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EVALUATION OF HAEMATOLOGICAL POLYMORPHIC CHARACTERISTICS OF *Clarias gariepinus* (Burchell, 1822) AND *Clarias angillaris* (Linnaeus, 1758) THROUGH ACETATE GEL ELECTROPHORESIS

***[1]Suleiman, S. B., [1]Diyaware, M. Y., [1]Idriss, I. M [1]Aliyu, M., [2]Dadile, M. A. and [3]Abdulkarim, M.**
[1]Department of Fisheries, University of Maiduguri, P.M.B. 1069, Maiduguri, Borno State
[2]Department of Biological Science, Yobe State University, P.M.B 1144, Damaturu
[3]Department of Animal Production, Abubakar Tafawa Balewa University, P.M.B. 0248, Bauchi
*Corresponding author: E-mail: suleimanbababala@gmail.com /+2347038365844

ABSTRACT
The study was carried out to investigate the blood groups, genotypes, allele frequency variation and test for conformity to Hardy-Weinberg Equilibrium in the Clarias anguillaris and Clarias gariepinus population of Lake Alau, Maiduguri. Blood samples were taken from the caudal vein of the fishes for determination of haemoglobin parameters through cellulose acetate electrophoresis. The result showed that both male and female species of the fishes had homozygous genotypes (AA, BB and CC). However, heterozygous genotypes of AB and BD were obtained in male C. anguillaris and female C. gariepinus respectively. The observed genotypes resulted in the allelic frequency within and among the fish species population to AA (0.4), BB (0.4), AB (0.1) and CC (0.1) for C. anguillaris while C. gariepinus had AA (0.4), BB (0.3) CC (0.2) and BD (0.1). The blood groups obtained in this study were 100% O ⁻ for the fishes. The mean values recorded for haemoglobin concentration were 8.500±0.767g/dl and 8.440±0.545g/dl for male and female C. anguillaris while that of male and female C. gariepinus were 9.180±0.274g/dl and 7.900±0.372g/dl respectively. There was no significant difference (P > 0.05) in the means. The test for conformity to Hardy-Weinberg Equilibrium was found to be 0.90 and 0.80 for C. anguillaris and C. gariepinus respectively.

Key words: Haemoglobin, Alleles, frequency, Genotypes, Blood group, Lake Alau

INTRODUCTION

Clarias anguillaris and *Clarias gariepinus* are among the cheapest and direct source of protein and micronutrients for many people worldwide (Ben and Heck, 2005). Thus, more than one billion people rely on fish as a primary source of animal protein. Fish accounts for about 30%, 10%, 20% and 55% of the total protein intake for people of Asia, Latin America, Africa and Nigeria respectively (Martini and Innes, 2018). The use of hematological polymorphic characteristics is imperative because of their important in the improvement of fish species growth and some polymorphic alleles may be connected with traits of economic importance due to heterozygosity. Variation in growth rate of larvae and fry are the characteristics of all fish species including *C. anguillaris* and *C. gariepinus*. These variations could be due to various environmental factors as well as genetics of fishes (Okafor and Chukwu, 2010; Allendorf *et al.*, 2010). Haematological parameters are those indexes that are related to the blood and blood forming organs such as the red blood cells (erythrocytes), white blood cells (leucocytes), and the thrombocytes (Merck, 2012). One of the important blood proteins in fish is haemoglobin (Bettati *et al.*, 2009). Hemoglobin is the predominant biochemical constituent of the vertebrate red blood cells (Guyton *et al.*, 1996). Haemoglobin is a group of transport protein and has the physiological functions to carry oxygen and to facilitate the return transport of carbon dioxide (Reece, 2005; Isaac *et al.*, 2013). Osterhoff (1964) and Agaviezor *et al.* (2013), defined polymorphism as the occurrence together of two or more varieties of blood protein or DNA in the same population at the same time in such proportions that the rarest of them cannot be maintained by mutation. Schork *et al.* (2000), reported the use of polymorphisms in modern initiative ultimately emanated from the study of physiological and biochemical variation exhibited by protein isoforms and blood group antigents. Protein and DNA polymorphisms by means of cellulose acetate electrophoresis can contribute to the field of aquaculture (Volckaert and agnese, 1996).

