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HAEMATOLOGICAL STUDIES AND DEOXYRIBONUCLEIC ACID (DNA) CONTENT OF DIPLOID AND TRIPLOID AFRICAN CATFISH *Heterobranchus bidorsalis* (Geoffroy St. Hilaire 1809)

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ABSTRACT

This study was carried out to compare some haematological parameters and Deoxyribonucleic acid (DNA) contents of triploid and diploid strains of *Heterobranchus bidorsalis*. The results showed that there were positive correlations among packed cell volume (PCV), Haemoglobin (HB), Red blood cell (RBC) and White blood cell (WBC) at 5% level of significance. Comparatively, diploids had higher values of WBC, lymphocytes, Mean corpuscular haemoglobin concentration (MCHC) and Mean corpuscular volume (MCV) but the triploid had higher values with respect to RBC, PCV and HB. These values showed the effects of the cold shock on the triploid strain. There were no significant differences ($p>0.05$) between diploid and triploid in the lymphocytes, MCV and MCHC. Variations in other haematological values of the diploids and triploids were very minimal. The above observation revealed that the blood components of diploid and triploid *H. bidorsalis* were apparently healthy. The DNA content values obtained in this study could be used as an index to establish ploidy level. Higher purity of DNA concentration (0.517µg) was found in triploid compared to the diploid concentration of (0.485µg). There was a significant difference ($p<0.01$) between the triploid and diploid DNA purity.

Keywords: Haematology, Triploid, diploid, Deoxyribonucleic acid, Heterobranchus bidorsalis

INTRODUCTION

The use of haematological values as indices of the state of fish health is receiving a lot of research efforts. Research direction has centred towards its application as a means of diagnosing disease and stress-induced conditions in fish (Rambhaskar and Rao, 1990). Many workers have stressed the need for the establishment of normal haematological values in fish with a view to the diagnosis of diseases and infections (other than by parasitism through injury). Fish live in the close contact with their environment; they are extremely dependent upon it and are affected by changes in it. Many of the agents causing diseases of fish (bacterial, fungal, protozoan, helminthal, and viral) are constantly present in the water surrounding the fish, which is the most important factor in establishing health of the fish.

A gene is a linear array of very specific subunit but a small segment of a much larger

molecule called deoxyribonucleic acid (DNA) (Tave, 1992). DNA synthesis is a prerequisite for cell division. DNA absorbs light preferentially in the ultraviolet (UV) wave range (200nm to 290nm) with a peak at about 257nm. The degree of absorbance is also called optical density (OD). However, a standard peak of 260nm is commonly used. The importance of C-value (that is, amount of DNA in grams that is present in one complete haploid set of an organism's chromosome) includes varieties of parameters among which are the following: firstly, the genome size which allows decision on probable distances between genes on a chromosome, the number of copies of genes that are repeated, the actual experimental conditions that must be used to dissect a genome and the probability of finding and isolating a particular gene. Secondly, it gives valuable clues to phylogeny and can expose all kinds of interesting aspects of genome evolution. Genome size is rather well related to cell volume for the bigger the genome, the bigger the cells. It is also clear that genome size must have considerable developmental significance because the larger the genome, the greater the time between cell divisions (mitosis).

However, reports on the haematological response and deoxyribonucleic acid level of diploid and triploid *H. bidorsalis* in the blood parameters of cultured fishes are non-existent. This study was undertaken to provide information on the blood parameters of the triploid and diploid *H. bidorsalis* cultured from the same system.

MATERIALS AND METHODS

Twenty-five juvenile *H. bidorsalis* (77.284 ± 4.913g) of diploids and triploids which were produced through cold-shock were sampled from the concrete rearing pond at HEPA Aqua farm, Abeokuta during the rainy season between May and June, 2006. The samples were transported in oxygenated polyethylene bag from the farm to the Department of Veterinary Pathology (Clinical Pathology Laboratory), University of Ibadan, South-West Nigeria.

Blood samples were collected from each of 20 diploid and triploid strains. The blood samples were obtained through incision made towards the caudal peduncle of each fish using microhaematocrit heparinized capillary tubes and were preserved in Ethylene diamine tetra-acetic acid (EDTA) bottles for the analysis.

Standard haematological procedures described by Blaxhall and Daisley (1973) were employed in the assessment of the various blood parameters. Haemoglobin (HB) concentration was done by the cyanomethaemoglobin method, packed cell volume (PCV) by micro haematocrit method and erythrocyte sedimentation rate (ESR) by the microwintrobe method. The white blood cell (WBC) was determined with the improved Neubauer counter and differential count was done on blood film stained with May Grumuald-Giemsa stain. The mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV) were calculated according to the method of Seiverd (1964).

