HAEMATOLOGICAL CHANGES IN THE BLOOD OF CULTURED *CLARIAS GARIEPINUS* STORED AT ROOM AND REFRIGERATOR TEMPERATURES

OKORIE-KANU, Christian Onwuchokwe, SOLOMON, Abasiofon Udo and NWAGBARA, Ngozi Dike

Department of Veterinary Pathology, Michael Okpara University of Agriculture, Umudike, POBox 3244, Umuahia, Abia State, Nigeria.

Corresponding Author: Okorie-Kanu, C. O. Department of Veterinary Pathology, Michael Okpara University of Agriculture, Umudike, POBox 3244, Umuahia, Abia State, Nigeria. **Email:** drcokoriekanu@yahoo.co.uk **Phone:** +234 8038993506

ABSTRACT

This study investigated the artifactual changes in the haematological values of Clarias gariepinus blood stored at room (32°C) and refrigerator (4°C) temperatures. Blood samples were collected from 12 apparently healthy fish weighing between 0.8 and 1kg. Samples were divided into two parts immediately after collection and baseline haematological values determined. Haematological determinations were thereafter carried out at 6 hourly intervals up to the 36th hour and then at the 48th hour. Results showed significant reduction (p<0.05) in PCV and RBC values at the 48th hour from the baseline value at room temperature and no significant variation (p>0.05) at the refrigerator temperature. There were significant increases (p<0.05) in the MCV and MCHC values from the baseline values at the 48th hour and MCH value at 36th hour at room temperature while no significant variations (p>0.05) were observed at refrigerator temperature. There were significant reductions (p<0.05) in the WBC count at the 36th and 48th hours when compared with the baseline value at both room and refrigerator temperatures while HBC values did not vary significantly (p>0.05) in both temperatures all through the duration of the study. Reliable RBC count can be obtained up to the 12th hour; WBC and MCH up to the 30th hour; PCV, MCV and MCHC up to the 36th hour and HBC up to the 48th hour at room temperature while at refrigerator temperature, reliable values can be obtained up to the 48th hour in all the parameters determined except WBC count that can be obtained up to the 30th hour of storage.

Keywords: *Clarias gariepinus,* Blood, Artifactual changes, Haematology, Duration of storage, Storage temperature

INTRODUCTION

Changes in the haematological values are reflection of conditions in the body (Schalm *et al*., 1975; Fry and McGavin, 2007) due to diseases or toxicities (Van Vuren *et al*., 1994; Velisek *et al*., 2013; Gadhave *et al,*. 2014) and determination of these parameters can give valuable information regarding physiological alterations in the body (Benjamin, 1978; Coles, 1986). However, changes sometimes may occur due to poor handling or storage of samples leading to misdiagnosis of diseases (Schalm *et al*., 1975; Cohle *et al*., 1981; Meyer and Harvey, 1998; Wood *et al*., 1999; Butarrello, 2004). Delay in analysis of blood samples may be due to restricted access to laboratories, due to the distance between where samples were collected (farm in remote villages) and laboratories located in the cities; delay in collection when large samples are involved; power outage especially in the developing countries; lack of test reagents; test kits and equipment at the time samples are submitted and inability to

finish sample analysis as soon as possible because of too many samples especially with manual procedures.

Refrigeration had been recommended to keep human blood sample in stable state and reduce artifactual changes (Goosens, 1994; Al-Ismail *et al*., 1995; Wood *et al*., 1999; Butarrello, 2004). Blood samples stored in room and refrigerator temperatures showed significant variations in the stability of blood sampled from different species (Clarke *et al*., 2002; Bluel *et al*., 2002; Ihedioha and Onwubuche, 2007). Contrary to the reports in human and some farm animals, Clarke *et al*. (2002) reported that cell counts in equine blood are more stable at room temperature (20 – 25^{o}C) than at refrigerator temperature (4^{o}C). Bluel *et al*. (2002) reported that refrigeration had a stabilising effect on erythrocyte count but led to reduction in WBC count after 24 hours of storage in cattle. Ihedioha and Onwubuche (2007) using manual procedures also reported more pronounced artifactual changes in blood samples stored at room temperature (30^{o}C) than those stored at refrigerator temperature (4^{o}C) in cattle, pigs and goats. In another study on avian blood samples, there were significant increases in the packed cell volume (PCV) and mean corpuscular volume (MCV) values after 18 and 12 hour storage, respectively with significant reduction in mean corpuscular haemoglobin concentration (MCHC) and white blood cells (WBC) count after 12 and 18 hours storage respectively in blood samples stored at room temperature (Ihedioha *et al*., 2008). Clinically significant increase in buffy coat percentage had also been reported in cattle and chicken blood samples stored at 30^{o}C and 37^{o}C at hour 72 and in pig blood samples stored at 30^{o}C and 37^{o}C as from the 48th hour of storage onwards (Ihedioha and Aba, 2010).