Several authors have studied the haemoglobin (Hb) polymorphism in animal and fishes as in cattle seems to be breed influenced of polymorphism with two alleles (AA and BB) and their possible phenotypes AA, AB and BB (Osterhoff, 1964), the existence of three major Hb types (AA, AB and BB) caused by Hb A and Hb B genes also in cattle as observed by Pal and Mummed, (2014), Akinyemi and Salako (2010) and Oladipo and Kikelomo, (2020) reported a higher frequency of Hb A in West African Dwarf sheep of Nigerian. There are two different Hb types in goat Hb A and Hb B as study by Schmid (1962). However, Agaviezor *et al.* (2013) documented three Hb types (Hb AA, Hb AB and Hb BB) in Red Sokoto goat, Hb AA and Hb AB in Sahelian goat, Hb AA in West African Dwarf goats sample in the Niger Delta area of Nigeria. Haemoglobin polymorphisms affect the growth performance and hatchability of an organism (Dimri, *et al.*, 1981). Hatchability was observed to be highest in AA

followed by AB and BB among Nigerian indigenous chicken breeds in the Niger Delta region (Ajayi *et al.,* (2013). Ross *et al.* (2013) reported two haemoglobin locus of HbI and HbII in *Gadua morhua* (Cod) and some gene products of HbI were polymorphic. The blood group of O^+ and O^- with AA genotype in male and female *Heterotis niloticus* (Ayorinde *et al.,* 2009), Odo *et al.* (2012) reported 88% AA and 12% AS genotype and blood group of O^+ (90%) and O^-(10%) in Parachanna *obscura*. Onyia *et al*. (2015) observed (83%) O^+ and 18% AB^+ the blood group and AA genotype from both males and females of *Clarias gariepinus* and *Clarias anguillaris* fish species from Lake-Geriyo, Adamawa State. Whereas, Diyaware *et al.* (2017) that work on wild and cultured populations of *Clarias gariepinus* reported the blood groups of wild male *C. gariepinus* were O^- and AB^+ with 10% each while the wild females had O^-, O^+, A^+, B^+ 10% each and AB^+ with 40%. Cultured male had O^+ 10%, A^+ 20% and AB^+ 20% while, the cultured female *C. gariepinus* were O^- 30%, A^- 10% and B^+ 10%. The author also documented that both wild and cultured *C. gariepinus* had AA, BB and CC genotypes in the males and females. However, he reported BD genotype in only one female wild *C. gariepinus*. A more genetics detailed knowledge of *C. anguillaris* and *C. gariepinus* is required for natural populations, which are threatened by habitat loss, natural hybridization and selection of suitable strains for aquaculture (Volckaert and agnese, 1996; Diyaware *et al.,* 2017).

Morphological features have been used for many genetics studies to identify both parental and hybrids stocks. The morphological characters and meristic counts may not be able to indicate the purity of the broodstock selected for breeding purposes and tend to overlap each other due to differences in the environment (Umaru *et al.*,2015; Diyaware *et al.,* 2017). The field of biotechnology has introduced new area at the molecular levels with the techniques like electrophoresis which can be employed for the detection of polymorphisms at protein and enzyme loci for the measurement of variation (Buth, 1984; Pujolar *et al.,* 2005). Electrophoresis is the useful technique devised for studying variability within and among populations of plants and animals (1980). It is the separation of a mixture of electrically charged molecules in an electric field (Aebersold *et al.,* 1986). Cellulose acetate electrophoresis is the type of electrophoresis used to determining genotype by using cellulose acetate strip in a buffer solution with blood being dropped and placed in an electrophoretic tank. Genotype is the part of the DNA sequence of the genetic makeup of a cell of an organism or individual, which determines a specific characteristic (phenotype) of that individual (Hulce and Liu, 2006). There are little or no researches carried out on haematological polymorphic characteristics of fish species in Lake Alau, Maiduguri using cellulose acetate electrophoresis technique. The information from this research could be used to determining genetic variations among the population of fish species studied. The aim of this study is to compare the blood group, genotype, gene frequency and haemoglobin polymorphism between population of C*. anguillaris* and *C. gariepinus* in Lake Alau.

