HAEMATOLOGICAL AND BLOOD BIOCHEMISTRY VALUES OF CULTURED *Heterobranchus longifilis* IN UMUDIKE, ABIA STATE, NIGERIA

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ABSTRACT

This study investigated the haematological and blood biochemistry profile of apparently healthy catfish (Heterobranchus longifilis) raised in concrete ponds in South East, Nigeria. A total of 20 fishes of two age ranges and equal sexes were used and determination of haematological and blood biochemical profile followed standard procedures. Results obtained for the parameters assessed for the young adults and adults respectively are summarized as follows: Packed cell volume - 29.67 ± 0.73% and 33.00 ± 0.87%, haemoglobin concentration - 10.26 ± 0.45 g/dl and 11.04 ± 0.32 g/dl, red blood cell count – 2.46 ± 0.04 x 10^6/µl and 2.39 ± 0.04 x 10^6/µl, mean corpuscular volume-120.68 ± 3.61 fl and 141.42 ± 2.83 fl, mean corpuscular haemoglobin – 41.97 ± 1.16 pg and 45.69 ± 1.15 pg, mean corpuscular haemoglobin concentration - 35.75 ± 1.63 g/dl and 32.93 ± 0.86 g/dl, white blood cell count - 17.94 ± 0.57 x 10^3/µl and 22.51 ± 0.95 x 10^3/µl, alanine amino transferase - 36.95 ± 0.09 IU and 38.86 ± 0.16 IU, aspartate amino transferase - 54.52 ± 0.10 IU and 49.55 ± 0.34 IU, alkaline phosphatase -5.04 ± 0.71 IU and 9.33 ± 0.55 IU, total proteins – 1.98 ± 0.09 g/dl and 3.86 ± 0.10 g/dl, albumin - 0.90 ± 0.01 g/dl and 1.53 ± 0.06 g/dl, globulin – 1.07 ± 0.09 g/dl and 2.40 ± 0.08 g/dl, plasma blood glucose – 136.25 ± 11.74 mg/dl and 171.67 ± 9.05 mg/dl, total cholesterol – 79.89 ± 2.72 mg/dl and 156.52 ± 5.69 mg/dl, calcium – 4.28 ± 0.32 mg/dl and 11.68 ± 0.71 mg/dl, blood urea nitrogen – 1.48 ± 0.24 mg/dl and 1.19 ± 0.12 mg/dl, body weight – 308.89 ± 22.26g and 666.67 ± 39.97g. Results obtained showed significant ($p<0.05$) changes due to age in all the parameters assessed except haemoglobin concentration, red blood cell count, mean corpuscular haemoglobin concentration and blood urea nitrogen. There were no significant changes ($p>0.05$) between the sexes in all the haematological and blood biochemistry parameters assessed. Data generated from this study was considered important as deviations in the normal haematology and serum biochemistry have a predictive value for general pathological and specific organ changes in the body.

Keywords: Catfish, *Heterobranchus longifilis,* Haematology, Blood biochemistry, Southeastern Nigeria

INTRODUCTION

Fish and fishery products provide protein and essential micronutrients for balanced nutrition and good health (FAO, 2012). Globally, fish accounted for 16.6% of animal protein intake and 6.5% of all protein consumed in 2009 (FAO, 2012). While world productivity of fish capture dropped slightly, there was a remarkable increase in aquaculture productivity in the last decade with Africa recording the highest annual increase in the number of people engaged in

fish farming followed by Asia, Latin America and the Caribbean respectively (FAO, 2012). In Africa, catfish has replaced tilapia as the most cultured fish since 2004 and Nigeria is ranked the highest producer of catfish in Africa (FAO, 2012).

The progressive dominance of this species in Nigeria and many African countries is due to its fast growth rate, high resistance to diseases and tolerance to environmental extremes and high consumer preference. Also, the increasing popularity of "point and kill' in relaxation centres and eateries in Nigeria where live fishes are displayed in small concrete ponds and customers make their choices before they are killed and prepared has led to a tremendous increase in small scale fish farming using concrete or tarpaulin ponds.

Diseases and adverse environmental conditions are the major constraints of aquaculture especially in the developing countries (Thien *et al.,* 2007; Chi *et al*., 2008; Phan *et al.,* 2010; FAO, 2012).