Fishes are reared most times in locations far away from diagnostic laboratories and blood samples collected from such locations at emergencies are prone to artifactual changes due to time lag between sample collection and analysis. Artifactual changes due to duration of storage and storage temperature had been reported in blood of cultured *Heteroclarias* hybrid (Okorie-Kanu and Solomon, 2015) but no reports exist for *Clarias gariepinus*.

This study therefore investigated the effect of time of storage and storage temperature on the packed cell volume, haemoglobin concentration, red blood cell and white blood cell counts and mean corpuscular values of blood samples collected from cultured *Clarias gariepinus*. The result of this study shall guide researchers and field veterinarians in proper handling of blood samples for haematological determinations to ensure the reliability and usefulness of haematological results as tools for diagnosis, prognosis and assessment of efficacy of therapeutic interventions in this species.

MATERIALS AND METHODS

Catfish: A total of 12 apparently healthy fish (*Clarias gariepinus)* weighing between 0.80 and 1.00 kg with an average weight of 0.92 kg were used for the study. They were obtained from the Michael Okpara University of Agriculture, Umudike Fish Farm raised in a 15 x 15 m^2 indoor concrete ponds under strict standard cultural practices. They were fed by point-feeding method with standard fish feed (Coppens, Germany) and water changed every week.

Blood: The blood samples were collected in the morning hours and were immediately divided into two parts and baseline haematological values determined. One part was kept on the laboratory bench at room temperature ranging between 29^{o}C and 35^{o}C (average 32^{o}C) and the other part was kept under refrigerator temperature ranging between 3^{0}C and 6^{o}C (average 4^{o}C). Haematological determinations were subsequently carried out at six hourly intervals for the first 36 hours and then at the 48th hour.

Haematology: Blood samples were collected through the caudal vein and put into bottles treated with appropriate quantity of ethylene diamine tetracetic acid (EDTA) Haematological determinations were carried out immediately after collection following standard procedures.

Packed cell volume (PCV) was determined by the microhaematocrit method, while haemoglobin concentration (HBC) was determined by the cyanomethaemoglobin method (Kachmar, 1970; Schalm *et al.,* 1975; Coles, 1986). Red blood cell (RBC) and total white blood cell (WBC) counts were carried out by the haemocytometer method. The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated using the standard formulae (Schalm *et al.,* 1975; Coles, 1986).

Data Analysis: Data from all the determinations were analyzed using Analysis of Variance (ANOVA) using SPSS 16.0 Statistical Package (SPSS 16.0 for Windows, SPSS Inc., Chicago, IL, USA). Different means were separated using the Least Significant Different (LSD) method and significant difference was accepted at $p<0.05$.

RESULTS

The mean PCV value recorded was significantly lower ($p<0.05$) at the 48th hour when compared with the baseline value at room temperature while there was no significant variation ($p>0.05$) at the refrigerator temperature all through the study (Table 1). The values recorded for haemoglobin concentration up to the 48th hour did not vary significantly ($p>0.05$) in both room and refrigerator temperatures (Table 1).

There was a significant reduction ($p<0.05$) in the RBC values recorded from 18th to 48th hour when compared with the baseline value at room temperature while there was a significant reduction ($p<0.05$) only at the 48th hour when compared with the baseline value at refrigerator temperature (Table 2).

Furthermore, there were significant reductions ($p<0.05$) in the WBC count recorded from the 36th hour at both room and refrigerator temperatures when compared with the baseline (Table 2).

The MCV values recorded at the 48th hour was significantly higher ($p<0.05$) when compared with the baseline value at room temperature but no significant variation ($p>0.05$) at refrigerator temperature all through the study (Table 3).

The MCH values recorded from the 36th hour was significantly higher ($p<0.05$) when compared with the baseline at room temperature while at refrigerator temperature, there was no significant variation ($p>0.05$) in the 48th hour period of the study (Table 3). The MCHC values recorded at the 48th hour was significantly higher ($p<0.05$) when compared with the baseline at room temperature while there was no significant variation ($p>0.05$) all through the study at the refrigerator temperature (Table 3).

