagbenro *et al.*, 1993; Erondu *et al.*, 1993; Etim *et al.*, 1999). Several authors have examined the haematology of fishes as in *Clarias heriensis* (Kori-Siakpere, 1985), *Clarias gariepinus*, *Heterobranchus longifilis*, *Heterobranchusbi dorsalis* and *Clarias nigrodigitatus* (Erondu *et al.*, 1993; Sowunmi, 2003), *Oreochromis niloticus* (Fagbenro, 1990), *Hemichromis fasciatus* and *Tilapia zilli* (Egwunyenga *et al.*, 1999), *Sarotherodon melanotheron* (Gabriel *et al.,* 2007), *Heterobranchus bidorsalis* (Fagbenro *et al.*, 1993; Onyia *et al.*, 2013). Meanwhile, there are no reports on the haematological profile of *Clarias anguillaris* and *Clarias gariepinus* from Lake Geriyo, Adamawa State, Nigeria to provide some useful information on their biology that will enhance their culture.

Few studies have been done on the blood group and genotype of fish. Ayorinde *et al.* (2009) worked on the blood group of *Heterotis niloticus* and discovered that the fish had 92% Rh-O+ and Rh-O-. The aim of this study is to compare the haematological profile, blood group, genotype of male and female *Clarias gariepinus* and *Clarias anguillaris*.

MATERIALS AND METHODS

Twelve adults live each of *C. gariepinus* and *C. anguillaris* (6 males and 6 females) were obtained from fish landing site at Lake-Geriyo, Jimeta Local Government Area of Adamawa State,

Nigeria. All fishes were considered healthy on the basis of their appearance and the absence of obvious signs of disease. Sexual selection was done by observation of the genital organs. Blood was collected from the caudal vein of each fish using separate heparinized disposable syringes and hypodermic needles.

Haematrocit (Hct): This was measured after centrifugation at 15 000 rpm using an MSE micro centrifuge.

Hemoglobin concentration (Hb): The indirect acid haematin (sahli) method was used. This involved the use of a special haemoglobinometer and pipette. Hemoglobin concentration was converted to acid haematin by the action of 0.1 M HCl using 0.02 mL pipette. The graduated tube was filled with 20 mL 0.1 M HCl 0.02 mL of blood sample was added. The mixture was allowed to stand for 5 min and then few drops of distilled water was added until the colour matched the standard. Hemoglobin concentration was estimated as:

$$Hbc = Values\ obtained \times 17.2\ g/100\ mL \div 100$$

Leucocyte count (Lc): The haemocytometer was also used for Lc determination with 0.8 cm objective of the microscope and large squares (area $= 1\ mm^2$, depth $= 0.1\ mm$) the expected volume was $0.1\ mm^3$ and the dilution factor was 20. Four squares was used and the total count per mm^3 was:

$$20 \times 1 \times L\ cells \div 0.4 = 50 \times L\ cells$$

where, L is number of leucocytes that was counted.

Erythrocytes (Ec): This was determined in heparinized blood diluted by Haymen solution at a ratio of 1:200. Neubauer improved haemocytometer placed on a compound microscope stage was used to count/estimate the erythrocyte population. The number of cells counted, R, (average of two fields) was multiplied by the dilution factor and the volume of $1/4000\ mm^3$ (area $= 1/400\ mm^3$, depth $= 1/10\ mm$) and counting was done in 80 squares with the sum total volume if $1/50\ mm^3$ the dilution factors was 200. The Ec was obtained as:

$$200 \times 50 \times R\ cells = 10.000 \times R$$

Mean cell volume: The mean cell volume was expressed in pico gram (pg) as:

$$(MCV) = Hbc \div Ec$$

Mean corpuscular hemoglobin concentration: This was calculated from the hemoglobin concentration values in g L^{-1} and from the haematrocit value using the equation:

$$MCHC = Hbc \div Hct \times 1000$$

Three determinations for each of the hematological indices was made for ten (10) specimens of each fish species (N = 12). The means and Standard Deviation (SD) was calculated for all the values obtained.

