https://dx.doi.org/10.4314/ijs.v19i2.9

HAEMOGLOBIN POLYMORPHISM IN WILD AND CULTURED AFRICAN CATFISH (*Clarias gariepinus* BURCHELL, 1822)

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(Received: 20th September, 2016; Accepted: 13th August, 2017)

ABSTRACT

Haemoglobin polymorphism, haemoglobin concentration, blood group and genotypes of wild and cultured *Clarias gariepinus* were investigated. Blood samples of *Clarias gariepinus* collected from Lake Alau (wild) and Dalori fish farm (cultured) were subjected to cellulose acetate electrophoresis to reveal the activities of hemoglobin (Hb). The result shows that both wild and cultured *C. gariepinus* had AA, BB and CC genotypes in the males and females. However, BD genotype was observed only in the female wild *C. gariepinus*. The percentage AA and BB genotypes of wild male *C. gariepinus* was 6.6% each. Wild females had AA, BB, CC and BD genotype with frequencies of 26.6, 33.3, 20 and 6.6% respectively. Cultured male *C. gariepinus* had AA, BB and CC with frequencies of 13.3, 26.6 and 6.6%, respectively, while the female *C. gariepinus* had AA, BB and CC with frequencies of 20, 13.3 and 20% respectively. Wild *C. gariepinus* had the highest (10.36 g/dl) haemoglobin concentration value while the cultured strain had 8.86 g/dl. The blood groups of wild male *C. gariepinus* were O^- (10% of the population) and AB^+ (10% of the population). The wild females had O^-, O^+, A^+, B^+ and AB^+ with 40, 10, 10, 10 and 10% of the population respectively, Cultured male had O^+, A^+ and AB^+ (10, 20 and 20% of the fish observed) respectively. The blood group observed in the cultured female *C. gariepinus* were O^- (30%), A^-(10%) and B^+ (10%). The BD genotype observed in a wild strain of *C. gariepinus* indicates incomplete (partial) dominance and rare allele. This could be an indication of natural hybridization.

Keywords: Haemoglobin concentration, polymorphism, genotype, blood group, wild and cultured, *Clarias*

INTRODUCTION

The African catfish *Clarias gariepinus* is the most widely cultured catfish in Africa and the third most cultured catfish species in the world (Garibaldi, 1996). Its aquacultural attributes include the highest growth rate at high stocking densities under culture condition, high fecundity rate, resistance to diseases, ability to tolerate a wide range of environmental extremes (Fagbuaro, 2014). African catfish provides food for the populace, allows improved protein nutrition because it has a high biological value in terms of high protein retention in the body, higher protein assimilation as compared to other protein sources, low cholesterol content (Anoop *et al.*, 2009). *C. gariepinus* production is important to the Nigerian economy because it serves as a source of income, reduces the rate of unemployment in the economy and increases the Gross Domestic Product (GDP) (Adebayo and Daramola 2013).

Polymorphism is a genetic variant that appears in at least 1% of the population. It is a discontinuous genetic variation where two or more forms, stages, or types exist in the same species within the same population, it can apply to biochemical, morphological, and behavioural characteristics, but must be discontinuous (Agaviezor *et al.*, 2013). Polymorphism is genetically useful to help determine the origin, phylogenetic relationships compiled among species and or groups within the species. Most of the blood protein polymorphism was genetically regulated by pair of alleles or sequence of alleles without dominance (Warwick *et al.* 1990; Abubakar *et al.* 2014).

Haemoglobin polymorphism have been used as marker to study evolutionary relationship in mammals (Chineke *et al.*, 2007). Evolutionary relationship between sheep and goat breeds, deer species and chicken genotype has been examined (Yang and Jiang 2005). According to Chineke *et al.* (2007), the study of biochemical polymorphism of blood protein is at present a useful tool to characterize livestock breed and fish population, hence it contributes to the knowledge of genetic

similarity and distance (Omitogun 2004). According to Fisher (1980), information on polymorphism of fish species can be used to enhance its capability to adapt to changing environment which is necessary for survival of the species. Polymorphism also increases adaptability thereby providing for the possibility of genetic change.

Biochemical diversity or polymorphism is the occurrence of varieties attributed to biochemical differences which are under genetic control (Egena and Alao 2014). It has created a leeway for the genetic improvement of farm animals. This is because it can be used as a useful tool for the characterization of livestock breeds and fish. This way, the degree of similarity or differences within and between species of fish can be ascertained using this simple biochemical diversity. This difference or similarity is an important raw material for genetic improvement of fish.