DISCUSSION

The significant reduction in the PCV at the 48th hour is in agreement with the result observed in Heteroclarias hybrid (Okorie-Kanu and Solomon, 2015) and also may be due to lysis of RBCs as there was also significant reduction in total RBC count. The result is in contrast to the values reported for humans (Cohle *et al.* 1981; Wood *et al.,* 1999; Buttarello, 2004), horses (Clarke *et al.,* 2002), rat, bovine, caprine and porcine blood stored at room and refrigerator temperatures and avian blood samples stored at varying temperatures (Bluel *et al.,* 2002; Ihedioha and Ibeachu, 2005; Ihedioha and Onwubuche, 2007; Ihedioha *et al.,* 2008). This could be attributed to degenerative changes that resulted in widening of the pores on the surfaces of RBCs, permitting ingress of water into the cells (Jandl, 1965; Schalm *et al.,* 1975; Coles, 1986). This may be that the elastic limit of the fish RBCs is less compared to humans and other animals resulting in early cell lysis even though there was initial cell swelling.

The lysis of RBCs led to the significant reduction in the RBC values recorded far earlier than humans and many farm animals. The result followed the same trend with what was reported in avian blood samples although the reduction was not significant (Ihedioha *et al.,* 2008). The significant increases in the erythrocytic indices were due to the reduction in RBC and PCV values as the two parameters were the denominators in the calculation of the

Table 1: Packed cell volume and haemoglobin concentration of *Clarias gariepinus* kept at different temperatures for fourty eight hours

Hour	Packed cell volume		Haemoglobin concentration	
	Room	Refrigerator	Room	Refrigerator
0	27.20 ± 1.93	27.30 ± 1.83	10.97 ± 0.81	10.96 ± 0.68
6	26.40 ± 1.44	26.70 ± 1.77	10.86 ± 0.79	11.07 ± 0.71
12	26.20 ± 1.82	27.20 ± 2.01	10.55 ± 0.72	11.07 ± 0.74
18	24.50 ± 1.76	25.50 ± 1.62	10.76 ± 0.62	10.76 ± 0.58
24	25.42 ± 1.29	25.50 ± 1.71	10.43 ± 0.54	10.76 ± 0.50
30	25.42 ± 1.42	25.90 ± 1.82	10.35 ± 0.42	10.71 ± 0.52
36	22.80 ± 1.74	25.20 ± 1.93	11.07 ± 0.83	10.60 ± 0.58
48	21.20 ± 1.74[b]	24.80 ± 1.81	10.76 ± 0.76	10.81 ± 0.68

*Different superscripts in a column indicate significant difference between the time intervals ($p<0.05$)

Table 2: Red blood cell and white blood cell counts of *Clarias gariepinus* kept at different temperatures for fourty eight hours

Hour	Red blood cell counts		White blood cell count	
	Room	Refrigerator	Room	Refrigerator
0	2.17 ± 0.12[a]	2.15 ± 0.09	17.53 ± 0.87[a]	17.24 ± 0.82[a]
6	1.99 ± 0.05[a]	2.05 ± 0.08	16.32 ± 0.98[a]	16.77 ± 0.95[a]
12	1.97 ± 0.07[a]	2.03 ± 0.11	16.01 ± 0.90[a]	16.45 ± 0.87[a]
18	1.76 ± 0.11[b]	1.83 ± 0.17	14.47 ± 1.01[a]	15.34 ± 1.42[a]
24	1.82 ± 0.10[b]	2.09 ± 0.10	14.25 ± 1.42[a]	14.34 ± 0.98[a]
30	1.83 ± 0.04[b]	2.09 ± 0.08	14.59 ± 0.89[a]	15.26 ± 0.99[a]
36	1.52 ± 0.15[c]	2.06 ± 0.13	9.93 ± 1.18[b]	13.49 ± 1.63[b]
48	1.24 ± 0.20[d]	2.20 ± 0.25	6.82 ± 1.53[b]	10.28 ± 1.28[b]

*Different superscripts in a column indicate significant difference between the time intervals ($p<0.05$)

Table 3: Mean corpuscular volume (fl), mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration of *Clarias gariepinus* kept at different temperatures for fourty eight hours

