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Influence of Sex on Haematological and Biochemical Profiles of Pond Reared *Heterobranchus longifilis* (Valenciennes, 1840)

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ABSTRACT

Haematological and biochemical parameters of male and female samples of 24-week old F_1 *Heterobranchus longifilis* were determined using standard methods, in order to establish baseline information and check for significant differences ($P \leq 0.05$) in the haematological and biochemical values of both sexes. Female fish samples had significantly higher ($P < 0.05$) mean values of white blood cell ($220.33 \pm 1.36 \times 10^3/\mu L$), red blood cell ($1.81 \pm 0.12 \times 10^6/\mu L$) and haemoglobin ($11.00 \pm 1.11$ g/dL) than male fish. Lower red blood cells ($1.48 \pm 0.06 \times 10^6/\mu L$) were observed in male than female ($1.81 \pm 0.12 \times 10^6/\mu L$). The fish may indicate quantitative protein inadequacy per feeding time to meet its nutrient requirements. Alkaline phosphatase (ALP) was found to be significantly ($P < 0.05$) higher (30.00 ± 1.73 IU/L) for male than female fish. This is probably associated with higher viscerosomatic index in male fish than in female fish as established by previous research, since ALP originates from the liver, bone and intestine. Sex has a varying influence on the haematological and biochemical parameters of 24 weeks old *H. longifilis.* Thus, serum biochemical parameters of the F_1 of *H. longifilis* could serve as good biomarkers of sex.

Key words: Biochemical, haematological**,** *Heterobranchus longifilis*, sex

INTRODUCTION

There is increased global attention on aquaculture because of the need to augment fish production from the wild. This is particularly noticeable in populous countries like Nigeria where there is high protein demand (Owodeinde *et al*., 2011). Aquaculture is acknowledged as the most efficient means of providing food which is rich in protein, minerals, vitamins and valuable lipids. It is also a source of income and employment opportunities to the populace (Ojutiku, 2008). *Heterobranchus longifilis* is among the cultured Clariid fishes in Nigeria. It has been reported to have high feed efficiency and utilization (Adebayo and Olanrewaju, 2000). Qualities that make it suitable for aquaculture include: fast growth rate, hardiness, high yield potential, high fecundity and palatability (Offem *et al*., 2008). *Heterobranchus longifilis* is a harmless freshwater fish that inhabits large rivers, deep pools and lakes. The common names include: Catfish, *Sampa*, *Ramboshi* and *Vundu* (Froese and Pauly, 2012). *Clarias gariepinus* and *H. longifilis* belong to the Family Clariidae; air breathing catfishes, considered to most important catfish species for aquaculture. They have an almost Pan African distribution (Solomon and Udoji, 2011).

Historically, biochemical parameters have been studied and baseline values have been established for fish species used in research or as food sources in Europe and North America. Data on haematological, biochemical and biological parameters of fish species, which are admitted to aquaculture in the developed countries, are available (Camfield, 1998). Information on the interaction of blood biochemical parameters and fish health, may prove useful as part of an integrated management system for fisheries, especially in predicting the onset of disease, thereby allowing for the employment of appropriate intervention strategies to mitigate fish loss. Adeyemo *et al*. (2007) reported that

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haematological indices are important parameters for evaluating the physiological status of fish. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) belong to the family of non-plasma-specific enzymes that are localized in the tissues of the liver, heart, gills, kidney, muscles and other organs. When present in blood plasma, they may provide specific information on the organ dysfunction (Anver, 2004).

Haematological and biochemical parameters are valuable tools for monitoring fish health, confirming maturation and monitoring any change in the quality of water and related soil (Satheeshkumar *et al*., 2011). The degree of haematological indices depends on fish species, age, cycle of sexual maturity and health conditions (Hubrec *et al*., 2001). Many haematological parameters can be used to assist in providing evidence and possible identification of an abnormality or a disease process, for instance; haemoglobin estimation, erythrocyte count and haematocrit values in the detection of anaemias, leukocyte count as an indication of possible types of infections or organic disease (Walencik and Witeska, 2007). Research on the link between reproduction and non-plasma specific enzymes is sparse; pawpaw seed extract was recommended as a natural reproductive inhibitor for Nile tilapia and was shown to influence ALT and AST levels in plasma alongside the histological structure of the gonads (Fish Site, 2014).

Bhasker and Rao (1989) recommended the ranges of 2-3 (1.7 to 4.00) $10^6/mm^2$ for RBC, 43 (22-48%) for PCV, 5.15 to7.5 g/100ml for Haemoglobin and 17.2 (10.9 – 38.1) % for MCHC. for normal, healthy fish. While Wedemeyer and Yasutake (1977) recommended a range of 0.77 – 1.58 x $10^6/mm^2$ for RBC, 5.4 – 9.3 g/100ml for Haemoglobin and 23 – to 43 % of PVC. Diyaware *et al*. (2013) reported 27.87% as PCV, 9.63g/dl, Hb, 16.67x10^3 as PLT, 190.40 x10^3 as LYM, 2.46 x10^3 RBC and 193.70 x10^3 as for juvenile F_1 Heteroclarias (*C. anguillaris* x *H. bidorsalis*) in the Northeast Nigeria. Diyaware (2012) documented higher blood parameters in male *C. anguillaris*, *H. bidorsalis* and their F_1 hybrids.