Blood typing have been applied intensively to study variation in fishes (Sindermann, 1967). According to Sindermann (1967), it has also been determined that, as in humans, the frequency of different blood types vary, depending on the population sampled and the variation is of course an important tool to study intermixing of fish population.

Morphological description and morphometric analyses were the first tools used to define fish species (Pante *et al*., 1988). But these techniques are polygenic, quantitative or continuous characters and their expression is influenced by environmental conditions (Majolagbe *et al*., 2012). However, many biochemical and molecular markers technique such as allozyme analysis, types of restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), microsatellite typing etc. can reveal better diagnostic genetic variations and are usually free from genotype and environmental interactions (Lombard *et al*., 2001; Torkpo *et al*., 2006).

The study of haemoglobin patterns provides a very good means of identifying distinct populations or sub-populations of fish (Sick, 1961). Analysis of haemoglobin patterns may be a valuable tool in taxonomy of different species of fish and their hybrids (Perez and Rylander, 1984). Haemoglobin and haemocrit are related to oxygen binding ability of blood and changes in both values, are related to stress.

Animals with genotype Hb and BB have been reported to have better body weight (Arora *et al*., 1971). In sheep, with Hb AB and Hb BB genotypes had been observed to have an effect on their performance (Dally *et al*., 1980; Arora, 1984; Dratch *et al*., 1986). Sick (1965) reported haemoglobin polymorphism in Cod and found that the frequencies of the Hb *I'* allele range from 55 to 72 %. Braman *et al*. (1980) investigated haemoglobin polymorphism in a population of adult Cutthroat trout (*Salmo clarkii*) by employing starch gel electrophoresis and reported 12 haemoglobin components in the species. Haemoglobin polymorphism has been reported in cattle by Pal and Mummed (2014), in sheep Tsunoda *et al*. (2010) and Al-Samarrae *et al*. (2010), in goat Elmaci (2001) and in poultry (Dimri, 1978). Onyia *et al*. (2015) documented the blood group and genotype of *Clarias gariepinus* and *C. anguillaris*. Eighty-three percent (83%) of males were O+, while 18% of the females were AB+. Both males and females were reported to have AA genotype for both fish species.

A population is said to be polymorphic when two or more distinctly inherited varieties coexist in the same individual (Das and Deb, 2008; Egena and Alao, 2014). This type of polymorphism is being increasingly used to study genetic differences within and between populations and to estimate genetic divergence (Lee *et al*., 1995). The study of haemoglobin polymorphism in fish has become imperative, because of its importance in the improvement of farm animals due to the fact that some polymorphic alleles may be linked with traits of economic importance (Egena and Alao, 2014). Arora *et al*. (1971) reported marginally but not significant better body weight in Hb BB type animals.

Morphological parameters have been used in the past to identify fish, but more specific tool is needed for a more concise differentiation as the morphological variations have been documented

to underestimate the true levels of genetic variability. Morphological features have been used in many genetic and breeding studies to identify both parental and hybrid stocks, the morphological characters tend to overlap each other due to differences in environment or mixed up in the population during sample collection (Diyaware *et al.,* 2012).

Molecular markers have recently been used to characterize Clariid species, but these tools are expensive and requiring huge upfront work for their development. There is a paucity of information on haemoglobin polymorphism of Clariid fish. The blood group and genotype of *Clariids* are yet to be exploited. This study, therefore investigated haemoglobin polymorphism, blood group and genotypes of wild and cultured *C. gariepinus*.

MATERIALS AND METHODS

Study Area

Fish samples from the wild were collected from Lake Alau in Konduga Local Government Area of Borno State, Nigeria, which is located at latitude 11^{0} and 42' 13" N and longitude 130 16' 02" E (Google Map, 2017). The study area has two distinct seasons: the dry season, which starts from October to April, while the rainy season commences from July to September.

Experimental Fish

A total of 15 each of wild and cultured *C. gariepinus* (330.50 - 900.00 g weight and 296.90-500.00 mm total length) were procured from fishermen at Lake Alau and fifteen (15) five months old cultured *C. gariepinus* (463.00 - 700.00 g weight and 374.50 - 40.05 mm total length) were procured from a private fish farm in Maiduguri and were used for the study. All the fish were transported live in 50 litre capacity plastic Jerry-cans half-cut horizontally, to fish hatchery complex of the Department of Fisheries, University of Maiduguri

Sample Collection

Blood samples were collected from each of the fish samples via the caudal vein according to the method described by Whitman (2004) using separate thermodermic syringes fitted with 2G needle and emptied into an EDTA bottle containing anticoagulant to prevent the blood from clotting. The labelled blood samples were transferred to the Animal Science laboratory of the University of Maiduguri for determination of haemoglobin concentration, blood group, and cellulose acetate electrophoresis.

