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Research Article
Differences in the Haematological Profile and Nutrients Value of Pigmented and Albino *Oreochromis niloticus* (Linnaeus, 1758)

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Abstract

Background and Objective: The awareness on the haematological values, genotype and blood group of Albino *O. niloticus* is unknown, however such information is optimal for the culturing of the species, to discover the health condition of the fish and it will be beneficial in genetic study, hence, enhance the growth and survival of the fish. The main objective of the current study was to evaluate the haematology, genotype, blood group and nutrients value of Pigmented and Albino *Oreochromis niloticus* of both male and female. **Materials and Methods:** The samples were obtained from Mautech fish farm and Upper Benue River with mean average weight and length for male Pigmented 176 g and 18.00 cm and the female 112.10 g and 16.20 cm whereas Albino male had 203.11 g and length 18.20 cm while the female had the mean weight of 186.5 g and a length of 18.93 cm. The fish was acclimatized in MAU earthen pond for 2 weeks before the analysis. The blood group was ascertained using test tube techniques and the genotype was done through hemoglobin electrophoresis. The nutrients value was analyzed based on Association of Official Analytical Chemist Methods (AOAC). **Results:** The results exposed that the haematological profile were significantly different among the specie. The hematocrit value was higher in Albino fish 32.30 and 39.20% for male and female Albino while Pigmented *O. niloticus* had lower values. Other values were WBC, RBC and haemoglobin for male and female Albino 106.64, 111.0, 4.68 and 8.10 µL and 8.78 and 10.26 g dL^{-1}, respectively. The blood group of the male Albino were 30% B^+ and 70% O^-, with the genotype of 80% AC and 20% AF, while the female had the blood group of 30% B^+ and 70% O^+, with the genotype of 90% AC and 10% AF. However, the males of Pigmented *O. niloticus* had 100% O^+ while the females were 20% B^+ and 80% O^- and the genotypes were 100% AC. The Albino had significant higher values in crude protein 59.3 and 65.9% compared to Pigmented with 55.8% CP. **Conclusion:** The significance of the haematological indices in fish cannot be over emphasized, it is a determinant factor for the wellbeing of the fish. The genotype and blood group correlate with the genetic status of the fish which can be ascertained to enhance the fish heterosis and survival. Finally, it can be denoted that Albino fish is profitable.

Key words: Haematology, nutrients, Pigmented, Albino *Oreochromis niloticus*

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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

Oreochromis niloticus (Nile tilapia) are fast-growing fish, prolific and produce good fillets, it is found in variety of fresh water ponds, lakes, rivers, reservoirs, sewage canals and brackish water[1]. They are one of the most important warm water fishes effectively used for aquaculture production. It is an African fresh water cichlid and generally accepted throughout the world[2,3]. *Oreochromis niloticus* feed on a wide variety of natural food organisms found in their habitat or organically fertilized ponds which includes phytoplankton, benthic algae, insect larvae, detritus and making them herbivorous in nature as well as artificial feeds[4].

Haematology is the study of the morphology of the blood, blood forming organs and diseases. However, haematology deals with the physiology and the pathology of the cellular elements of the blood, which includes the erythrocytes, leukocytes and platelets[5]. Blood is essential for proper functioning of the organs in the body of the fish which will facilitate aquaculture development[6]. It aids in the circulation of oxygen and nutrients in every organ. Blood aid in the removal of waste and defense against infection[7]. However, the water body in which the fish inhabit has an impact in the blood components of the fish considering the physical and chemical qualities of the water. Some of the fish have been exposed to toxins, chemical compounds and has generally influenced the health of the fish[6]. The health of the fish is important because it correlate with the wellbeing, growth and survival of the fish[8].

Several factors can directly or indirectly influence haematological profile of the fish[9], environmental factors such as water temperature, genetic makeup of the fish, specie of the fish[10], handling stress, sex, age, size and physiological conditions[6,11]. Significant knowledge of hematological parameters is an important tool that can be used to monitor the health status of the cultured fish.