Deoxyribonucleic acid level was determined using the method described by Adegoke (1984). DNA was extracted by means of a spectrophotometer using an ultraviolet source. The lysing solution containing 0.45M EDTA had the viscosity which persisted for as long as they were kept free of contamination. Deproteinization and purification of the DNA followed similar treatment for other animal cell lysates, except that all the

extraction buffers contained 0.01M EDTA. The homogenizing solution was plain Standard Sodium Citrate (SSC) without $MgCl_2$. Proteins generally have a maximum absorbance at 280nm where the DNA absorbance is about half its absorbance at 260nm. Therefore, the ratio OD_{280}/OD_{260} was used to check the purity of the DNA sample, or more specifically degree of protein contaminant. A ratio of 0.48 to 0.52 is usually acceptable for pure DNA. By knowing the absorbance of a DNA sample at 260nm, it is possible to compute the approximate amount of DNA in the sample. From calibration studies, DNA of 1µg/ml has an approximate absorbance value of 0.02 at 260nm. Hence from the OD_{260} and volume of sample, an estimated amount of DNA can be extracted.

Physico-chemical parameters of the ponds were monitored before the fish were harvested. Water temperature was determined with mercury in glass centigrade thermometer, while pH and dissolved oxygen concentrations were determined by means of pH and oxygen meters. The mean values of physico-chemical parameters of the water before fish harvest in the tank were monitored using APHA (1980) method.

Data obtained from the experimental fish were subjected to t-test analysis at 0.05% probability level.

RESULTS

Table 1 shows the results of haematological studies of diploid and triploid *Heterobranchus bidorsalis.* There were significant differences ($p<0.05$) in the haematological values of erythrocytes in the triploid and diploid strains. There was an inverse relationship between the erythrocyte and the leucocytes mean values of both strains. All the haematological values were higher in diploids except for the PCV (%), HB and RBC.

Comparatively, diploids had higher values of WBC, lymphocytes, MCV and MCHC. The reverse was the case with respect to RBC, PCV and HB content.

Mean values for triploid PCV, HB, RBC, WBC were higher than corresponding values PCV, HB, WBC for diploid specimens. (Table 1). The parameters were significant at $p<0.05$. Diploid leucocyte Platelet, MCV, MCHC, Lymphocytes were significantly higher ($p<0.05$) than mean values leucocyte Platelet, MCV, MCHC and Lymphocytes for triploid strain, as indicated in Table 1.

The monocytes and eosinophils maintained equal mean values (2.00) in both strains. Values for monocytes and eosinophils in the triploids were fairly different being (1.333 ±0.422) and (2.000 ±0.516) respectively. There was no significant difference ($p>0.05$) in the eosinophils counts between the two strains while heterophil count was significantly ($p<0.05$) higher in triploid than in the diploid specimens.

The deoxyribonucleic acid (DNA) values are shown in Table 2.

There were significant differences ($p<0.001$) between the triploid and diploid DNA contents.

The triploid fish had the highest amount (6337µg) of DNA compared with value (3675µg) recorded for diploids. The Optical density value (0.658µg) at 260nm was higher in triploids than value (0.588µg) recorded in diploid. The OD value (0.375µg) at 280nm in triploid was also higher than value (0.285µg) recorded in diploid.

Table 1: Haematological parameters of diploid and triploid *H. bidorsalis*

Parameters	Diploid	Triploid
PCV(%)	27.00 ± 2.55	33.60±7.44
HB (mg/dl)	8.90 ± 0.71	10.62 ± 2.25
RBC (x10^3/µl)	2.49 ± 0.58	3.17 ± 0.80
WBC (n/µl)	19160 ± 645.56	19020 ± 640.90
Platelet	100000 ± 25729.36	94600 ± 13352.90
MCV	111.20 ± 18.44	106.80 ± 8.47
MCHC	33.20 ± 0.84	31.60 ± 0.55
Lymphocytes (n/µl)	70.40 ± 2.70	65.60 ± 3.91
Heterophils (n/µl)	25.25 ± 2.99	32.33 ± 3.21
Monocytes (n/µl)	2.00 ± 0.82	1.33 ± 0.58
Eosinophils (n/µl)	2.00 ± 0.82	2.00 ± 1.00

Table 2: DNA contents of liver sample for diploid and triploid Heterobranchus bidorsalis