Reports with the normal haematological and serum biochemical parameters of many fish species farmed in northern Nigeria, such as *H. longifilis* with respect to sex are scarce to come by. The determination of variation in haematological analytes, diagnostic and prognostic values of serum biomarkers by sex in F_1 generation of *H. longifilis* might provide some useful baseline information to enhance further studies on mechanisms of sex, hormonal changes and effect of sexual processes such as oocyte maturation and spermiation.

MATERIALS AND METHODS

Description of the study area

The breeding and culture exercise was carried out at Sama fish farm, Mando. Mando is situated in Igabi Local Government Area of Kaduna, Kaduna State, which falls between latitude 10º 49′ 06″ N and longitude 6º 42′ 00″ E. The annual rainfall in Kaduna varies from 0.0 – 825.0 mm per month, reaching its peak in August. Temperature during peaks of breeding season (June to September) ranges from 22-29 ºC and as low as 17 ºC in the dry season (Google Imagery, 2013).

Determination of haematological indices of the F_1 generation of *H. longifilis*

Haematological analysis was conducted at 24 weeks old, male and female artificially spawned and concrete-pond reared *H. longifilis*, using automated Sysmex Hematology and Coagulation Systems (Model KX-21N) in the Haematology unit, Ahmadu Bello University Teaching Hospital, Zaria. Samples of 24 weeks old male and female *H. longifilis* were conveyed to the Necropsy Laboratory, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. Blood samples were collected and pooled from 3 live adults per treatment in triplicates. The sampled fish were rinsed thoroughly in clean water and wiped dry with clean towels. Blood samples were collected from the intraperitoneal vein in adults, using 2 ml plastic syringes with 22-gauge needles treated with the anti-coagulant ethylene diamine tetra acetate acid (EDTA) and stored in labelled sample bottles in the refrigerator at 2-4°C for

a few hours before analysis. Results for white blood corpuscles, red blood corpuscles, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and platelet counts; number and percentage lymphocytes, monocytes, neutrophils were printed automatically.

Determination of prognostic and diagnostic values of serological markers of *H. longifilis*

Serum biochemical assay was conducted via an automated Serological Roche Hitachi (Model 902) machine in the Chemical Pathology Laboratory, Ahmadu Bello University Teaching Hospital, Zaria. Pooled whole blood samples were put in triplicate labelled sample bottles per treatment and centrifuged (Triac Clinical Centrifuge Saitexiangyi, model TG12MX) at 1,400 rpm. The sera obtained were kept for some hours in a deep freezer at -20°C. The preserved sera were then fed into the automated Serological machine to obtain values for aspartate aminotransferase, alanine aminotransferase, bilirubin, alkaline phosphatase (ALP) and total cholesterol, following the automated methods recommended by the International Federation of Clinical Chemistry (Bergmeyer *et al*., 1986).

Statistical analyses

Statistically significant differences were determined by setting the aggregate type I error at 5% ($P \leq 0.5$) for each comparison. The Student's t-test was used to test for significant differences between treatments for various haematological and biochemical values of male and female *H. longifilis*.

RESULTS

Haematological values of 24 weeks old female and male *Heterobranchus longifilis*

The mean haematological values for female and male table-size *H. longifilis* F_1 with respect to white blood cell, red blood cell, haemoglobin, haematocrit and mean corpuscular volume ranged from 9.3 to 11.1 × 10^3/µL, 1.42 to 1.9/µL, 8.2 to 12g/dL, 25.8 to 35%, and 179.2 to 189.6fL, respectively for both female and male fishes. Female fish samples had significantly higher ($P \leq 0.05$) mean values for white blood cell (220.33 ± 1.36), red blood cell (1.81 ± 0.12), haemoglobin (11.00 ± 1.11), haematocrit (33.33 ± 2.08), platelet (7.33 ± 1.15) and neutrophil/µL (0.53±0.12) than male fish samples (Table 1).