MATERIALS AND METHODS

Description of Study Site

Lake Alau is located in the northeast arid zone of Nigeria along Maiduguri Bama road, 29 km south of Maiduguri metropolitan which lies between latitudes $11^0$39"84'N and $11^0$40" 02'N and longitudes 13^0 39" 92'E and 13^0 40" 12'E (Google map, 2019). The Climate in Lake Alua is Sahelian with two distinct seasons. The rainy season starts from June and ends in September with a mean annual rainfall of about 600mm while dry harmattan period start from October to February (Uwah and Ogugbuaja, 2012).

Source of Experimental Fish

A total of 20 fish samples with an average weight of 68.5g and 7.35cm were used for the experiment which comprises 5 males and 5 females of *Clarias gariepinus* and 5 males and 5 females *Clarias anguillaris* respectively. The fish for the experiment were obtained from fishermen in Abbari fish landing site of Lake Alau. All the fish were transported live in 25 liters' capacity plastic Jerry-cans half-cut horizontally, to fish hatchery complex of the Department of Fisheries, University of Maiduguri

Blood Sample Collection

Blood sample were collected from each of the fish according to method described by Diyaware *et al*., (2017). The labelled blood samples were transported to Animal Science laboratory of University of Maiduguri for determination of haemoglobin concentration, blood group, genotype and cellulose acetate electrophoresis.

Determination of haemoglobin (Hb)

The haemoglobin was estimated using Cynomethaemoglobin procedure according to Kelly, (1979). Twenty microliters (20µl) of blood were diluted in 5ml of Drabklin's solution then it was shake to ensure that, the mixture is homogenous. It was then left to stand for 15 minutes and placed in a haemoglobin cytometer, and the absorbencies were recorded. Hemoglobin concentrations was estimated as $\text{Hbc} = \frac{\text{values obtained} \times 17.2\text{g}}{100\text{ML}100}$ (Diyaware *et al*., (2017).

Acetate gel Electrophoresis

Cellulose acetate electrophoresis was prepared from the whole blood samples using Helena's Hemoglobin Electrophoresis procedure (Catalog No. 4093, Beaumount, Texas, USA). This involved the use of Supre-Heme Buffer at pH 8.5 for haemoglobin as described by the kit.

The direct gene counting method was used to score the resulting haemoglobin polymorphism bands as

five zones of migration were detected after electrophoresis as described by Ajayi *et al.* (2013).

Genotype frequency were estimated after modification of Ajayi *et al.* (2013) formulae as follows:

AA frequency = $NAA/N \times 100$
AB frequency = $NAB/N \times 100$
BB frequency = $NBB/N \times 100$
CC frequency = $NCC/N \times 100$
BD frequency = $NBD/N \times 100$
Where: N = total number of individuals sampled
NAA= Observed genotype number for AA
NAB= Observed genotype number for AB
NBB= Observed genotype number for BB
NCC= Observed genotype number for CC
NBD= Observed genotype number for BD

The calculation of Gene frequency, the following Hardy Weinberg equilibrium P + Q = 1
Equation was followed:
P = (2NAA + NAB) / 2N and Q = (2NBB + NAB) / 2N. Where:

P = gene frequency for allele A
Q = gene frequency for allele B
N = total number of individuals sampled

NAA = Observed genotype number for AA
NAB = Observed genotype number for AB
NBB = Observed genotype number for BB (Ajayi *et al.*, 2013)

Fish blood group analysis

Blood groups were analyses through a standard test tube agglutination techniques described by Prasad (2013) as reported in Diyaware *et al*., (2017). 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 (Prasad, 2013; Diyaware *et al*., 2017). Blood groups were recorded based on coagulation of blood with modification following the methods of Daramandya and Davenport (1985); Svobodova *et al.* (1991) and Diyaware *et al*. (2017).

Scoring the genotype

The bands representing genotype at the polymorphic protein were manually scored as binary data, which is present as 1 and absence as 0 and further designated as A, B, C, D, E, F from the top of the gel using ruler according to Laloei *et al.* (2013) and Diyaware *et al*. (2017).

Statistical analysis

The data (1 and 0) obtained for haematological characteristics were subjected to Analysis of Variance (ANOVA) and differences between means were separated using LSD with SPSS Version 2016 of Windows at 95% confidence level. The blood group, genotype and gene frequency were analysed using descriptive statistics.

