Select Hematological Values of the African Catfish (*Clarias gariepinus*) Raised in a Water Recirculating Aquaculture System

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ABSTRACT

Clinical evaluation of blood parameters is routinely used to assess the health of wild and domestic animals. The commercial catfish industry in Nigeria has undergone rapid expansion in recent years. An understanding of normal hematology values for healthy fish and the identification of predictors of the onset of health problems may enable fish health specialists to intervene before major losses occur. This paper reports values for selected hematological parameters of normal healthy African catfish (*Clarias gariepinus)* (n=120) raised in a recirculating aquaculture water recirculation system, including hemoglobin (Hb), red blood cells (RBC), packed cell volume (PCV), white blood cells (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), thrombocyte count, and leukocyte differential (lymphocytes, neutrophils and monocytes). Significant differences ($p< 0.05$) were observed between males and females in the values of Hb, PCV, and thrombocytes. This work provides hematological baselines for selected values for *Clarias gariepinus* in recirculation systems, and is intended to enhance production performance through early detection and identification of infectious diseases.

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INTRODUCTION

The African catfish, (*Clarias gariepinus)*, belongs to the family Claridae, and is the most popular fish cultured in Nigeria, next to the tilapine fishes (FAO 1997, Adeogun *et al*. 2007). Aquaculture production in Nigeria was concentrated on tilapia culture before the clariids began to assert special importance in fish production in many parts of the country (AIFP 2004). According to De-Graaf and Janssen (1996), the reasons for preference of the clariids in tropical aquaculture includes hardiness to adverse environmental conditions, fast growth rates in captivity, easy procurement of fingerlings, adaptation to artificial feed, and high consumer preference. Additional attributes of this species, relevant to culture include high fecundity, potential for year-round induction of final oocyte maturation, remarkable nutrient conversion efficiency, and tolerance of high culture density (Legendre *et al*. 1992, Ayinla and Nwadukwe 2003).

With the recent expansion of the catfish industry in Nigeria, many farmers are now raising their fish using recirculating aquaculture systems. This is a clear departure from the traditional earthen pond culture system, which makes fish production seasonal and unreliable, to a more advanced, reliable, intensive, and results-oriented culture system (Akinrotimi *et al.* 2007a, Gabriel *et al.* 2009). With this level of intensive stocking density of fish, there is therefore the need to monitor the health status of cultured fish to prevent the outbreak of devastating diseases.

One of the difficulties in assessing the health of fish has been the scarcity of reliable references describing the normal condition. To achieve this goal, fish physiologists have employed hematology assessments to characterize the physiological status of fish (Kori-Siakpere *et al.* 2005). According to Wedemeyer *et al.* (1983), hematological studies are carried out in fish to ascertain the normal values in relation to age, sex, and culture system. Therefore the establishment of reference values with accepted limits is important for comparison of data obtained from a wild population with that of fish maintained in aquaculture conditions. With this information, significant changes in these values can be used to interpret the metabolic condition and overall health of fish (Gabriel *et al.* 2007a, Akinrotimi *et al.* 2007b).

Previous studies have determined blood parameters for this species in various culture systems such as reservoirs (Sowunmi 2003; Ezeri *et al.*

2004) and earthen ponds (Erondu *et al.* 1993, Omitoyin 2006, Akinrotimi 2008), but reports from recirculating aquaculture systems are lacking, thus necessitating the need for this work. The objective of this study was to report the hematological reference values for selected parameters of *Clarias gariepinus* reared in a recirculating system using sufficient numbers of fish to provide representative baseline values.

MATERIALS AND METHODS

Post-fingerlings of *Clarias gariepinus* were stocked in production recirculating aquaculture systems at Watershed Fish Farms, Nigeria Limited, Rumuodara, Port Harcourt, Rivers State, Nigeria and reared to market size (average 1,800.00g ± 1.01SD) over 5 months. The recirculating system in this farm measured 25m x 10m x 7m. The rate of water flow was 40,000 liters/day and the water exchange was continuous, with a stocking density of150 fish/m^2. For this study, 200 apparently healthy fish were carefully moved to other holding tanks to avoid stress from crowding and maintained for ten days. The fish were later sexed following the methods described by De-Graaf and Janssen (1996). The following water quality parameters were determined daily for a period of 10 days, and included temperature, pH, ammonia, nitrite, nitrate and dissolved oxygen using methods described by APHA (1998).