Haematological and blood biochemistry parameters are important in diagnosis, monitoring of therapeutic interventions and well being of fishes (Schalm *et al.,* 1975; Coles, 1986; Fry and Mc Garvin, 2007). Determination of reference values of these parameters in this species is of utmost importance since so many factors including species, age, sex, season and management system had been reported to have effects on the haemato-clinical chemistry values in fishes. This study was therefore designed to evaluate the normal haematological and blood biochemistry profile of cultured *Heterobranchus longifilis* raised in concrete ponds in Umudike, Abia State, Nigeria.

MATERIALS AND METHODS

A total of 20 apparently healthy *Heterobranchus longifilis* of two age ranges, and equal sexes were used for the study. They were obtained from the Michael Okpara University of Agriculture, Umudike fish farm between January and March, 2014. The fish were raised in a 15 x 15 m^2 in-door concrete ponds of 1 m depth under standard cultural practices. They were fed by point-feeding method with standard fish feed (Coppens, Germany) and water changed every week.

Umudike is located within the tropical rain forest zone on 5^0 28´ North and 7^0 31´ East and the environment lies on 122 mm above sea level. The environment is characterized by an average annual rainfall of 2177 mm and daily temperature of between 27^oC and 35^oC (FMANR, 2005).

The fishes were caught with nets and about 2.5ml of blood was collected through the caudal vein and 0.5ml was put into a bottle containing EDTA for haematology and 2ml put into clean test tubes, allowed for 30 minutes to clot and centrifuged at 2000g for 10 minutes and the serum harvested (Tomlinson *et al.,* 2013). Haematological and clinical chemistry determinations were carried out immediately after collection and standard procedures were followed in all the determinations. All the biochemical parameters except plasma glucose level were determined using serum sample and Quimica Clinica Aplicada test kits (Quimica Clinica Aplicada, Spain) and a Cole Parmer 1200 spectrophotometer (Cole-Parmer Instrument Company, USA). Packed cell volume (PCV) was determined by the microhaematocrit method, while haemoglobin concentration (HbC) was determined by the cyanomethaemoglobin method (Schalm *et al.,* 1975; Coles, 1986). Red blood cell (RBC) count 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). The serum alanine amino transferase (ALT) and serum aspartate amino transferase (AST) were determined by the Reitman-Frankel method (Reitman and Frankel, 1957). The serum alkaline phosphatase (ALP) was determined by the phenolphthalein monophosphate method (Klein *et al.,* 1960; Babson *et al.,* 1966).The total serum proteins were determined by the direct Biuret method (Librans, 1978), while the serum albumin was determined by the bromocresol green method (Doumas *et al.,* 1971; Doumas and Peters, 1997). The serum globulin was calculated as the

difference between the serum total protein and serum albumin (Colville, 2002).

The serum cholesterol was determined by the enzymatic colorimetric method (Allain *et al.,* 1974). The serum calcium was determined by the O-cresolphthalein direct method (Kessler and Wolfman, 1964; Gitelman, 1967; Biggs and Moorehead, 1964), while blood urea nitrogen (BUN) was determined by the modified Berthlot-Searcy method (Fawcett and Scott, 1960; Searcy *et al.,* 1967) The blood glucose level was measured using ACCU-CHEK Glucometer (Roche Diagnostics GmbH, Nannheim, Germany) based on the glucose oxidase method (ADA, 2003; D'orazio *et al.,* 2005). The body weights were measured using a weighing balance (Hana, China), while sex determination was by *in-situ* gonad examination. Catfishes below six months of culture were classified as young adults and those above eight months as adult.

Data for all the determinations were presented as means ± standard error (SE) with the minimum and maximum values. The results were subjected to descriptive statistics, while Student's t-test was used to compare the differences due to age and sex. Statistics were carried out using SPSS 16.0 Statistical Package (SPSS 16.0 for Windows, SPSS Inc., Chicago, IL, USA). Significant difference was accepted at probability level of $p<0.05$.

RESULTS AND DISCUSSION

The results of haematological and blood biochemistry parameters of the young adults and adult *H. longifilis* indicated that adults *H. longifilis* had significantly higher ($p<0.05$) values when compared to the young adults in all the parameters determined except HBC, RBC, MCHC and BUN (Table 1). There were no significant ($p>0.05$) differences in all the parameters between the sexes in both the young adults and adult *H. longifilis* (Tables 2 and 3). The results of PCV, HBC, MCH, MCHC and WBC counts recorded for both the young and adult *H. longifilis* lie within the reference ranges reported by Adeyemo *et al.* (2014) in cultured *C. gariepinus* in the lower Benue River Basin, North central, Nigeria but lower (173.75 ± 41.93fl) and higher (87.01 ± 14.37fl) MCV for the young adults and adults respectively.