Results

The blood groups from two species of *Clarias* in Lake Alau are shown in table 1.The blood groups obtained in both sexes of *Clarias anguillaris* and *Clarias gariepinus* samples was O^- (100%).

Table 1: Mean blood groups from two species of *Clarias* in Lake Alau, Maiduguri

Fish Species	♂ Blood group	Blood group %	♀ Blood group	Blood group %
C. anguillaris	O^-	100	O^-	100
C. gariepinus	O^-	100	O^-	100

Key: ♂ = Male ♀ = Female

Plates 1 and 2 show some samples of cellulose acetate gel with genotypes photographed after electrophoresis of *C. anguillaris* and *C. gariepinus* respectively. The mean values of genotypes and haemoglobin concentration of *C. anguillaris* and *C. gariepinus* in Lake Alau were shown in table 2. The genotypes distributions of haemoglobin polymorphic characteristics for both *C. anguillaris* male and female are AA (2), BB (1), CC (1) and AA (2), BB (3) respectively. While male and female *C. gariepinus* in this study showed AA (3), BB (1), CC (1) and AA (2) BB (1), CC (1) respectively. However, AB genotype was obtained in male *C. anguillaris* and BD genotype was recorded in female *C. gariepinus* sampled. The mean haemoglobin concentration ranged from (7.900g/dl) to (9.180 g/dl). The highest (9.180 g/dl) Hb concentration was observed in male *C. gariepinus* followed by male *C. anguillaris* with the value of (8.500 g/dl) and (8.440 g/dl) for that of *C. anguillaris* female. The least (7.900 g/dl) mean value of Hb concentration was obtained in female *C. gariepinus*. There was no significant difference ($p>0.05$) among the species in Hb concentration.

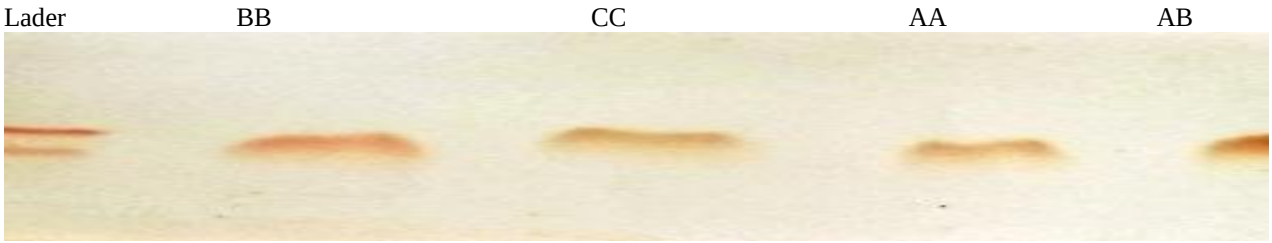

Plate 1: Sample of cellulose acetate gel with genotypes photographed after electrophoresis of *C. anguillaris*

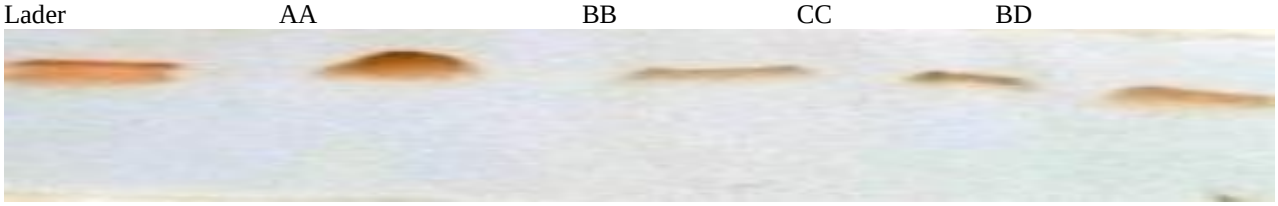

Plate 2: Samples of cellulose acetate gel with genotypes photographed after electrophoresis of *C. gariepinus*

Table 2: Effect of species and sex on genotypes and Haemoglobin concentrations from two species of *Clarias* in Lake Alau, Maiduguri

