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Full Length Research Paper

Influence of sex, source, health status and acclimation on the haematology of *Clarias gariepinus* (Burch,1822)

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The influence of sex, source (pond and wild) acclimation and health status on some blood parameters of *C. gariepinus* was studied. There were no significant differences between the blood parameters (haemoglobin (Ht), packed cell volume (PCV), erythrocyte sedimentation rate (ESR), red blood cells (RBC), RBC indices (MCHC;, MCH: and MCV), white blood cells (WBC) and differential counts (neutrophils, lymphocytes, basophils and eosinophils) of the males and females among the apparently healthy and sick group of fish under sex, source and acclimation. Differences in blood parameters in fish before and after acclimation were noted in the WBC ($p<0.001$), neutrophils ($p<0.001$) and lymphocytes ($p<0.001$). Interactions between sex, acclimation and health status did not significantly influence all the parameters studied; however, various degrees of significant differences were produced by the interactions of health status and source of fish in the WBC ($p<0.05$), neutrophils ($p<0.001$), lymphocytes ($p<0.001$) and monocytes ($p<0.05$). Pooled data for males and females, apparently healthy and sick fish, respectively, showed there were significant differences between the WBC, neutrophils and lymphocytes of males and females under acclimation as well as monocytes of apparently healthy and sick fish under source and neutrophils of the same under acclimation. Results from this study suggest that sex, source of fish, and period of acclimation have some degrees of influence on the blood parameters of *C. gariepinus* and hence the need to reckon with them when reporting haematological parameters of this fish species.

Key words: Sex, source, acclimation, *Clarias gariepinus*, haematology.

INTRODUCTION

It is a recommended practice to subject fish species to be used in laboratory experiments to a minimum acclimation period of seven days. Generally, it is believed that during the quarantine period, the fish may manifest symptoms of diseases that may aid in the separation of apparently healthy fish from sick individuals thereby ensuring that only healthy individuals are used for any trial. This ensures that results thus obtained are not influenced by the state of health of the subject.

During the acclimation period, the fish may undergo some degree of stress similar to that characteristic of high density stocking in intensive culture systems. Such overcrowding conditions have the likelihood of increasing the incidence of diseases that may produce a number of measurable changes in the physiological processes of the fish. Such changes include serum enzymes levels, stress hormones and haematological parameters (Heath, 1987).

According to Fernades and Mazon (2003), haematological parameters are closely related to the response of the animal to the environment, an indication

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Table 1. Morphometric characteristics (mean ± SEM) of experimental fish.

Parameter	Wild		Cultured	
	Male	Female	Male	Female
Total length (cm)	36.1±0.4	34.8±0.7	34.6±0.9	33.1±0.7
Weight (g)	360.25±15	348.34±26	333.05±17	272.41±13
Girth (cm)	4.8±.0.2	5.0±0.4	5.20±0.2	4.9±0.2

Table 2. Mean (±SEM) water quality parameters in the dam, pond and acclimation tanks.

Parameter	Wild (dam)	Pond	Fibre tanks
Temperature (^{0}C)	25.8±1.16	27.64±1.06	26.8±1.14
pH	6.79±0.18	6.68± 0.52	6.06±0.25
Dissolved oxygen (mg/l)	6.88±0.14	6.68±0.22	5.84±0.14

that the environment where fish lives could exert some influence on the haematological characteristics (Kori-Siakpere, 1985). Sex of the fish may also influence the blood parameters. Studies on sexually matured gold fish.(*Carassius auratus)* (Summerfelt, 1967), brook trout (*Salvelinus fontinalis) and* brown trout *(Salmo gairdneri)* (Sniezsko, 1960) showed that males consistently had higher packed cell volume values than the females and this has been proposed as means of sexing fish.

Changes in the blood characteristics of *Clarias gariepinus* caused by stress because of exposure to environmental pollutants, diseases or attack by pathogens have been studied by a number of authors (Onusiriuka and Ufodike, 2000; Ezeri, 2001; Gabriel, *et al*., 2001). These indices have been employed in effectively monitoring the responses of the fish to the stressors and thus its health status under such adverse conditions. Report on the influence of sex, source and acclimation on the blood parameters of *C. gariepinus,* an important aquaculture species in many tropical and subtropical countries is non-existent. This study assessed the influence of these factors on the haematology of *C. gariepinus* with the hope of providing some useful information on this aspect of its biology.