Only the RBC counts of males and MCHC of the young adults lie within the values reported by Gabriel *et al.* (2004) in young cultured *C. gariepinus* with higher HBC, MCV and far lower WBC count in Rivers State, Nigeria. Also, the haematological parameters fell within the reference ranges reported in cultured short nose sturgeon but with slightly lower HBC and far higher MCV, MCH and WBC count (Knowles *et al.,* 2006). The PCV and WBC also fell within the reference ranges (30 - 34%, 19.8-28.1 x 10^3/µl) reported by Tripathi *et al.* (2004) in Koi (*Cyprinus carpio*) but higher HBC and RBC (6.30 - 7.60 g/dl and 1.70 - 1.90 x 10^6/µl). All the haematological parameters determined lie within the reference ranges reported by Owolabi (2011) in upside-down catfish (*Synodontis membranacea*) from Jebba Lake, Nigeria (PCV - 22.00 - 38.00 %, HBC - 5.30 - 12.06 g/dl, RBC - 1.20 - 8.50 10^6/µl, MCV – 68.99 - 156.33fl, MCH - 9.26 - 64.41pg, MCHC - 6.94 - 34.17 g/dl). The haematological parameters determined except MCHC that was slightly higher and WBC that nearly fell into the reference range lie within the reference ranges reported for young adult cultured tilapia, *Oreochromis* hybrid (Hrubec *et al.,* 2000)

The ALT activity was far higher while total proteins, albumin and globulin were lower when compared to the values (ALT- 0.00 - 29.00 IU/L, Total proteins - 2.99 - 6.11, Albumin-0.81-1.67 and globulin- 2.18-4.44 g/dl) reported by Myburgh *et al.* (2008) in *C. gariepinus* in South Africa. The total proteins (2.40 - 4.80 g/dl), albumin (1.9 - 3.4 g/dl), ALP (12.00 - 108.00 IU/L) and BUN (5.88 - 28.24 mg/dl) values recorded in this study are lower than the reference ranges reported for *S. membranacea* and higher than the ranges for AST (6.00 - 28.30 IU/L) but within the ranges for ALT (3.50 - 50.20 IU/L), plasma glucose (21.43 - 375 mg/dl) and total cholesterol (37.60 - 603.85 mg/dl) reported by Owolabi (2011). The total proteins, albumin, globulin, calcium, ALP recorded were higher in both low and high density systems, higher cholesterol only in high density system but lie within the ranges for AST

Table 1: Age differences in the haematological and blood biochemistry profile of apparently healthy *Heterobranchus longifilis*

Parameters	Young adults	Range	Adults	Range
PCV (%)	29.67 ± 0.93[a]	27.00 - 32.00	33.00 ± 0.87[b]	27.00 - 36.00
HBC (g/dl)	10.26 ± 0.45	9.31 - 11.90	11.04 ± 0.32	9.31 - 11.90
RBC (10^6/µl)	2.46 ± 0.04	2.38 - 2.63	2.39 ± 0.04	2.20 - 2.57
MCV (fl)	120.68 ± 3.61[a]	112.17 - 133.89	141.42 ± 2.83[b]	128.40 - 152.54
MCH (pg)	41.97 ± 1.16[a]	39.12 - 47.04	45.69 ± 1.15[b]	40.66 - 50.42
MCHC (g/dl)	35.75 ± 1.63	30.72 - 39.67	32.93 ± 0.86	29.09 - 36.06
WBC (10^3/µl)	17.94 ± 0.57[a]	14.85 - 20.25	22.51 ± 0.95[b]	18.70 - 27.80
ALT (IU)	36.95 ± 0.09[a]	36.64 - 37.25	38.86 ± 0.16[b]	38.19 - 39.40
AST (IU)	54.52 ± 0.10[a]	54.03 - 54.98	49.55 ± 0.34[b]	48.08 - 51.51
ALP (IU)	5.04 ± 0.71[a]	3.53 - 7.06	9.33 ± 0.55[b]	6.49 - 10.54
PGL (mg/dl)	136.25 ± 11.74[a]	71.00 - 173.00	171.67 ± 9.05[b]	147.00 - 235.00
T. Proteins (g/dl)	1.98 ± 0.09[a]	1.60 - 2.40	3.86 ± 0.10[b]	3.39 - 4.29
Albumin (g/dl)	0.90 ± 0.01[a]	0.87 - 0.96	1.53 ± 0.06[b]	1.25 - 1.73
Globulin (g/dl)	1.07 ± 0.09[a]	0.73 - 1.44	2.40 ± 0.08[b]	2.12 - 2.75
Calcium (mg/dl)	4.28 ± 0.32[a]	3.16 - 5.26	11.68 ± 0.71[b]	8.97 - 14.48
T. Cholesterol (mg/dl)	79.89 ± 2.72[a]	69.57 - 91.30	156.52 ± 5.69[b]	130.43 - 173.91
BUN (mg/dl)	1.48 ± 0.24	0.67 - 2.67	1.19 ± 0.12	0.71 - 1.43
Body weight (g)	308.89 ± 22.26[a]	200.00 - 400.00	666.67 ± 40.00[b]	550.00 - 900.00