Haemoglobin Concentration (Hb)

The indirect acid haematin (sahli) method Louis-Berman (1919) was used. This involved the use of a special haemoglobinometer and a pipette. The graduated tube was filled with 20 ml of 0.1 M HCl and 0.02 ml of blood sample was added. The mixture was allowed to stand for 5 min and then a few drops of distilled water were added until the colour matched the standard. Haemoglobin concentration was estimated as follows: Hbc = coloured matching values ×17. 2 g/100 mL÷100 (Cook, 1985; Balasubramaniam and Malathi, 1992)

Determination of Fish Blood Group

Blood group was determined using a standard test tube agglutination techniques described by Prasad (2013). The blood was dropped on a clean white porcelain tile (Prasad, 2013) using syringe in three different places, then on each of the blood samples, 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 the coagulation of blood according to Daramandy and Davenport (1985) and Svobodovaet al. (1991).

Cellulose Acetate Electrophoresis

Haemoglobin typing was accomplished using cellulose acetate electrophoresis. The cellulose acetate strips were soaked in EDTA borate buffer of pH 8.6 and blotted slightly with filter paper to remove excess buffer. The samples were placed on the cellulose acetate paper and then placed into the electrophoresis tank. The samples were then allowed to separate for about 10-15 minutes. After the separation, the cellulose acetate papers were blotted dry using filter paper and then dried in open air for some minutes and then the numbers of bands were taken. The direct gene counting method was used to score the resulting haemoglobin bands after electrophoresis.

The haemoglobin polymorphism was pointed out by detection of three migration zones as described

by Agaviezor *et al.* (2013): a single faster band was designated as the AA homozygote; the presence of a single slower band was designated as BB homozygote and the presence of both bands designated AB heterozygote. Genotype frequency was estimated according to Agaviezor *et al.* (2013) formulae as follows:

Gene frequency AA = No. of AA ÷ No. of samples x 100 ÷ 1

Gene frequency AB = No. of AB ÷ No. of samples x 100 ÷ 1

Gene frequency BB = No. of BB ÷ No. of samples x 100 ÷ 1

CC frequency = No. of CC ÷ No. of samples x 100 ÷ 1

BD frequency = No. of BD ÷ No. of samples x 100 ÷ 1

Estimation of Gene Frequency

Gene expression frequencies were estimated using Hardy Weinberg equation (Oghanje *et al.*, 2015): $P = 2N_{AA} + N_{AB} \div 2N$ and $Q = 2N_{BB} + N_{AB} \div 2N$, where, P = gene frequency of allele A, N = Total number of individual sampled, N_{AA} = observed genotype number for AA, N_{AB} = observed genotype number for AB and N_{BB} = observed genotype number for BB and Q = Gene frequency of allele B

Scoring of the Genotype on the Cellulose Acetate Gel Electrophoresis

The cellulose acetate gel electrophoresis representing genotype at the polymorphic protein were manually scored based on their clarity and designated as A, B, C, D, E, F and G from the bottoms to the top of the gel using a ruler as described by Laloei e*t al.* (2013).

Data Analysis

The haemoglobin data were analysed using One - Way Analysis of Variance (ANOVA). The differences between the means were determined using LSD at 95% confidence level (p=0. 05). The blood group, genotype and gene frequency were analysed using descriptive statistics (percentage).

RESULTS

Genotypes of Wild and Cultured *Clarias gariepinus*

Plate 1 and 2 show the representative of acetate electrophoresis of culture and wild strains of *C. gariepinus* respectively.

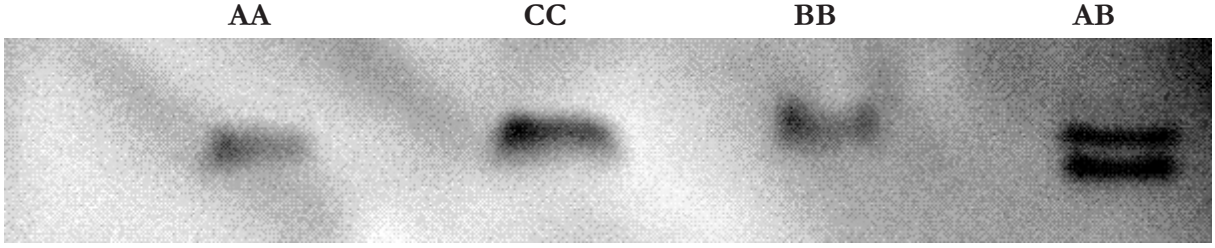

Plate 1: Representative of cellulose acetate electrophoresis gel of cultured *Clarias gariepinus*