Authors have established haematological profile of some tilapia species, mono sex *Oreochromis niloticus*[12], Nile tilapia fed with ginger[13], *Tilapia zillii*[14], *Sarotherodon melanotheron*[15], Nile tilapia and hybrid catfish[16], hematological changes in tilapia *Oreochromis niloticus* exposed to mercury and selenium[17], Nile green tilapia[18], Haematological profile of *O. niloticus* exposed to various concentrations of cadmium chloride[1]. However little or no knowledge is known on haematological and nutrient values of Pigmented and Albino *Oreochromis niloticus* from the upper Benue River Basin in Adamawa State of Nigeria. This research will provide information on the haematological profile, blood group, genotype and nutrients value of Albino and Pigmented *O. niloticus*, this beneficial knowledge will enhance the culture of the specie in the region.

MATERIALS AND METHODS

The research was conducted from 22nd August-September, 2020 in the laboratory of the Department of Fisheries, Modibbo Adama University, Yola Adamawa State, located at latitude 9°13′48″N and longitude of 12°27′36″ E.

Sample collection: Forty live *Oreochromis niloticus* Pigmented male and females (176.50±59.08, 112.10±9.20) and Albino (203.11±39.49, 186.55±2.12) was collected from Upper Benue River Basin landing site in Adamawa State and transported in an aerated plastic trough to the University earthen pond and acclimatized for 7 days before the commencement of the analysis. To ensure quality research, the fish health was monitored based on their feeding capacity, further observation was carried out by observing the appearance of the fish to ensure there was no visible indication of disease or wounds. The fishes were separated based on their sex by viewing their genital organ, males had 2 openings and female's 3 openings.

Blood collection: Twenty adults of *O niloticus* was used for the blood collection. Ten males and 10 females for the Pigmented and Albino. The blood for the analysis was amass from the caudal vein with different 5 mL of heparinized disposable syringes and hypodermic needles containing 0.2 mL heparin solution as anticoagulant. The blood sample collected from the adult fish was placed in an Ethylene Diamine Tetra Acetic acid (EDTA) container for analysis on the haematological values.

Followings were analyzed

Haematocrit (Hct): it is a test method used to ascertain the volume of the red blood cell that are present in the blood of a fish, it is expressed in percentage. The MSE micro centrifuge was used, it was evaluated after centrifugation at 15000 rpm.

Hemoglobin concentration (Hb): it is the amount of hemoglobin in a volume of blood. The indirect acid Haematin method (Sahli technique) was utilized. It was determined using haemoglobinometer and pipette. The concentration of the Hemoglobin was converted to acid haematin by the use of 0.1 M HCl using 0.02 mL pipette. The graduated tube added with 20 mL 0.1 M HCl 0.02 mL of blood sample. It was

placed to stand for 5 min before few drops of distilled water was added until the color matched the standard. Hemoglobin concentration are calculated as:

$$\text{Hbc} = \frac{\text{Values obtained} \times 17.2 \text{ g}}{100 \text{ mL}} \div 100$$

Leucocyte count (Lc): it is a test that measure the number of white blood cells in the blood of a fish. The haemocytometer was used to determine the Lc. 0.8cm objective of the microscope and large squares was used (area = 1 mm^2, depth = 0.1 mm) while the expected volume was 0.1 mm^3 and the 20 represent the dilution factor. Four squares were utilized for while the total count per mm^3 is:

$$\frac{20 \times 1 \times \text{L cells}}{0.4} = 50 \times \text{L cells}$$

where, L is number of leucocytes that was counted.

Erythrocytes (Ec): Are a nucleate, biconcave cells that contain hemoglobin, which transport oxygen and carbon dioxide between the lungs and tissues. RBC are produced in the red bone marrow by a process called erythropoiesis. This was determined in heparinized blood diluted by Hayman solution at a ratio of 1:200. The 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 2 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: 200×50×R cells = 10.000×R Mean cell volume: The mean cell volume was expressed in pico gram (pg) as:

$$\text{MCV} = \frac{\text{Hbc}}{\text{Ec}}$$

Mean corpuscular hemoglobin concentration: it is the quantity of haemoglobin concentration present in a volume of the packed red blood cell. It is calculated by dividing the haemoglobin and the haematocrit:

$$\text{MCHC} = \frac{\text{Hbc}}{\text{Hct}} \times 1000$$

Determination of fish blood group: The different blood types in a fish are evaluated by the presence of or absence of proteins known as antigens on the cell membrane of the red blood cells. The test tube techniques based on tests agglutination was used. The fish blood was collected with a syringe and drop on the tile in three different places. The blood s ample from each treatment replicated thrice, the anti-sera A, B and D was dropped on each, respectively and mixed. The tile was rocked for about 3-5 min. Blood groups were recorded based on coagulation of blood according to[6,11].