**Different superscripts in a row indicate significant difference between the groups ($p<0.05$), PCV - packed cell volume, HBC - haemoglobin concentration, RBC - red blood cell, MCV - Mean corpuscular volume, MCH - Mean corpuscular haemoglobin, MCHC - Mean corpuscular haemoglobin concentration, WBC - white blood cell, ALT - alanine amino transferase, AST - aspartate amino transferase ALP - alkaline phosphatase, BUN- blood urea nitrogen, PGL- plasma glucose level*

Table 2: Sex differences in the haematological and blood biochemistry profile of apparently healthy young adult *Heterobranchus longifilis*

Parameters	Male	Range	Female	Range
PCV (%)	30.83 ± 0.73	29.00 - 32.00	28.50 ± 0.87	27.00 - 30.00
HBC (g/dl)	10.17 ± 0.62	9.31 - 11.38	10.35 ± 0.79	9.31 - 11.90
RBC (10^6/µl)	2.47 ± 0.08	2.39 - 2.63	2.45 ± 0.04	2.38 - 2.53
MCV (fl)	125.08 ± 6.60	112.17 - 133.89	116.28 ± 1.50	113.45 - 118.58
MCH (pg)	41.79 ± 0.74	40.96 - 43.27	42.15 ± 2.47	39.12 - 47.04
MCHC (g/dl)	33.67 ± 2.47	30.72 - 38.58	37.82 ± 1.67	34.48 - 39.67
WBC (10^3/µl)	17.75 ± 1.11	14.83 - 20.25	18.11 ± 0.50	17.25 - 19.55
ALT (IU)	36.93 ± 0.12	36.64 - 37.25	36.98 ± 0.18	36.64 - 37.25
AST (IU)	54.36 ± 0.13	54.03 - 54.62	54.68 ± 0.11	54.50 - 54.98
ALP (IU)	5.30 ± 1.01	3.53 - 7.06	4.71 ± 1.18	3.53 - 7.06
PGL (mg/dl)	146.25 ± 14.08	109.00 - 173.00	126.25 ± 19.46	71.00 - 155.00
T. Proteins (g/dl)	2.00 ± 0.14	1.80 - 2.40	1.95 ± 0.13	1.60 - 2.20
Albumin (g/dl)	0.91 ± 0.02	0.87 - 0.96	0.88 ± 0.01	1.35 - 1.63
Globulin (g/dl)	1.09 ± 0.13	0.89 - 1.44	1.05 ± 0.17	0.73 - 1.29
Calcium (mg/dl)	4.08 ± 0.45	3.16 - 5.26	4.47 ± 0.50	3.16 - 5.26
T. Cholesterol (mg/dl)	80.44 ± 4.52	69.57 - 91.30	79.35 ± 3.71	69.57 - 86.96
BUN (mg/dl)	1.17±0.17	0.67 - 1.33	1.73 ± 0.40	0.67 - 2.67
Body weight (g)	262.50±23.94	200.00 - 300.00	346.00 ± 25.81	250.00 - 400.00