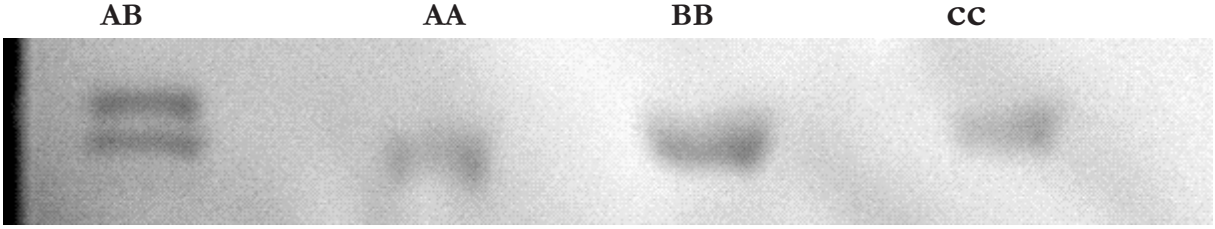

Plate 2: Representative of cellulose acetate electrophoresis gel of wild *Clarias gariepinus*

Table 1 shows the genotypes of wild (Lake Alau) and cultured *Clarias gariepinus* in Maiduguri. Both the wild and cultured *C. gariepinus* had AA, BB and CC genotypes in the male and females. However, BD genotype was observed only in the female wild *C. gariepinus*, the percentage AA and BB genotypes of wild male *C. gariepinus* was 6.6% each. Wild females had AA, BB, CC and BD genotypes with frequencies of 26.6, 33.3, 20 and 6.6% respectively. Cultured male *C. gariepinus* had AA, BB and CC with frequencies of 13.3, 26.6 and 6.6% respectively. The female *C. gariepinus* had AA, BB and CC with frequencies of 20, 13.3 and 20% respectively.

Table 1: Genotypes of wild and cultured *Clarias gariepinus*

Fish strain	Male	Percentage (%)	Female	Percentage (%)
Wild *C.gariepinus*	AA	6.6	AA	26.6
	BB	6.6	BB	33.3
			CC	20
			BD	6.6
Cultured	AA	13.3	AA	20
C.gariepinus	BB	26.6	BB	13.3
	CC	6.6	CC	20

Gene Frequency of Wild and Cultured *Clarias gariepinus* in Maiduguri

Table 2 shows the gene frequency of the wild and cultured *C. gariepinus*. Wild strain of *C. gariepinus* had A, B, C and D alleles, while the cultured strain had allele of A, B and C but had no D allele. The A, B, C and D alleles of wild *C. gariepinus* had frequencies of 0.33, 0.2, 0.1 and 0.03 respectively. The cultured *C. gariepinus* allele of A, B and C had frequency of 0.33, 0.2 and 0.13 respectively.

Table 2: Gene frequency of wild and cultured *Clarias gariepinus*

Fish strain	Genotype				Total
	AA	BB	CC	BD	
WCg	5	6	3	1	15
CCg	5	6	4	-	15
Gene frequency					
WCg	0.33	0.2	0.1	0.33	0.66
CCg	0.33	0.2	0.13	-	0.66

Key: WCg = Wild *Clarias gariepinus*, CCg = Cultured *C. gariepinus*

Genotyping (Allelic) Variation between Wild and Cultured *Clarias gariepinus*

Table 3 shows genotyping (Allelic) variation within the wild (Lake Alau) and cultured *C. gariepinus* in Maiduguri. The total number of genotypic alleles per population was 15 genotypes and mean of 1.0 per population for each strain

Table 3: Genotyping (Allelic) variation between wild and cultured *C. gariepinus*

FS	Population number															Total	Mean
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
WCg	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	15	1.0
CCg	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	15	1.0

Key: FS= Fish species, WCg = Wild *Clarias gariepinus*, CCg = Cultured *C. gariepinus*

Haemoglobin Concentrations in Wild and Cultured *Clarias gariepinus* **from Lake Alau**

Wild *C. gariepinus* had significantly ($p<0.05$) higher Hb concentration (10.36 g/dl) compared to the cultured *C. gariepinus* (8.86 g/dl). Sex-wise, cultured *C. gariepinus* males had higher (10.46 g/dl) Hb concentration followed by that of the female CCg with (10.30 g/dl). The least (8.70 g/dl) Hb concentration was observed in the male WCg. No significant variation ($p>0.05$) was observed sex wise (Table 4).