Table 1: Haematological Values of Female and Male Table-size *Heterobranchus longifilis* F_1
Mean of triplicate samples each pooled from 5 fish, *Significant difference at 95% confidence interval

Haematological Parameter	Mean Female	Mean Male	t-value	*P*	Std. Dev. Female	Std. Dev. Male	F-ratio - Variances
WBC × 10^3/µL	11.00	9.46	16.05	0.00*	1.36	3.06	5.04
RBC × 10^6/µL	1.81	1.48	4.34	0.01*	0.12	0.06	3.78
Hb(g/dL)	11.00	8.93	2.78	0.05*	1.11	0.64	3.00
Ht (%)	33.33	26.60	5.29	0.01*	2.08	0.72	8.33
MCV (fL)	185.20	180.50	1.49	0.21	5.38	0.87	38.11
MCH (pg)	60.87	60.03	1.15	0.32	0.31	1.22	16.00
MCHC (g/dL)	33.03	33.10	-0.05	0.96	1.50	1.61	1.15
PLT (fL)	7.33	3.33	5.37	0.01*	1.15	0.58	4.00
Lym (%)	97.73	97.90	-0.15	0.89	1.90	0.30	40.15
Neut (%)	2.13	2.17	-0.45	0.68	0.12	0.06	4.00
Mon (%)	0.23	0.13	2.12	0.10	0.06	0.06	1.00

Key: WBC=White Blood Cells, RBC=Red Blood Cells, Hb=Haemoglobin, Ht=Haematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Platelet (PLT), Lymphocyte (Lym), Neutrophil (Neut) and Monocyte (Mon).

Biochemical values of female and male 24 weeks *Heterobranchus longifilis*

Biochemical values ranged from 359.8 to 1285.6, 70.8 to 251.2, 0.04 to 0.12, 12 to 31, 3.0 to 4.8 for AST, ALT, bilirubin, ALP and total cholesterol, respectively, for both female and male table size *H. longifilis* F_1. Mean values for female and male are presented in Table 2. Female fish had significantly higher ($P \leq 0.05$) AST (1282.2 ± 2.94), ALT (250.10 ± 1.15) and bilirubin (0.10 ± 0.02) mean values than male fish samples. However, ALP was found to be significantly higher ($P \leq 0.05$) (30.00 ± 1.73) for male fish than female fish samples. No significant difference ($P > 0.05$) was observed between the sexes with respect to total cholesterol (Table 2).

Table 2: Serological Values of Female and Male *Heterobranchus longifilis* F1

Serological Parameter	Female Mean	Male Mean	t-value	*P*	Female Std.	Male Std.	F-ratio – Var.	p – Var.
AST (IU/L)	1282.20	361.20	482.07	0.0000*	2.94	1.51	3.80	0.42
ALT (IU/L)	250.10	72.10	133.67	0.0000*	1.15	2.00	3.00	0.50
Bil (mg/dL)	0.10	0.05	3.273	0.0306*	0.02	0.02	1.33	0.86
ALP (IU/L)	13.00	30.00	-14.72	0.0001*	1.00	1.73	3.00	0.50
TC (mg/dL)	4.30	3.80	0.83	0.4534	0.50	0.92	3.36	0.46

Mean of triplicate samples each pooled from 5 fish, *Significance difference at 95% confidence interval.
Key: AST=Aspartate aminotransferase, ALT=Alanine aminotransferase, Bil=Bilirubin (Bil), ALP=Alkaline phosphatase, TC=Total Cholesterol.

DISCUSSION

Lower red blood cells (RBC x 10^6/µ L) in male (1.48 ± 0.06) than female (1.81 ± 0.12) fish may be due to quantitative protein inadequacy per feeding time to meet its nutrient requirements, which might have inhibited erythrocyte production. Lower haemoglobin level according to Joshi *et al*. (2002) might decrease the ability of fish to enhance its activity in order to meet occasional demands. Higher white blood cell count in female fish in this study does not necessarily initiate leucopomia (a condition of increase in total white blood cell) in the haematopoietic tissue of the kidney and perhaps the spleen as suggested by Omitoyin (2006) but it is possibly linked to the higher spleenosomatic index observed in female fish. The mean white blood cell count of 220/µl for fish with mean weight of 1029.1g in this study is higher than 212 ± 25.87 × 10^9 cells reported by Gabriel *et al*. (2010) for catfish hybrid (*H. longifilis* × *C. gariepinus*) with mean weight of 207.83 ± 12.63g. All the haematological parameters recorded in this study fall with the ranges recommended by Bhasker and Rao (1989) for healthy fish.

The significantly higher values of AST, ALT in female 24-weeks old fishes than their male counterparts suggest a probable link between AST, AST and sex-linked physiological processes (Fish Site, 2014). Higher values of bilirubin in female fish may be linked to their higher red blood cell values as bilirubin is a metabolite of haemoglobin degradation in the body. This theory is further buttressed by the higher spleenosomatic index observed in female *H. longifilis* compared to their male counterparts (Suleiman, 2015). The higher values of ALP in male fish compared to the females may be associated with higher viscerosomatic index in male fish than in female fish as reported by Suleiman (2015), since increased ALP in serum could be associated with liver and osteoblastic activities and or conditions (CDC, 2006). Sex has a varying influence on the haematological and biochemical parameters of 24-week *H. longifilis.* Serum biochemical parameters of the F_1 of *H. longifilis* are good biomarkers of sex.

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