Hours	Mean corpuscular volume		Mean corpuscular haemoglobin		Mean corpuscular haemoglobin concentration	
	Room	Refrigerator	Room	Refrigerator	Room	Refrigerator
0	124.65 ± 7.25[a]	126.74 ± 4.20	51.03 ± 4.59[a]	50.95 ± 2.35	42.25 ± 3.67[a]	40.41 ± 1.96
6	132.52 ± 6.56[a]	130.38 ± 6.16	54.63 ± 4.11[a]	54.04 ± 2.37	41.05 ± 1.89[a]	41.55 ± 2.05
12	133.52 ± 8.76[a]	133.42 ± 5.35	53.70 ± 3.96[a]	54.29 ± 2.19	40.41 ± 1.73[a]	41.13 ± 2.82
18	134.24 ± 5.14[a]	124.47 ± 2.67	65.90 ± 5.53[a]	51.11 ± 7.27	44.32 ± 2.56[a]	42.71 ± 2.56
24	134.17 ± 2.09[a]	121.80 ± 6.23	66.08 ± 4.59[a]	51.51 ± 1.67	44.31 ± 3.25[a]	43.33 ± 2.19
30	139.23 ± 7.10[a]	127.22 ± 4.31	57.56 ± 2.26[a]	51.33 ± 2.75	41.57 ± 1.80[a]	41.72 ± 2.53
36	139.14 ± 4.04[a]	123.12 ± 7.93	78.13 ± 10.39[b]	52.65 ± 5.04	49.34 ± 4.90[a]	43.03 ± 4.27
48	162.20 ± 10.56[b]	128.54 ± 9.10	87.42 ± 7.51[b]	61.06 ± 4.17	54.44 ± 5.05[b]	45.03 ± 1.97

*Different superscripts in a column indicate significant difference between the time intervals ($P < 0.05$)

mean corpuscular values (Thrall and Weiser, 2002). The lack of significant changes in the HBC values was in agreement with reports of previous studies in humans, cattle, goats, horses and pigs (Cohle *et al*., 1981; Goosens, 1984; Al-Ismail *et al.,* 1995; Wood *et al.,* 1999; Clarke *et al.,* 2002; Buttarello, 2004; Ihedioha and Onwubuche, 2007). This could be due to the fact that haemoglobin fractions released from the lysed RBCs are still available in the sample unlike when they are in circulation where they are degraded into their various components where bilirubin is conjugated and excreted and iron returned to the storage pool to be reused for haemoglobin production (Benjamin, 1978; Coles, 1986).

The reduction in the WBC counts recorded at both room and refrigerator temperatures are in agreement with the reports of Ihedioha *et al.* (2008) in chickens but contrasts with the findings in studies with blood of mammals including humans (Cohle *et al.,* 1981; Goosens, 1984; Al-Ismail *et al.,* 1995; Wood *et al.,* 1999; Clarke *et al.,* 2002; Buttarello, 2004; Ihedioha and Onwubuche, 2007). This may be because the WBC of birds and fishes are nucleated and therefore degenerate earlier making them unable to take up stains and therefore are not seen and counted (Campbell and Coles, 1986; Dein, 1986).

Conclusion: Based on the results of this study, it was concluded that reliable RBC count can be obtained up to the 12th hour; MCH up to the 30th hour; PCV, MCV and MCHC up to the 36th hour and HBC up to the 48th hour at room temperature while at refrigerator temperature, reliable values can be obtained up to the 48th hour in all the parameters determined. Also, reliable WBC count can be obtained up to the 30th hour in both room and refrigerator temperatures. The significant reduction in RBC as early as the 18th hour and significant reduction in WBC count in both room and refrigerator temperatures are worthy of note, therefore blood sample from this species must be analysed within 12 hours at room temperature and 30th hours for both room and refrigerator temperatures for the two parameters to be useful in disease diagnosis, prognosis and monitoring of therapeutic processes.

ACKNOWLEDGEMENT

We are grateful to the Pond supervisor, Mr. Candidus I. Obodagu for his assistance throughout the period of the study.

REFERENCES

Al-ISMAIL, S. A., BOND, K. and CARTER, A. B. (1995). Two centre evaluation of the Abbott Cell-DYN 3500 blood counter. *Clinical Laboratory Haematology*, 17: 11 – 21.

BENJAMIN, M. M. (1978). *Outline of Veterinary Clinical Pathology*. 3rd Edition, Iowa State University Press, Ames, Iowa, U.S.A.

BLUEL, U., SOBIRAJ, A. and BOSTEDT, I. I. (2002). Effects of duration of storage and storage temperature on cell counts of bovine blood samples as determined by an automated haematology analyser. *Comparative Clinical Pathology,* 11: 211 – 216.

BUTTARELLO, M. (2004). Quality specification in haematology: the automated blood cell count. *Clinica Chemica Acta,* 346: 45 – 54.

CAMPBELL, T. W. and COLES, E. H. (1986). Avian clinical pathology. Pages 279 – 301. *In:* COLES, E. H. (Ed.) *Veterinary Clinical Pathology.* 4th Edition, W. B. Saunders Company, Philadelphia.

CLARKE, P., MOGU, P. V., TVEDTEN, I. I. W. and KOREAL, D. (2002). Artifactual changes in equine blood following storage detected using Advia 120 haemoanalyser. *Veterinary Clinical Pathology,* 31: 90 – 94.