MATERIALS AND METHOD

Male and female *C. gariepinus*, (mean weight, 320 ± 13.25g; mean TL, 34.2±12.3cm) respectively were sampled from the wild (a reservoir, Oyan Dam) and culture pond (S&D Aderopoko Farms) all in Abeokuta, Nigeria. They were weighed, sexed and apparently healthy and sick fishes were identified. Health status was based on the presence or absence of lesions and other physical injuries on their bodies such as eroded barbels, mouth, skin, and haemorrhages, ulcerations and other morphological diagnostic symptoms as suggested by Wedemeyer et al. (1977a). Blood samples were collected from five males and females, five sick and apparently healthy fish from the respective sources at the sampling site. The collection was done by cardiac puncture using a 21 gauge hypodermic needle at the site of collection and preserved in disodium salt of ethylene diamine tetra-acetic acid (EDTA) bottles for analysis.

Twenty-eight fish of the sampled population, fourteen males and females from each source were transported in oxygenated bags to the laboratory of the Department of Aquaculture and Fisheries Management, Abeokuta, Nigeria. Fish from the two sources were acclimated separately in fibreglass tanks (1.06 m^3) with an effective water depth of 0.3 m for 7 days. They were given pelleted feed (35% crude protein) at 1% body weight daily. The daily ration was split into two and dispensed at 800 and 1700 hours, respectively. Half of the water volume in the tanks was exchanged on the third day. At the end of the acclimation period, blood was sampled from the fish and preserved for analysis as was done at the site. Physico-chemical parameters (pH, water temperature and dissolved oxygen in the reservoir, pond and tanks) were determined *insitu* with Horiba U-7 water checker.

Standard haematological procedures described by Blaxhall and Daisley (1973) were employed in the assessment of the various blood parameters. Haemoglobin (Hb) was done by the cyanomethaemoglobin method, packed cell volume (PCV) by microhaematocrit method and erythrocyte sedimentation rate (ESR) by the micro-Wintrobe method. WBC was determined with the improved Neubauer counter; differential count was done on blood film stained with May Grumwald-Giemsa stain. RBC was estimated using the relationship between Hb and PCV (Mirale, 1982). The following indices: mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV) were calculated according to Seiverd (1964).

Data obtained from the experimental fish were subjected to analysis with the General Linear Model (GLM) of ANOVA at 0.05% probability, unless otherwise indicated, and differences among means were separated with the least significant difference using SAS® software.

RESULTS

The morphometric characteristics of the experimental fish are shown in Table 1. Physico-chemical characteristics of the water in the reservoir, pond and acclimation tanks were not significantly different (Table 2). One fish died during the acclimation period. There were no significant differences in all the parameters between males and

females among the apparently healthy and sick group of fish under sex, source and acclimation (Table 3). Differences in blood parameters in fish before and after acclimation were recorded in the WBC ($p<0.001$), neutrophils ($p<0.001$) and lymphocytes ($p<0.001$) only. Interactions between sex, acclimation and health status did not have any significant influence on all the parameters studied. However, significant differences were produced with the interactions of health status and source of fish in the WBC ($p<0.05$), neutrophils ($p<0.001$), lymphocytes ($p<0.001$) and monocytes ($p<0.05$, Table 4). Basophils and eosinophils were not recorded in the fish studied. When the data were pooled for males and females, apparently healthy and sick fish, respectively, there were significant differences between the WBC, neutrophils and lymphocytes of males and females under acclimation, as well as monocytes of apparently healthy and sick fish under source and neutrophils of the same under acclimation (Table 5).