Determination of fish genotype: Genotype is ascertained by the makeup of alleles, pairs of genes that are responsible for a particular trait. However, an allele can be made up of dominant genes (AA) or a recessive gene (aa) which are homozygous or heterozygous genes (Aa). The combination of the 2 and the dominant alleles, determines the traits expressed by the alleles. The fish blood that was collected were placed in a sterile EDTA container, with a known control AS. With the test sample and the cellulose acetate paper was deep inside distilled water and removed, it was laid on the work bench, a lancet was used to take a small portion of a known AS and kept vertically on the acetate paper, also the test control was placed 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[19].

Proximate composition analysis: The nutrients value of the Pigmented and Albino *Oreochromis niloticus* were determined using Association of Official Analytical Chemist Methods (AOAC). Four samples replicated 3 times, comprising of male and female of both Albino and Pigmented *O. niloticus* were thoroughly washed, gutted and sundried for 4 hrs to reduce the moisture contents. It was further processed using local smoking kilns under low heat. The percentage crude protein, fiber ash, lipid and Nitrogen Free Extract were determined.

Statistical analysis: The data obtained from the analysis was subjected to one way analysis of variance, while the differences between the means were determined using Least Significant Differences (LSD) at 95% confidence level (p = 0.05) with the aid of SPSS20.

RESULTS

The results of the haematological value of male Albino and Pigmented *O. niloticus* were shown in Table 1. Haematocrit value was in Albino male 32.30% and Pigmented 29.90%. Other values were WBC 106.64 µL, RBC 4.68 µL and haemoglobin 8.78 g dL^{-1} while male Pigmented had 29.90% for haematocrit, WBC 95.30 µL, RBC 5.15 µL and hemoglobin 9.45 g dL^{-1}. The weight of the male Albino was 203.11 g and Pigmented had 176.50.

The results of the haematological value of the female Albino and Pigmented *O. niloticus* were represented in Table 2. The female Albino had 39.29% in haematocrit, WBC 111.0 µL, RBC 8.10 µL and hemoglobin 10.26 g dL^{-1}, respectively. While the female Pigmented had 32.20%, WBC 98.20 µL, RBC 4.51 µL and hemoglobin 9.04 g dL^{-1}, respectively. The weight in g was for Albino 186.55 and Pigmented 112.10.

The blood group, genotype of male and female Albino *Oreochromis niloticus* were presented in Table 3. Male Albino was 30% B^+ and 70% O^-, with the genotype of 80% AC and 20% AF, while the female had the blood group of 30% B^+ and 70% O^+, with the genotype of 90% AC and 10% AF.

The blood group, genotype of male and female Pigmented *O. niloticus* was shown in Table 4. The males of Pigmented *O. niloticus* were 100% O^+ while the females had 20% B^- and 80% O^-. The genotype of the male and the female were 100% AC.

The results of the proximate composition of both male and female Pigmented and Albino *O. niloticus* were presented in Table 5. The percentage crude protein was higher in all the samples. The female Albino had the highest percentage crude protein levels of 65.9, followed by the male Albino with 54.3% and the male and the female Pigmented had 55.8 and 55.0%, respectively. The percentages of Ash contents were 19.5%, followed by 18.0% for female Pigmented and 16.0%, male Albino, which is the least. The percentage of Lipid of male Albino and female Pigmented were 11.5 and 11.0%, respectively. The female Albino has 9.5% while male normal has about 80%. The percentage of

Table 1: Comparison of hematological indices of male Albino and male Pigmented *O. niloticus*