Fish species	N	Sex	N	AA	AB	BB	CC	BD	Hb(g/dl)
C. anguillaris	10	♂	5	2	1	1	1	0	8.500±0.767[a]
		♀	5	2	0	3	0	0	8.440±0.545[a]
		Total	10	4	1	4	1	0	
C. gariepinus	10	♂	5	3	0	1	1	0	9.180±0.274[a]
		♀	5	2	0	1	1	1	7.900±0.372[a]
		Total	10	5	0	2	2	1	

Key: ♂ = Male ♀ = Female

Table 3 indicates the percentage of genotype heamoglobin of *C. anguillaris* and *C. gariepinus* in Lake Alau. Among all the fish species, the highest AA (50%) percentage of genotype was recorded in *C. gariepinus* followed by AA and BB with 40% each for *C. anguillaris* and BB (20%), CC (20%) for *C. gariepinus*. The least value of genotype percentage AB (10%) and CC (10%) were contained in *C. anguillaris* and also ten percentage BD (10%) genotype was recorded in *C. gariepinus*.

Table 3: Percentage genotype heamoglobin from two species of *Clarias* in Lake Alau, Maiduguri

Fish species	N	AA%	AB%	BB%	CC%	BD%	Total%
C.anguillaris	10	40	10	40	10	00	100
C. gariepinus	10	50	00	20	20	10	100
Sex							
♂	10	50	10	20	20	00	100
♀	10	40	00	40	10	10	100

Key: ♂ = Male ♀ = Female

Alleles frequency of *Clarias anguillaris* and *Clarias gariepinus* in Lake Alau are presented in table 4. The following alleles AA, AB, BB and CC with frequencies of 0.4, 0.1, 0.4 and 0.1 were observed among *Clarias anguillaris* species respectively. While, *Clarias gariepinus* had AA, BB, CC and BD alleles with frequencies of 0.4, 0.3, 0.2 and 0.1 respectively.

Table 4: Alleles frequency from two species of *Clarias* in Lake Alau, Maiduguri

Fish species	Genotype					Total
	AA	AB	BB	CC	BD	
C.anguillaris	4	1	4	1	0	10
C.gariepinus	4	0	3	2	1	10
Gene frequency						
C.anguillaris	0.4	0.1	0.4	0.1	0.0	1.1
C. gariepinus	0.4	0.0	0.3	0.2	0.1	1.1

The test for significant deviation or conformity to Hardy-Weingberg Equilibrium (HWE) using equilibrium P + Q = 1. This equation was followed as P = (2NAA + NAB) / 2N and Q = (2NBB + NAB) / 2N is shown in Table 5. The result recorded for this study indicate that high A (0.60) allele frequency value was for *C. gariepinus* species while A and B alleles frequencies had 0.45 each as obtained for *C. anguillaris* species. The least B (0.20) number of allele was observed in *C. gariepinus* species.

Table 5: Test for deviation or conformity to Hardy-Weingberg Equilibrum from two species of *Clarias* in Lake Alau, Maiduguri

Fish species	N	A	B	Total
C. anguillaris	10	0.45	0.45	0.90
C. gariepinus	10	0.60	0.20	0.80

The genetic variation between the two species of *Clarias anguillaris* and *Clarias gariepinus* in Lake Alau (Table 6). The total and mean number of alleles for each species of the fish was 10 and 1.0 respectively.

Table 6: Alleles frequency variation between *C. gariepinus* and *C. anguillaris*

	Population number										Total	Mean
Fish species	1	2	3	4	5	6	7	8	9	10		
C. anguillarias	1	1	1	1	1	1	1	1	1	1	10	1.0
C. gariepinus	1	1	1	1	1	1	1	1	1	1	10	1.0