COHLE, S. D., ABDUS, S. and MAKKOUI, D. E. (1981). Effects of storage on stability of haematological parameters. *American Journal of Clinical Pathology,* 76: 67 – 69.

COLES, E. H. (1986). *Veterinary Clinical Pathology.* 4th Edition, W. B. Saunders Company, Philadelphia.

DEIN, F. J. (1986). Haematology. Pages 174 – 179. *In:* HARRISON, G. J. and HARRISON, L. R. (Eds.), *Clinical Avian Medicine and Surgery.* W. B. Saunders Company, Philadelphia.

FRY. M. and MCGAVIN, M. D. (2007). Bone marrow, blood cells and lymphatic system. Pages 743 – 832. *In:* MCGAVIN, M. D. and ZACHARY, J. F. (Editors), *Pathologic Basis of Veterinary Disease,* 4th Edition, Mosby Incorporated, USA.

GADHAVE, P. D., BRAR, R. S., BANGA, H. S. and DHAWAN, A. (2014). Studies on acute toxicity of synthetic pyrethroid λ-cyhalothrin on freshwater fish, *Labio rohita. Veterinary World*, 7(1): 7 – 9.

GOOSENS, W. (1994). Pre-analytical effects on automated differential counts. *Nouvelle Revue Francaise D'hematologie,* 36: 114 – 117.

IHEDIOHA, J. I. and ONWUBUCHE, R. C. (2007). Artifactual changes in PCV, haemoglobin concentration and cell counts of bovine, caprine and porcine blood stored at room and refrigerator temperatures. *Veterinary Clinical Pathology,* 36: 60 – 63.

IHEDIOHA, J. I., IDIKA, I. K., OGAMBA, G. N. and AKAM, C. J. A. (2008). Changes in the haematological values of avian blood samples stored at varying temperatures for a period of up to 72 hours. *Comparative Clinical Pathology,* 17: 77 – 79.

IHEDIOHA, J. J. and IBEACHU, C. O. (2005). Time related quantitative changes in the haematological values of rat blood kept at room temperature. *Proceeding of the 30th Annual Conference of Nigerian Society of Animal Production (NSAP),* 30: 41 – 44.

JANDL, J. H. (1965). Leaky red cells - an analytical review. *Blood*, 26: 367 – 374.

KACHMAR, J. F. (1970). Determination of blood haemoglobin by the caynomet-haemoglobin procedure. Pages 268 – 269. *In:* TIETA, N. W. (Ed.), *Fundamentals of Clinical Chemistry,* W. B. Saunders Company, Philadelphia.

MEYER, D. J. and HARVEY, J. W. (1998). *Veterinary Laboratory Medicine Interpretation and Diagnosis.* 2nd Edition, W. B. Saunders, Philadelphia.

OKORIE-KANU, C. O. and SOLOMON, A. U. (2015). Artifactual changes in the haematological values of cultured *Hetroclarias* hybrid blood stored at room and refrigerator temperatures. *Comparative Clinical Pathology,* DOI: 10.1007/s00580-015-2095-y.

SCHALM, O. W., JAIN, N. C. and CAROLL, E. J. (1975). *Veterinary Haematology*. 3rd Edition, Lea and Fabriger, Philadelphia.

THIEN, P. C., DALGAARD, A. D., THANH, B. N., OLSEN, A. and MURRELL, K. D. (2007). Prevalence of fish borne zoonotic parasites in important cultured fish species in the Mekong Delta, Vietnam. *Parasitology Research,* 101: 1277 – 1284.

THRALL, M. A. and WEISER, M. G. (2002). Haematology. Pages 29 – 74. *In:* HENDRIX, C. M. (Ed.), *Laboratory Procedures for Veterinary Technicians*. 4th Edition, Mosby Publisher, St. Louis, USA.

VAN VUREN, J. H. J., VAN DER MERWE, M. and DU PREEZ, H. H. (1994). The effect of copper on the blood biochemistry of *Clarias gariepinus* (*Clariidae*). *Ecotoxicology and Environmental Safety*, 29: 187 – 199.

VELISEK, J., STARA, A., ZUSKOVA, E. and SVOBODOVA, Z. (2013). Use of biometric, haematologic and plasma biochemical variables and histopathology to assess the chronic effects of the herbicide prometryn on common carp. *Veterinary Clinical Pathology,* 42(4): 508 – 515.

WOOD, B. L., ANDREWS, J., MILLER, S. and SABBATH, D. E. (1999). Refrigerated storage improves the stability of complete blood count and automated differential. *American Journal of Clinical Pathology,* 112: 687 – 695.