Treatment	Wt. of liver (g)	OD 260	OD 280	Purity of DNA (µg/ml)	Amount of DNA (µg)
Triploid	4.5	0.658	0.375	0.517	6337
Diploid	4.5	0.588	0.285	0.485	3675

Table 3: Summary of range and mean values of physico-chemical parameters of the water before fish harvest in the tank

Parameters	Range	Mean
Temperature (^{0}C)	25.00- 27.80	26.47 ± 0.89
Dissolved oxygen (mg/l)	7.60 – 8.00	7.80 ± 0.64
PH	6.22 – 6.86	6.54 ± 0.43
Ammonia (mg/l)	0.05 – 0.09	0.07 ± 0.02

Higher purity of DNA concentration (0.517µg) was recorded in triploid than value (0.485µg) recorded diploid strains. There was a significant increase ($p<0.01$) in DNA content of (0.032µg) in triploids over the diploids. This increase was a significantly increase ($p<0.01$) of triploids over diploids.

Table 3 reveals physico-chemical temperature, dissolved oxygen, pH and Ammonia average values respectively as 26.47 ± 0.89^{0}C, 7.80 ± 0.64mg/l, 6.54 ± 0.43, 0.07 ± 0.02mg/l.

DISCUSSION

Comparatively, diploids had higher values of WBC, lymphocytes, MCV and MCHC, but the reverse was the case with respect to RBC, PCV and HB content. This suggests that diploid fish would respond to stress and injuries better than triploid fish since changes in WBC and differential counts were reported to play important roles in the state of health of *C. gariepinus* (Ezeri 2001; Omoregie and Oyebanji, 2002).

There were no significant differences among the lymphocytes, MCV and MHCH while variations in the haematological values of the strains were not very wide. The above observation reveals that using the blood components, the diploid and triploid *H. bidorsalis* were apparently healthy. There were positive correlations between eosinophils and monocytes of diploid and triploid at ($p<0.05$). This study recorded eosinophils in the stained blood of specimen samples (Table 1) while Ezeri (2001), and Gabriel *et al.* (2004) did not record eosinophils in the stained blood of *Clarias gariepinus*. Results from this study do not conform with previous reports of Ezeri (2001); Kelenyi and Neimeith (1969) who reported that eosinophils are usually rare in fish but that their occurrence was commonly reported in haemopoietic tissues, such as fish kidney. Findings from this study corroborated the reports of Blaxhall (1972) on gold fish, *Carassius auratus.* The value range (0.78 – 2.06%) of the eosinophils obtained in gold fish was closer to the value range (0.82 – 2.00%) obtained in this study. The record of eosinophils obtained in this study indicate that eosinophils were not occasionally seen in fish species as reported by Weinereb (1958) in rainbow trout. The value range of eosinophil in both diploid and triploid *H. bidorsalis* were within acceptable haematology range and this indicates that they were free from infection.

The DNA values (3675µg and 6337µg) recorded in the diploid and triploid *H. bidorsalis* forms respectively were expected because the amount of DNA in diploid was significantly lower than the amount obtained in triploid (Table 2). These recorded values could be used as an index to establish ploidy level (Tave, 1992). Furthermore, DNA and the genes form structures called chromosomes which are located in the nucleus of a cell, the number of chromosomes varies from species to species but, remains constant within a species. In most fish species, diploid number of chromosome (2n) occurs. However there are exceptions to the rule in that some fishes have become triploids (3n) during their evolution (Tave, 1992) and are sometimes generated through experimental procedures such as cold and heat shock.

C-value is the amount of DNA in grams that is present in one complete haploid set of an organism's chromosomes (Macgregor 1993). Therefore the C-value is bound to increase in triploid set of organism's chromosome, because, of the additional unreleased polar body which confers the triploid state (Macgregor, 1993).

The higher values of haematological parameters namely PCV, HB, RBC and deoxyribonucleic acid content of triploids of African catfish over the diploids showed the effects of the cold shock undertaken during their production. The cold shocking confers a relative level of stress which enables the triploid strain to withstand other environmental hazards after recovery better than their diploid counterparts (Tave, 1992).

CONCLUSION

In conclusion, this study has provided valuable baseline data on the haematology and deoxyribonucleic acid content of diploid and triploid African catfish *Heterobranchus bidorsalis* fish species in Nigeria. Because of the increased interest in aquaculture in general and fish farming in the world, and especially in Nigeria in recent times, there is need to establish normal haematological values and deoxyribonucleic acid content in different fish species for the monitoring of health and production parameters as done for pet and other food animals.

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