DISCUSSIONS

The overall blood group of both male and female *Clarias gariepinus* and *Clarias anguillaris* in Lake Alau was found to be O^- (100%). Our finding was appear to be well substantiated by Diyaware *et al*. (2017), who reported the wild females and males *Clarias gariepinus* had O^-, O^+, A^+, B^+ and AB with 50, 10, 10, 10 and 20% respectively. However, the result of the present study differs from Ayorinde *et al.* (2009), Odo *et al.* (2013), and Onyia *et al*. (2013) who had reported O^+(90%) and O^-(10%) for *Heterotis niloticus*, O^+(90%) and O^- (10%) for *Parachanna obscura* and O^+ (83%) and AB^+ (16.7%) for *Heterobranchus bidorsalis* respectively. Onyia *et al*. (2015) recorded O^+ (83%) and AB^+ (16.7%) each for *C. gariepinus* and *C. anguillaris* from Lake-Geriyo. Those differences might be due to the differences in the fish species. The blood group of O^- ' it is an advantageous to the fish in terms of risk of death of fingerlings in case of fertilization compared to positive blood group (Odo *et al.,* 2013). The current study revealed that there were five heamoglobin genotypes, three homozygotes (AA, BB and CC) and two heterozygous (AB and BD). *Clarias gariepinus* indicated the existence of small number of heterozygote genotypes when compared to homozygote genotypes and such heterozygosity confers better growth performance (Ola-Oladimeji, 2021). This finding agrees with the general observation of A and B alleles with their corresponding genotypes AA, BB, CC, AB and BD in different species (Ajayi *et al.,* 2013; Ross *et al.,* 2013). Also similar findings were reported by Diyaware *et al*. (2017) of AA, BB, CC and BD genotypes from *C. gariepinus* and AA, AB and BB in Nigerian indigenous chicken breeds in the Niger Delta region, reported by Agaviezor *et al.* (2013). However, the result was contradicted by the findings of Ayorinde *et al.* (2009) who reported AS and SS genotype in *Heterotis niloticus* and AA and AS genotype in *P. obscura* as documented by Odo *et al.* (2013). The heterozygous genotype AB and BD obtained in this study agrees with the result of Ajayi *et al.* (2013) and Diyaware *et al*. (2017) who had documented AB genotype in Nigerian indigenous chicken breeds and BD genotype in wild *Clarias gariepinus* respectively. Suleiman *et al.*(2020) reported 76.12% of polymorphic bands while 21.39% were monomorphic from *C. gariepinus*. Heterozygosity is related to the polymorphic nature of each locus and could be expected to correlate with high levels of genetic variation at loci with critical importance for adaptive response to environmental changes or natural hybridization (Kotzé and Muller, 1994; Diyaware *et al*., 2017). The Hb concentration value obtained of this study for males *C. gariepinus* and *C. anguillaris* were 9.180±0.274g/dl and 8.5000±767g/dl but in females were 7.900±0.372g/dl and 8.440±0545g/dl respectively. This result differs with the values documented by Osman *et al.* (2018) and Kefas *et al*. (2015). Higher Hb recorded in males compared to

females might be due to differences in sex or males are more active and aggressive. Oluwalola *et al.*(2020) reported the haemoglobin concentration of 12.6±012-7.83±0.19 in Nile Tilapia *Oreochromis niloticus* from different culture enclosures. The gene frequencies of allele AA in both species were higher than that of AB, BB, CC and BD respectively. This is similar with the findings of Tella *et al*. (2000) and Ajayi *et al.* (2013). This genus of fish has been reported to have the ability to withstand harsh and disease resistance (Agbebi *et al.*, 2009; Ikpeme *et al.*, 2015; Diyaware *et al*., 2017)). The value for Allele A in both species is higher than that for Allele B and the result of this finding is within the threshold of 1 that is considered the value for the population to confirm Hardy Weinberg's equilibrium (P + Q = 1). This conformity also indicates that there is heterozygosity in the population as indicate in table 2.

Conclusion

It can be concluded from the findings of this study that, the presences of AB and BD genetic genotypes is an indication of the level of genetic diversity at the heamoglobin (Hb) locus in the *C. anguillaris* and *C. gariepinus* populations. Polymorphic characteristics in *C. anguillaris* and *C. gariepinus* in terms of allele is defined by two alleles, 'A' and 'B' with the A being 0.90 while the B was 0.80. The total values for alleles A and B in both species from this study is within the threshold of 1 that is considered the value for the population to confirm Hardy Weinberg's equilibrium. This conformity also indicates that there is heterozygosity in the populations. The degree of heterozygosity value, suggesting that the fishes populations could be undergoing assortative mating.

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