DISCUSSION

The haematological characteristics of a number of culturable fish species have been studied with the aim of establishing normal value ranges, and any deviation from it may indicate a disturbance in the physiological processes Rainza-Paiva et al. (2000), O'Neal et al. (2001). Several of these studies were attempts to determine if significant variations from normal values of these parameters exist that could be attributable to some internal or external factors (Munkittrick and Leatherland, 1983; Gabriel et al., 2001). In the assessment of the blood parameters of goldfish, *Carassius auratus*, Summerfelt (1967) observed that males consistently had significantly higher haematocrit values than the females and suggested the need to separate blood component data on the basis of sex to avoid attributing sex differences to other factors. His observation is not consistent with the results obtained in this study, with regards to the source and health status of the fish (Tables 3 and 5). The observation in this study agrees with that of Kori-Siakpere (1985) who noted wide variations in the Hb, PCV and RBC indices of *Clarias ishierensis* from the wild. Furthermore, we recorded variations in the values of the various blood parameters within the same sex. Similar observations had been made in other fish species and were attributed to intrinsic factors (Burton and Murray, 1979; Etim et al. (1999). However, pooled data (Table 5) indicates that after acclimation, males consistently had higher values of WBC, neutrophils and monocytes than the females (Table 5), but the reverse was the case with lymphocytes. It appears then that the males are more responsive to the stress of acclimation than the females.

The source of fish (wild or cultured) may influence its state of health. This could be revealed by changes in the haematological parameters due to variations in the physico-chemical parameters of habitats, exposure to aetiological agents and environmental pollution among others (Das, 2003). Interactions between sex of *C. gariepinus*, source (wild or cultured), acclimation and health status indicated that source of fish had a highly significant impact on the health status (Table 5). This is shown in the values of WBC neutrophils, lymphocytes and monocytes and differential counts. Besides, fish from the wild had higher ESR values after acclimation than cultured. The significant interactions recorded between source of fish and health status seem to suggest that the source of fish plays an important role in the health status when adjudged by changes in WBC and differential counts. Changes in WBC and differential counts have been reported to play important roles in the assessment of the state of health of *C. gariepinus,* and leucopaenia and leukocytosis has been reported in the fish under exposure to pathogens, heavy metals and chemotherapeutants (van Vuren, et al. (1994), Ezeri, 2001; Omoregie and Oyebanji, 2002).

In this study the number of fish adjudged sick rose from three before acclimation to ten after acclimation (Table 3). The fish may have developed morphological injuries due to abrasion from the holding tanks and pectoral fins of other fishes under the crowded conditions. The role of blood parameters in the assessment of the health status of fish is underscored by the observation of Omoregie (1998) who noted the possibility that changes in the blood will reveal conditions within the body of the fish long before any outward manifestation of diseases.

Although there were no significant differences between the blood characteristics of apparently healthy fish before and after acclimation, the values of some of the parameters (Hb, PCV, RBC, MCV, WBC and neutrophils) were higher in the acclimated fish. The above observation reveals that the period of acclimation may influence the blood components of apparently healthy *C. gariepinus* (Tables 3 and 4). Sick fish had higher ESR, WBC and neutrophil values after acclimation than the apparently healthy and sick ones before acclimation. In addition, pooled data (Table 5) showed that sick fish had higher ESR than the apparently healthy ones. ESR is a non-specific haematological parameter that may indicate the presence and intensity of a disease state. The values are usually raised with increased tissue destruction as in acute infections and heavy metal poisoning among others (Blaxhall and Daisley, 1973). Furthermore, Onusiriuka and Ufodike (2000) reported that raised ESR values in *C. gariepinus* under exposure to a toxicants [Akee apple (*Blighia sapida)* and sausage plant (*Kigella africana*)] indicated polycythemia, dehydration and stress. Diseased fish may also show a variety of changes in their blood parameters. Ogbulie and Okpokwasili (1999) noted that apparently healthy *C. gariepinus* and *H. bidorsalis* had significantly higher Hb than the diseased

Table 4. Interactions between sex, source, health status and blood parameters of apparently healthy and sick *Clarias gariepinus* (mean ± SEM).