No difference between the groups ($p>0.05$), PCV - packed cell volume, HBC - haemoglobin concentration, RBC - red blood cell, MCV - Mean corpuscular volume, MCH - Mean corpuscular haemoglobin, MCHC - Mean corpuscular haemoglobin concentration, WBC - white blood cell, ALT - alanine amino transferase, AST - aspartate amino transferase, ALP - alkaline phosphatase, BUN- blood urea nitrogen, PGL- plasma glucose level

Table 3: Sex differences in the haematological and blood biochemistry profile of the adult *Heterobranchus longifilis*

Parameters	Male	Range	Female	Range
PCV (%)	32.20 ± 1.36	27.00 - 35.00	34.00 ± 0.91	32.00 - 36.00
HBC (g/dl)	11.07 ± 0.39	9.83 - 11.90	10.99 ± 0.61	9.31 - 11.90
RBC (10^6/µl)	2.40 ± 0.09	2.20 - 2.57	2.38 ± 0.04	2.29 - 2.46
MCV (fl)	139.92 ± 4.54	128.40 - 150.08	142.92 ± 3.93	134.15 - 152.54
MCH (pg)	45.23 ± 0.91	42.92 - 47.01	46.15 ± 2.29	40.66 - 50.42
MCHC (g/dl)	32.81 ± 1.13	31.03 - 36.06	33.05 ± 1.46	29.09 - 36.06
WBC (10^3/µl)	23.43 ± 1.86	18.70 - 27.80	21.60 ± 0.42	20.45 - 22.45
ALT (IU)	38.92 ± 0.21	38.19 - 39.40	38.80 ± 0.28	38.19 - 39.40
AST (IU)	49.68 ± 0.55	48.31 - 51.51	49.40 ± 0.44	48.08 - 49.91
ALP (IU)	9.73 ± 0.57	8.11 - 10.54	8.92 ± 0.99	6.49 - 10.54
BGL (mg/dl)	184.80 ± 13.84	159.00 - 235.00	155.25 ± 3.20	147.00 - 162.00
T. Proteins (g/dl)	3.89 ± 0.10	3.66 - 4.11	3.82 ± 0.19	3.39 - 4.29
Albumin (g/dl)	1.54 ± 0.10	1.25 - 1.73	1.52 ± 0.06	1.35 - 1.63
Globulin (g/dl)	2.42 ± 0.06	2.30 - 2.57	2.39 ± 0.19	2.12 - 2.75
Calcium (mg/dl)	11.79 ± 0.91	8.97 - 14.48	11.49 ± 1.40	8.97 - 13.79
T. Cholesterol (mg/dl)	161.74 ± 6.51	139.13 - 173.91	149.83 ± 10.04	130.43 - 165.22
BUN (mg/dl)	1.29 ± 0.14	0.71 - 1.43	1.07 ± 0.21	0.71 - 1.43
Body weight (g)	600.00 ± 27.39	550.00 - 700.00	750.00 ± 64.55	600.00 - 900.00

No difference between the groups (p>0.05), PCV - packed cell volume, HBC - haemoglobin concentration, RBC - red blood cell, MCV - Mean corpuscular volume, MCH - Mean corpuscular haemoglobin, MCHC - Mean corpuscular haemoglobin concentration, WBC - white blood cell, ALT - alanine amino transferase, AST - aspartate amino transferase, ALP - alkaline phosphatase, BUN- blood urea nitrogen, PGL- plasma glucose level

in both the low and high density systems in tilapia (Hrubec *et al.,* 2000). Also there were lower calcium and higher plasma glucose level when compared with the ranges reported for cultured short nose sturgeon while the cholesterol level lie within the reported range (total proteins - 2.7 - 5.3 g/dl, albumin- 0.8 - 1.7 g/dl, globulin - 1.80 - 3.70 g/dl, calcium - 6.60 - 12.10 mg/dl, AST- 90.00 – 311.00 IU/L, ALP – 47.00 – 497.00 IU/L, plasma glucose- 37.00 - 74.00 mg/dl and cholesterol - 42.00 - 133.00 mg/dl) (Knowles *et al.,* 2006)

Species, age, sex, stress and different management systems (Hubrec *et al.,* 2000; Gabriel *et al.*, 2004; Owolabi, 2011; de Souza Neves *et al.,* 2014) had been reported to have effects on haematological and blood biochemical values of fishes.

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