The fish were individually restrained manually, then blood samples (5.0 mL) were collected from the caudal vessels of male and female fish (60 each), using a heparinized plastic syringe fitted with a 21 gauge hypodermic needle, and immediately transferred to EDTA tubes. After collection of the blood samples, the fish were weighed (Sartorius model H112, Portugal). Measurement of each blood parameter was repeated for all 120 animals. Total RBC counts were obtained using a hemacytometer (Improved Neubauer, Model BS-713, Weber Scientific Limited, Middlesex, UK) using the method of Wintrobe (1934). Packed cell volume (PCV) was determined by filling heparinized hematocrit capillary tubes with blood, which was centrifuged for 5 minutes at 500×*g* in a microhematocrit centrifuge (Model TDL60B, Hunan Xingke Scientific Instruments Co. Ltd, Hunan, China), following the methods of Serveid (1983). Hemoglobin was determined using the cyanmethemoglobin method (Blaxlall and Daisley 1973). The total WBC counts (WBC) were later enumerated in a hemacytometer (Improved Neubauer, Model BS-

713, Weber Scientific Limited, Middlesex, UK) using Shaw's diluting fluid (Miale 1982). Blood smears were stained with Grumwald-Giemsa for differential examination (Tavares Dias *et al.* 1999).

Statistical analysis was performed using SAS Software package (SAS Institute Inc., Cary, NC, USA). One-way analysis of variance (ANOVA) was applied to check for significant changes between male and female fish. Statistically significant differences were determined by Tukey's multiple comparison test. The reference values were calculated based on the minimum and maximum values of blood parameters (Zar 1996).

RESULTS AND DISCUSSION

The water quality parameters examined in this study indicated values characteristic of recirculation systems (Hrubec and Smith 2004). All parameters were within an acceptable range to enhance production performance of cultured catfish (Table 1).

In fish medicine, hematological profiles are one of the most frequently used methods to predict levels of disease and the impact of stressors in fish. Hematological characteristics of a number of cultured fish species have been studied, with the aim of establishing reference intervals useful in cases where significant deviations may indicate a disturbance in the physiological process (Raiza-Piava *et al.* 2000, Gabriel *et al. 2004*; Akinrotimi *et al.* 2007c). Many of these studies were attempted to determine if significant variations from normal values could be attributed to internal factors, or to factors external to the culture environment (Gabriel *et al.* 2007b).

Several factors have been reported to affect hematological parameters of teleost fish; these include species, sex, age, size, and environmental and culture conditions (Sowunmi 2003; Akinrotimi *et al.* 2009). In the assessment of the blood profile of black jaw tilapia, (*Sarotherodon melanotheron),* Akinrotimi *et al.* (2007d) observed that results from the female fish were consistently higher in all parameters examined, and suggested the need to separate blood component data on the basis of sex.

In this present work, significant differences ($p < 0.05$) were found in female fish for Hb, PCV, and total thrombocyte count (Table 2). Similar findings were reported by Kori-Siakpere and Egor (1997) in *Clarias*

buthapogun and Kori-Siakpere (1985) in *C. isheriensis*. The gender differences may be due to the larger size of females (1900.00g ± 1.02SD) and higher hormonal interaction compared with the males (1700.00g ± 1.02SD) (Sowunmi 2003). The higher values of Hb observed in the female fish corroborate the reports of Akinrotimi *et al.* (2010) in *Tilapia guinnensis*. The higher values of blood parameters associated with oxygen transport suggest that under adverse environmental conditions that impact negatively on available oxygen, the females may be better equipped to handle such stressors than the males.

Values for hematological reference values determined in the 120 samples (Table 3) are comparable to those reported previously for hybrid striped bass (*Morone chrysops x Morone saxatilis)* raised in recirculating systems (Hrubec *et al.* 2004). The results were within the same range except in the value of Hb content. The reference interval of Hb in *Clarias gariepinus* (10.02-18.64 g/dL) was higher than that of hybrid striped bass (4.2-8.4 g/dL). The difference may be due to species-specific variation of the fish (Nikinmaa 2001). Hemoglobin may also show wide variability in sensitivity to effectors like organic phosphate, environmental conditions, and various fish management procedures in aquaculture (Angelids *et al.* 1987, Brauner and Randall 1999, Pelster 2001). However, the blood reference values obtained in this study contradict those reported for yellow perch reared in recirculating systems (Hrubec and Smith 2004). This difference may be due to species-specific hematological characteristics in teleost fish. Mauel *et al.* (2007) reported that species origin and breeding systems can influence hematological reference values in fish, as observed in *Tilapia* species maintained in recirculating systems. The hematological variables observed in this work were lower than those obtained previously for *C. gariepinus* cultured in freshwater tidal earthen ponds (Akinrotimi 2008). The higher values of *C. gariepinus* in tidal earthen ponds may be due to relatively high physical and metabolic activity in the fish raised in tidal systems, which are known to elicit a higher erythrocyte to plasma ratio in response to tidal shifts, which occur every six hours (Akinrotimi *et al.* 2010b).

Hence, the data reported in this study and those published previously indicate that these values can be a useful tool for veterinarians and aquaculturists in evaluating the health of cultured African catfish in recirculating aquaculture systems.

Table 1. Water quality parameters in the recirculating aquaculture system for African catfish, (Clarias gariepinus), over 10 days.