Parameter	Hb	Ht	ESR	RBC	RBC indices			WBC	Differential count		
					MCHC	MCH	MCV		Neutrophils (%)	Lymphocytes (%)	Monocytes (%)
SEX											
Apparently healthy males	6.53±1.10	22.43±3.66	4.29±0.52	2.4±0.4	37.70±4.20	35.1±4.0	93.0±0.7	32.86±5.84	43.43±2.46	55.00±3.44	3.16±0.54
Apparently healthy females	8.10±1.20	26.8±3.16	10.83±3.94	2.9±0.3	34.5±0.8	32.2±0.8	93.5±0.5	40.33±17	43.0±4.55	55.5±4.50	1.6±0.24
Sick males	7.04±0.97	23.86±2.80	8.0±2.27	2.5±0.3	34.3±1.2	32.4±1.1	94.3±0.6	34.14±5.97	47.78±3.4	48.42±3.11	3.17±0.79
Sick females	9.20±1.05	21.14±2.90	7.86±2.41	2.3±0.3	33.47±3.56	31.0±0.4	93.2±1.0	28.29±5.17	37.85±7.69	60.57±8.1	2.2±0.8
SOURCE											
Apparently healthy wild	8.26±1.13	23.40±1.26	6.0±1.31	2.5±0.4	35.47±0.92	32.9±0.8	93.5±0.4	25.8±7.62^{a}	39.40±3.4^{ab}	58.80±3.38^{ab}	2.60±0.6^{b}
Sick wild	7.73±1.08	22.63±3.13	11.0±3.4	2.4±0.3	±34.15±0.63	32.2±0.6	94.3±0.9	38.63±4.9^{b}	49.38±2.63^{a}	48.88±3.11^{b}	2.6±0.68^{b}
Apparently healthy cultured	7.92±0.93	23.0±1.26	4.56±0.63	2.5±0.3	36.16±3.56	33.4±3.3	93.3±0.6	37.78±4.27^{a}	49.11±1.97^{a}	48.89±2.50^{b}	3.63±0.56^{a}
Sick cultured	8.42±1.03	25.60±3.04	6.4±1.03	2.7±0.3	32.75±0.56	30.8±0.5	94.2±0.9	26.2±5.34^{a}	25.6±7.30^{b}	73.2±7.28^{a}	1.2±0.21^{c}
ACCLIMATION											
Apparently healthy before	8.40±1.00	8.40±1.00	6.2±1.28	2.6±0.3	34.96±1.01	32.96±0.9	93.8±0.6	17.80±1.11	39.4±3.4	59.2±3.20	2.20±0.49
Sick before	8.01±0.87	8.13±1.79	7.0±1.73	2.6±0.6	33.42±0.05	31.7±0.1	93.8±0.6	21.67±6.93	21.67±6.65	77.0±6.67	1.33±0.33
Apparently healthy after	7.92±0.98	7.92±0.98	6.11±1.84	2.4±0.3	36.57±3.36	34.2±3.2	84.1±9.3	42.22±3.71	49.11±1.97	48.67±2.45	3.63±0.56
Sick after	8.01±0.87	8.01±0.87	9.90±2.79	2.5±0.3	33.91±0.53	31.7±0.1	93.8±0.6	37.40±4.15	45.8±4.19	52.6±4.45	2.14±0.56

Key; Hb: Haemoglobin (g/dl), Ht: Aematocrit (l/l), ESR: Erythrocyte Sedimentation Rate (mm/hr), RBC: Red Blood Cells (cellx10^{12} /l), MCV: Mean corpuscular volume (fl), MCHC: Mean corpuscular haemoglobin concentration (g/dl), MCH: Mean corpuscular haemoglobin concentration (pg), WBC: White blood cells (cellsx10^{9}/l).

Table 5. Pooled data for the influence of sex and health status on haematological parameters of apparently healthy and sick Clarias *gariepinus (*mean ± SEM).