Parameters	Albino *O. niloticus*	Pigmented *O. niloticus*
Haematocrit (%)	32.30±4.16^a	29.90±4.33^b
WBC (µL)	106.64±22.84^a	95.30±14.32^b
RBC (µL)	4.68±0.73^b	5.15±1.98^a
Hb (g dL^{-1})	8.78±1.27^b	9.45±1.63^a
MCHC (g dL^{-1})	27.13±3.45^b	31.87±3.64^a
MCH (pg)	1.92±0.42	2.05±0.61
MCV (fl)	70.97±14.59^a	64.02±18.89^b
Weight (g)	203.11±39.49^a	176.50±59.08^b
Standard length (cm)	18.20±1.99	18.00±2.54
Total length (cm)	21.75±2.34	21.15±2.78

Means with different superscript are significantly different ($p<0.05$) and values are Mean±SE

Table 2: Comparison of hematological indices of female Albino and Pigmented *O. niloticus*

Parameters	Albino *O. niloticus*	Pigmented *O. niloticus*
Haematocrit (%)	39.20±1.93^a	32.20±2.49^b
WBC (µL)	111.00±10.09^a	98.20±13.99^b
RBC (µL)	8.10±1.94^a	4.51±1.27^b
Hb (g dL^{-1})	10.26±0.99^a	9.04±2.97^b
MCHC (g dL^{-1})	26.18±2.67^b	31.43±4.01^a
MCH (pg)	1.44±0.43^b	2.38±0.60^a
MCV (fl)	54.08±17.37^b	76.18±20.99^a
Weight (g)	186.55±2.12^a	112.10±9.20^b
Standard length (cm)	18.93±2.12^a	16.20±0.92^b
Total length (cm)	22.32±2.12^a	19.02±1.34^b

Means with different superscript are significantly different ($p<0.05$) and values are Mean±SE

Table 3: Blood group, genotype of male and female Albino *Oreochromis niloticus*

Male blood groups	Blood groups (%)	Female blood groups	Blood groups (%)
A^-	-	A^-	-
A^+	-	A^+	-
B	30	B	30
O^+		O^+	70
O^-	70	O^-	-
Male genotypes	Genotypes (%)	Female genotypes	Genotypes (%)
AC	80	AC	90
AF	20	AF	10

Table 4: Blood group, genotype of male and female Pigmented *Oreochromis niloticus*

Male blood groups	Blood groups (%)	Female blood groups	Blood groups (%)
A^-	-	A^-	-
A^+	-	A^+	-
B	-	B^-	20
O^+	100	O^+	-
O^-		O^-	80
Male genotypes	Genotypes (%)	Female genotypes	Genotypes (%)
AC	100	AC	100
AF	-	AF	-

Table 5: Proximate composition of Albino and Pigmented *Oreochromis niloticus*

	Albino		Pigmented	
Fish species	Female	Male	Female	Male
CP (%)	65.9[a]	59.3[b]	55.0[c]	55.8[c]
ASH (%)	19.5[a]	16.0c	18.0[b]	19.5[a]
Lipid (%)	9.5[b]	11.5[a]	11.0[a]	8.0[c]
Fibre (%)	0.21	0.20	0.63	0.82
Moisture (%)	1.5[a]	1.0[b]	1.0[b]	1.5[a]
NFE	3.39[c]	12[b]	14.37[a]	14.38[a]
Dry matter	98.5[a]	99[a]	99[a]	98.5[a]

Means with different superscript are significantly different ($p<0.05$)

fiber content were 0.21, 0.21, 0.63 and 0.82% for female and male Albino, female and male Pigmented, respectively.

The moisture content has a slim variation, the percentage for female Albino and that of male Pigmented were 1.5 each while male Albino and female Pigmented had 1.09. The Nitrogen Free Extract (NFE) was least in female Albino with 3.39% followed by 12.0% for male Albino and both female and male Pigmented had 14.37% each. The percentage of dry matter showed that female Albino and male Pigmented had 98.5% while that of male Albino and female Pigmented have 99% each.