Table 4: Mean Haemoglobin Concentrations of Wild and Cultured *C. gariepinus*

Fish Strains	Hb (g/dl)	SEM
Wild *Clarias gariepinus*	10.36[a]	0.36
Cultured *Clarias gariepinus*	8.86[b]	
Sex		
Wild male *Clarias gariepinus*	9.30[a]	0.74
Wild Female *Clarias gariepinus*	8.70[a]	
Cultured male *Clarias gariepinus*	10.46[a]	0.65
Cultured female *Clarias gariepinus*	10.30[a]	

Mean values in the same column with the same superscripts are not significantly different from each other ($P \leqslant 0.05$)

Blood Groups of wild (Lake Alau) and Cultured *Clarias gariepinus* in Maiduguri

Table 5 shows the blood groups of the two strains of the *C. gariepinus*. The males of wild *C. gariepinus* had O^- and AB^+ blood groups with 10% each, while the females had O^-, O^+, A^+, B^+ and AB^+ blood groups with 40, 10, 10, 10 and 10% respectively. The males of the cultured had O^+, A^+ and AB^+ with 10, 20 and 20% respectively, while the females had O^- A^- and B^+ with 30, 10 and 10% respectively.

Table 5: Blood Groups of Wild and Cultured *Clarias gariepinus*

Strain	Male group	Blood group (%)	Female blood group	Blood group (%)
WCg	O^-	10	O^-	40
	AB^+	10	O^+	10
			A^+	10
			B^+	10
			AB^+	10
Total				100
CCg	O^+	10	O^-	30
	A^+	20	A^-	10
	AB^+	20	B^+	10
Total				100

Key: WCg = Wild *Clarias gariepinus*, CCg = Cultured *Clarias gariepinus*

DISCUSSION

The O^+ and AB^+ blood groups recorded in this study for males of wild and cultured species differs from (O^- and A^+) for *C. gariepinus* and *C. anguillaris* documented by Onyia *et al.* (2015). This variation could be due to the differences in the species. The O^+, A^+ and AB^+ blood group in male cultured *C. gariepinus* differs from O^+ and AB^+ recorded by Onyia *et al.* (2015) for the same fish species. The variation in the blood groups of wild female *C. gariepinus* observed in this study (O^-, A^- and B^+) differs from those (O^+ and AB^+) reported by Onyia *et al.* (2015). The O^-, A^- and B^+ blood groups observed in this study for female cultured *C. gariepinus* differs from (O^+ and AB^+) reported by Ayorinde *et al.* (2009).

The occurrence of two co-dominant genotypes (AA and BB) in both wild and cultured strains of *C. gariepinus* has also been reported in farm animals by Pal and Mummed (2014). Tell (2000) reported that after electrophoresis on cellulose acetate, the Hb genotype that migrated faster from the point of application at pH of 8.5-9.0 was labelled AA, the slow-moving fraction was identified as BB while the heterozygote was AB. The results of this study varied with AA genotype reported by Chuku and Uwakwe (2012) for the same fish species and *H. bidorsalis* reported by Onyia *et al.* (2013).

A genetic character is known to be polymorphic when the rarest genotype has a frequency greater than one percent (Das and Deb, 2008). Allele B had the highest frequency in both strains followed by allele A. Based on the electrophoretic separation of proteins in the present study, three different alleles, encoding three different genotypes were found on the locus of haemoglobin with higher B, than A frequency. The AA gene has been reported to confer resistance to helminths on the carriers and the degree of resistance is dose-dependent (FAO, 1988).

The higher Hb values reported in this study differs from 18.43 g/dl and 16.5 g/dl reported by (Onyia, 2013). This variation may be due to environmental factors and the size of the fish. According to Lenfant and Johansen (1976) haemoglobin concentration is higher in the fishes capable of aerial respiration. The BD genotype recorded in the wild strain of *C. gariepinus* caught from Lake Alau indicates incomplete (partial) dominance and is a rare allele. Incomplete (partial) dominance refers to a heterozygous condition in which both alleles at a gene locus are partially expressed, often producing an intermediate phenotype. However, rare alleles according to Underwood (2017) are caused by mutation. The presence of BD genotype in this population could be an indication of possible natural hybridization.

CONCLUSION

The wild and cultured populations of *C. gariepinus* showed genetic variation. The BD genotype observed in the wild population of *C. gariepinus* caught from Lake Alau indicates incomplete (partial) dominance and rare allele, and could be an indication of natural hybridization.

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