Parameter	Hb	Ht	ESR	RBC	RBC indices			WBC	Differential count		
					MCHC	MCH	MCV		Neutrophils (%)	Lymphocytes (%)	Monocytes (%)
SEX											
Females	8.6±0.77	24.46±2.43	7.31±1.98	2.60±0.30	36.20±2.30	33.80±2.1	93.20±0.4	36.31±3.94	42.86±4.27	55.23±2.66	2.45±0.39
Males	6.79±0.72	22.50±1.97	7.93±1.59	2.40±0.20	33.80±0.60	31.70±0.6	93.70±0.6	2.40±0.20	43.23±2.36	54.5±4.48	2.72±0.56
Apparently healthy	7.31±0.80	23.14±2.22	6.14±1.23	2.50±0.20	36.00±2.20	33.80±2.0	93.70±0.5	33.50±4.01	45.64±2.11	51.71±2.40	3.17±0.46
Sick	8.00±0.75	23.77±2.21	9.23±2.18	2.50±0.20	33.80±0.4	31.60±0.5	93.30±0.6	33.85±3.91	40.23±4.52	58.23±4.68	1.90±0.41
SOURCE											
Females	7.93±0.77	22.92±2.23	9.08±2.21	2.50±0.2	34.66±0.54	32.5±2.1	93.60±0.4	33.69±4.39	45.54±2.44	52.69±2.62	2.60±0.43
Males	8.10±0.69	23.93±2.20	5.21±0.58	2.60±0.2	34.94±2.29	32.5±0.5	93.60±0.4	33.69±4.39	40.71±4.14	57.57±4.33	2.69±0.49
Apparently healthy	8.04±0.70	23.14±2.22	5.07±0.62	2.50±0.2	35.91±2.26	33.2±2.1	93.40±0.5	33.50±4.01	45.64±2.11	52.43±0.43	3.23±0.43^{a}
Sick	7.99±0.75	23.77±2.21	9.23±2.18	2.50±0.2	33.61±0.47	31.7±0.5	94.3±0.6	33.84±3.92	40.23±4.52	58.23±4.68	1.90±0.41^{b}
ACCLIMATION											
Females	8.30±0.84	24.13±2.45	6.50±0.96	2.60±0.3	34.39±0.66	32.4±0.6	94.2±0.4	19.25±2.47^{a}	32.75±4.40^{a}	65.88±4.36^{a}	1.88±0.35
Males	7.97±0.63	23.16±1.96	8.11±1.72	2.50±0.2	35.17±1.60	32.9±1.5	89.0±4.4	39.68±2.78^{b}	47.37±2.57^{b}	50.74±2.57^{b}	2.93±0.43
Apparently healthy	8.09±0.71	23.14±2.22	6.14±1.23	2.50±0.2	35.99±2.15	33.7±2.0	87.5±6.0	33.5±4.01	45.64±2.11^{a}	52.43±2.34	3.07±0.43
Sick	8.04±0.75	23.77±2.21	9.23±2.17	2.50±0.2	33.8±0.41	31.7±0.5	93.8±0.3	33.77±3.92	40.23±4.52^{b}	58.23±4.68	1.9±0.41

Key; Hb: Haemoglobin (g/dl), Ht: Aematocrit (l/l), ESR: Erythrocyte Sedimentation Rate (mm/hr), RBC: Red Blood Cells (cellx10^{12} /l), MCV: Mean corpuscular volume (fl), MCHC: Mean corpuscular haemoglobin concentration (g/dl), MCH: Mean corpuscular haemoglobin concentration (pg), WBC: White blood cells (cellsx10^{9}/l).

ones, whereas the reverse was the case with WBC and ESR. However, Ezeri (2001) observed no significant differences in the Hb, ESR, WBC and PCV of healthy *C. gariepinus,* those infected with *Pseudonomas florescens* and the infected treated with chloramphenicol. Available information on the influence of health status on the haematological indices of fish seems contradictory. This may due be explained by the fact that different stress factors in some cases elicit different physiological responses.

Eosinophils and basophils were not recorded in the fish studied. Reports of the presence of these cells in teleost fish are somewhat contradictory. Basophils were not found in the blood of plaice (Ellis, 1977), rainbow and brown trout (Blaxhall and Daisley, 1973). However, they were not recorded in some species like salmon pink (Ostroumova, 1960). Eosinophils are usually rare in fish and their occurrence has been commonly reported in haemopoietic tissues, for example in the kidney (Kelenyi and Neimeith, 1969).

This study revealed that the source of fish (wild or pond), sex, period of acclimation may exert some degrees of influence on some of the haematological characteristics of *C. gariepinus* and hence the need to reckon with these factors in the assessment and reporting of the haematological indices of this fish species.

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