Parameter	**Mean ± SD**	**Range Min-Max**
Temp (^{o}C)	28.66 ± 4.21	26.44 – 30.64
pH	7.64 ± 1.21	6.81 – 8.12
Dissolved oxygen (mg/L)	6.91 ± 1.02	5.81 – 7.99
Ammonia (mg/L)	0.016 ± 0.02	0.004 – 0.027
Nitrite (mg/L)	0.014 ± 0.01	0.006 – 0.024
Nitrate (mg/L)	3.64 ± 0.61	2.01 – 5.67

Table 2. Hematological parameters (Mean ±SD) for male and female African catfish, (Clarias gariepinus), reared in recirculating aquaculture system.

Parameter	Male (n=60) (W=1700.00g ± 1.01SD)	Male Reference Range	Female (n=60) (W=1900.00g ± 1.02SD)	Female Reference Range
Hemoglobin (g 1D/dL)	14.86 ± 2.42[a]	10.02 – 16.74	16.99 ± 3.26[b]	11.22 – 18.64
Red blood cell ($x10^{12}$/L)	4.98 ± 0.81[a]	3.05 – 6.99	7.38 ± 1.22[b]	4.26 – 8.64
Packed cell volume (%)	36.21 ± 4.11[a]	32.64 – 40.70	41.31 ± 1.21[b]	36.71 – 45.74
White blood cell ($x10^{9}$/L)	21.68 ± 3.41[a]	18.66 – 23.98	22.74 ± 3.66[a]	19.10 – 25.61
Mean corpuscular volume (f1)	72.71 ± 10.32[a]	72.11 – 78.66	82.95 ± 9·74[b]	75.14 – 82.95
Mean corpuscular hemoglobin (pg)	33.92 ± 4.61[a]	30.21 – 36.22	34.11 ± 5.22[a]	34.18 – 46.74
Mean corpuscular hemoglobin concentration (g/dL)	41.63 ± 7.11[a]	38.21 – 46.72	41.12 ± 6.34[a]	38.28 – 46.68
Thrombocytes ($x10^{9}$/L)	102.64 ± 1.11[a]	92.01 – 114.68	142.61 ± 7.11[b]	110.34 – 158.74
Lymphocytes (%)	64.22 ± 6.71[a]	51.14 – 70.10	64.64 ± 5.11[a]	55.22 – 70.16
Neutrophils (%)	32.14 ± 3.66[a]	28.21 – 40.14	31.58 ± 3.76[a]	27.64 – 39.78
Monocytes (%)	3.64 ± 1.14[a]	1.86 – 3.92	3.78 ± 1.31[a]	1.99 – 4.01

Where W = average weight

Means within the row are denoted with different superscripts where significant ($P < 0.05$)

Table 3. Hematological reference values of African catfish, Clarias gariepinus, reared in a recirculating aquaculture system (n = 120).

Parameter	Mean ± SD	Reference range
Hemoglobin (g/dL)	15.93 ± 4.61	10.02 - 18.64
Red blood cell ($x10^{12}$/L)	4.68 ± 1.71	3.051 - 8.64
Packed cell volume (%)	38.76 ± 8.42	32.64 - 45.74
White blood cell ($x10^{9}$/L)	22.21 ± 6.46	18.66 - 25.61
Mean corpuscular volume (f1)	82.81 ± 9.66	72.11 - 91.34
Mean corpuscular hemoglobin (pg)	34.02 ± 6.01	30.21 - 46.74
Mean corpuscular hemoglobin concentration (g/dL)	41.38 ± 7.11	38.21 - 46.74
Thrombocytes ($x10^{9}$/L)	122.63 ± 12.61	92.01 - 158.74
Lymphocytes(%)	64.43 ± 9.64	51.14 - 70.16
Neutrophils (%)	31.86 ± 6.42	27.64 - 40.14
Monocytes (%)	3.71 ± 1.02	1.86 - 4.01
Hemoglobin (g/dL)	15.93 ± 4.61	10.02 – 18.64
Red blood cell ($x10^{12}$/L)	4.68 ± 1.71	3.051 – 8.64
Packed cell volume (%)	38.76 ± 8.42	32.64 – 45.74
White blood cell ($x10^{9}$/L)	22.21 ± 6.46	18.66 – 25.61
Mean corpuscular volume (f1)	82.81 ± 9.66	72.11 – 91.34
Mean corpuscular hemoglobin (pg)	34.02 ± 6.01	30.21 – 46.74
Mean corpuscular hemoglobin concentration (g/dL)	41.38 ± 7.11	38.21 – 46.74
Thrombocytes ($x10^{9}$/L)	122.63 ± 12.61	92.01 – 158.74
Lymphocytes (%)	64.43 ± 9.64	51.14 – 70.16
Neutrophils (%)	31.86 ± 6.42	27.64 – 40.14
Monocytes (%)	3.71 ± 1.02	1.86 – 4.01

Means within the row are denoted with different superscripts where significant ($P < 0.05$)

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