DISCUSSION

The research compared the haematology, blood group, genotype and nutrients value of *O. niloticus*. Several factors influence the haematological values of aquatic organisms these include stress, sex, age[20], environmental and physiological conditions, nutrients composition of the consumed feeds[9,12] and disease[21]. Authors have not taken interest on Albino fish perhaps because of its low availability or non-interest of the researchers in the qualitative genetics aspect of aquatic organisms. This research revealed that the Albino both male and females had higher values in PCV, leucocytes, erythrocytes, MCV, hematocrit, HB, weight and nutrients value when compared with the Pigmented, which proved that Albino *O. niloticus* is advantageous over Pigmented *O. niloticus*. The high value of the leucocyte obtained in the research unveiled the health status of the fish and the ability of the fish to withstand pathogenic attack and inherently fight against disease, furthermore, with high leucocytes, the fish can resist pathogenic spike during the rearing period[6]. There were significant differences ($p\geq0.05$) between the Albinos and Pigmented of both male and females in this research which disagreed with[22]. The percentage value of hematocrit, hemoglobin value and erythrocyte significantly revealed the oxygen circulation capacity in the research fish, thereby exposing the health status of the fish[23].

The haematological characteristics in this study showed significant differences when compared between the male and females. The differences observed for separate sexes could be linked to the size[24], age, nutrients composition of the feeds[25], water chemistry and season of the year[26], hormonal interactions and disease. Disease affects the values of MCH, MCV and MCHC of fish[21]. Furthermore, the disease and infection free fish had higher values in his research. However, the values of MCHC and MCV obtained in this research was higher than what he reported in his disease-free fish while he recorded slightly higher values in MCH. The erythrocytes value obtained in this research was higher than the value previously reported by[26], however, it coincides with[27]. He further reported that RBC of *O. niloticus* ranged from 4.20 ± 0.70 to $9.0\pm2.9\times10^6$ mm^3. The erythrocytes (RBC) contain pigment known as Haemoglobin. Fish require adequate supply of oxygen in the tissues for oxidation process, this will provide the needed energy for its activities and survival[25]. Oxygen depends on the haemoglobin concentrates as respiratory pigments available in the blood. Haemoglobin counts also correlate with the number of the erythrocytes available.

Erythrocytes has a poor relationship with leucocyte and MCHC but are in positive correlation with haemoglobin and PCV[25]. In this research high haemoglobin concentration is found in female Albino with 10.26±0.99 and the least is in male Albino with 8.78±1.27 which shows high relationship with RBC[25,26]. The female Albino had the highest PCV value 39.20±1.93, followed by male Albino with 32.30±4.16, female Pigmented with 29.90±4.33.

The blood group of the samples were B^+, B^-, O^+ and O^-. This result slightly differs from the work previously reported by[27], who got only O and AB. It has shown that both Albino and Pigmented *O. niloticus* have similarity in terms of blood group. While the genotypes found in the sample were AC and AF. There was no significant difference in the genotype recorded across the fish samples. However, the result obtained in this article differed from[27], who recorded SS, SC, SD and CC in *O. niloticus*.

The nutrient evaluation revealed that the crude protein was highest in female Albino (65.9), followed by male Albino (59.3) and the Pigmented male and female samples had 55.8 and 55.0, respectively this is in agreement with the work of[28], who recorded 59.51-62.12 in crude protein. These results affirmed the report that fish is a useful key component in a healthy diet of human. The percentage composition of the ash shows that the sample fish could be a good source of minerals[29].

The major limitation was predatory attack on the Albino *O. niloticus* perhaps because of the color, this may be linked to other Albino species. More research can be carried out on ways to culture the specie in semi-intensive manner without losing it to predators. The awareness on the nutrients values of the specie needs to be created to encourage the farmers to engage in the culture of the specie.

CONCLUSION

The Pigmented and Albino *Oreochromis niloticus* are significantly different in their haematology, genotypes and nutrients composition. The study further exposed that haematological values of the fish are valuable components of determining the health status of the fish, it will enable fish geneticists, biologist and breeders to ascertain the quality fish to effectively use for selective breeding, hybridization, quantitative, qualitative genetics and culture purposes. Furthermore, the research exposed the value and quality of the Albino *O. niloticus* which have not received attention.

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