Determination of fish blood group: Blood group was determined with the test tube techniques based on tests agglutination.

The fish blood was collected with a syringe and drop on the tile in three different places, then on each of the blood sample, anti-sera A, B and D was dropped, respectively and mixed. The tile was rocked for about 3-5 min. Blood groups were recorded based on coagulation of blood according to Daramandy and Davenport (1985) and Svobodova *et al*. (1991).

Determination of fish genotype: The fish blood was collected and it was put in a sterile EDTA container, with a known control AS. With your test sample, the cellulose acetate paper, it was deep inside distilling water after which it was removed. After removing it, it was laid on the work bench, a lancet was used to take a small portion of a known AS and it was put vertically on the acetate paper and also the test control was put on line with the known AS then the acetate paper was put into electrophoresis and the machine was switch on for some times and the result was observed (Delany and Garratty, 1969).

Statistical analysis: Data obtained from the experiment was subjected to analysis of variance while differences between the means were determined using Least Significant Differences (LSD) at 95% confidence level (p = 0.05) with the aid of Statistix 8.0.

RESULTS

The haematological parameters of male *C. anguillaris* and *C. gariepinus* are shown in Table 1. There were significant differences in the values obtained. *Clarias anguillaris* had higher values in WBC (1733.33±65.90 µL), PCV (87.2±1.97%), Hb (29.77±1.20 g dL^{-1}) and MCV (365.97±6.16 pg) than *C. gariepinus*. Whereas *C. gariepinus* male had higher values of RBC than *C. anguillaris* male.

Among the females (Table 2), values of WBC and RBC were higher in *C. gariepinus* than *C. anguillaris*. While, the reverse was the case in PCV%, Hb and MCV as observed in *C. anguillaris* female. There was no significant difference (p<0.05) in MCHC obtained in both species.

From the results (Table 3) *Clarias gariepinus* and *Clarias anguillaris* had 83% Rh .O^{+} each and AB^{+} (17%), respectively. The genotypic traits of the two species were all AA. From the results, there was no significant difference (p>0.05) in the blood groups and genotypes in both males and females of the two species.

Table 1: Comparison of haematological parameters of male *Clarias gariepinus* and *Clarias anguillaris*

Parameters	C. anguillaris	C. gariepinus
Weight (g)	230.17±32.50	337.17±89.40
Length (cm)	34.08±1.40	37.5±2.88
WBC (µL)	7733.33±65.90[b]	10216.67±10[a]
RBC (µL)	2.60±0.45[b]	3.0833±1.23[a]
MCHC (g dL^{-1})	33±0.00[a]	33.15±0.10[a]
PCV (%)	87.2±1.97[a]	51.62±6.79[b]
Hb (g dL^{-1})	29.77±1.20[a]	18.62±1.87[b]
MCV (pg)	356.97±6.16[a]	172.12±43.03[b]

Means with different super script are significantly different (p<0.05), Values are Mean±SE

Table 2: Comparison of haematological parameters of female *Clarias gariepinus* and *Clarias anguillaris*

Parameters	*Clarias anguillaris*	*Clarias gariepinus*
Weight (g)	254.67±56.96	464.0±59.60
Length (cm)	34.2±3.66	39.17±2.07
WBC (µL)	8033.33±16.47[b]	9033.33±13[a]
RBC (µL)	2.60±0.44[b]	3.85±0.46[a]
PCV (%)	66.73±30.93[a]	65.02±18.64[b]
Hb (g dL^{-1})	22.47±10.13[a]	22.12±5.8[a]
MCHC (g dL^{-1})	33±0.00[a]	33.08±0.09[a]
MCV (pg)	274±92.60[a]	169.3±31.65[b]

Means with different super script are significantly different (p<0.05), Values are Mean±SE

Table 3: Mean blood groups and genotype of *Clarias gariepinus* and *Clarias anguillaris*

Species	Male blood group	Blood group (%)	Female blood group	Blood group (%)
C. gariepinus	O$^+$	83.3	O$^+$	83.3
	AB$^+$	16.7	AB$^+$	16.7
C. anguillaris	O$^+$	83.3	O$^+$	83.3
	AB$^+$	16.7	AB$^+$	16.7
	Male genotype	Percentage genotype	Female genotype	Percentage genotype
C. gariepinus	AA	100	AA	100
C. anguillaris	AA	100	AA	100

DISCUSSION

Haematological study of *Clarias gariepinus* and *Clarias anguillaris*: The result of the PCV obtained in male and female *C. anguillaris* (87.2±1.97 and 66.73±30.92) were higher than that obtained in *C. gariepinus*. The PCV results obtained in this work were higher than that obtained in *Heterobranchus bidorsalis* (Onyia *et al.*, 2013), *Heterobranchus longifilis* (Akinwande *et al.*, 2005), *C. gariepinus* (Adeyemo *et al.*, 2003; Omitoyin, 2006). From the results, *C. anguillaris* had higher WBC, Hb and MCV than *C. gariepinus*. White blood cell counts in an organism determines its ability to resist invasion of pathogens into the body. The high WBC in the two species in this study could be an indication of the well-being and ability to with stand pathogen invasion during culture period. Das (1964) stated that both RBC and Hb tend to increase with length and age but this assertion tend to agree with the results of this study. The *C. gariepinus* with higher length had higher RBC than *C. anguillaris* but disagree with the results of Hb. The high Hb value in *C. anguillaris* and *C. gariepinus* males and females is indicative of its air breathing character in activity (Odo *et al.*, 2012). There was no significant difference (p<0.05) in the MCHC obtained from the males of both species. The MCHC refers to the quantitative measurement of mean amount of hemoglobin per erythrocyte in biological organisms (Moses, 2007). The MCHC in this study was high in the species hence the ability to survive in low dissolved oxygen concentration in culture system. The results of MCHC were higher than what was obtained in *Heterobranchus longifilis* (Akinwande *et al.*, 2005), *C. gariepinus* from the wild and pond culture (Ayorinde *et al.*, 2009).

Haematological study of female *Clarias anguillaris* and *C. gariepinus*: The weight, Length and RBC of female *C. gariepinus* were higher than that of *C. anguillaris and* this agree with the result of Das (1964), that RBC and Hb increase with increase in length. However, it was not the same in Hb in this study. The high values of RBC and Hb concentration (Table 2) reflect high oxygen carrying capacity of the blood. According to Lenfant and Johansen (1976), hemoglobin concentration is higher in the fishes capable of aerial respiration. Therefore, the high RBC and Hb values in females are indicative of its air-breathing character and high activity. There was no significant difference (p<0.05) in the MCHC in the females as observed in the males also.

Blood group and genotype of *Clarias anguillaris* and *Clarias gariepinus*: The result showed that *Clarias gariepinus* and *Clarias anguillaris* had the same percentage level of blood groups and genotype (Table 3). The results do not agree with the blood group from *Heterotis niloticus* that had only blood group Rh-O$^+$ and Rh-O$^-$ and genotype AS and SS (Ayorinde *et al.*, 2009; Odo *et al.*, 2012). However, *Clarias gariepinus* and *Clarias anguillaris* species had the same genotype in both males and females. The results of the genotype in the two species agree with the work of Chuku and Uwakwe (2012), that worked on *C. gariepinus*. The authors reported AA genotype in all sampled fish. Whereas *Parachannaobscura* had genotypes 88% AA and 12% AS and blood groups O$^+$ (90%) and O$^-$(10%) according to Odo *et al.* (2012). The present work had 100% AA, in both species and 83.3% O$^+$ and 16.7 AB$^+$. This could be as a result of the population of fish sample used in this research.

CONCLUSION

From this study, there were differences in RBC, WBC, PCV and MCV between *C. gariepinus* and *C. anguillaris*. They had similar Hb and MCHC. The differences in the results in the two species could be attributed to differences in physiological differences. The results of genotype and blood groups in the two species of family *Clariidae* showed similarities indicating their predominance in the sampled fish. The blood group and genotype obtain in males and females of *C. anguillaris* and *C. gariepinus* could serve as baseline information for further studies of same species from other ecological zones of Nigeria.

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