

Reference Intervals for the Serum Biochemistry and Lipid Profile of Male Broodstock African Catfish (*Clarias gariepinus*: Burchell, 1822) at Varied Ages

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Abstract

The study established reference intervals and age variations in the serum biochemistry and lipid profile of cultured male broodstock African catfish. The evaluations were carried out on twenty-five (25) cultured male broodstock African catfish (*Clarias gariepinus*) of 6, 8, 9, 10 and 12 months of age. Standard biochemical procedures were carried out in all the serum biochemistry and lipid profile determinations. The overall mean \pm SD values for the serum biochemistry parameters and lipid profile were as follows: alanine aminotransferase 17.49 ± 8.30 U/L; aspartate aminotransferase 125.76 ± 28.59 U/L; alkaline phosphatase 12.42 ± 2.56 U/L; total proteins 3.70 ± 1.49 g/dl; albumin 1.60 ± 0.30 g/dl; globulin 2.10 ± 1.38 g/dl; albumin/globulin 1.53 ± 1.71 ; total bilirubin 1.71 ± 0.51 mg/dl; direct bilirubin 0.36 ± 0.43 mg/dl; indirect bilirubin 1.35 ± 0.52 mg/dl; creatinine 1.13 ± 0.64 mg/dl; blood urea nitrogen 2.18 ± 2.47 mg/dl; total cholesterol 128.18 ± 40.22 mg/dl; HDL-C 26.36 ± 3.73 mg/dl; LDL-C 75.44 ± 9.30 mg/dl; VLDL-C 32.00 ± 2.03 mg/dl and triglyceride 160.00 ± 10.15 mg/dl. The results showed significant ($P < 0.05$) variations in all the serum biochemistry parameters and lipid profile of the studied fishes, across the different age groups except in the serum alkaline phosphatase activity. Therefore, the current report offers information that shall be useful to all carrying out experimental studies involving assay of these parameters in male broodstock African catfish.

Keywords: serum biochemistry, serum lipid profile, male, broodstock, *Clarias gariepinus*

Introduction

Changes in blood biochemical profile reflect changes in the metabolism and biochemical processes of the internal milieu as well as the whole fish (Edsall, 1999). Thus, analyses of the blood biochemical profile could be of diagnostic importance in the evaluation of fish health. The blood biochemistry of apparently healthy fish is affected by season, sex, age, stressors and the environment (Hrubec *et al.*, 2000; Braun *et al.*, 2010; Acharya and Mohanty, 2014). Also, reproduction is one of the major factors that influence fish physiology (Nicula *et al.*, 2010; Nasari *et al.*, 2014), thus there is need to evaluate such variations. However, analysis

of biochemical profile of the fish is only bioindicative (Luskova, 1998) and not absolute, due to environmental, management, climatic variables, as well as differences in methodology. The need for prudent management and enhancement of the natural fish biodiversity necessitates establishment of the normal physiologic and serum biochemical values (Swanson *et al.*, 1996; Shahsavani *et al.*, 2010; Guzman *et al.*, 2015) under different climatic and management conditions. These will form the bases for health assessment and diagnoses (Stoskopf, 1993) and a solid base in selecting the healthiest broodstock, application, as well as development of assisted reproductive technologies for spawning in catfish hatcheries (Guzman *et al.*, 2015).

Reproduction being an intemperance, warrants fisheries to employ only broodstock fish in the best of health for spawning (Roberts, 2004). Spawning is a stress factor to fish, thus there is need to evaluate the serum biochemistry and lipid profile with a view to determining the healthiest individual for the best possible spawning outcome (Kocaman *et al.*, 2005). Furthermore, attainment of sexual maturity and selection of apparently healthy broodstock fish based on physical examination alone does not imply that the fish is healthy. Therefore, the need to ascertain beyond attainment of sexual maturity is of high interest, as well as to determine the best age of broodstock fish to be used as spawning candidates (Guzman *et al.*, 2015) based on their serum biochemistry and serum lipid profile (Nasari *et al.*, 2014).

Paucity of information in available literature on serum biochemistry and serum lipid profile of male broodstock catfish necessitated this study which evaluated age variations in the serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) activities, as well as serum levels of total proteins, globulin, albumin, albumin-globulin ratio, total bilirubin, direct bilirubin, indirect bilirubin, creatinine, urea nitrogen, cholesterol, high density lipoprotein-cholesterol, very low density lipoprotein, low density lipoprotein cholesterol and triglyceride of 6, 8, 9, 10, 12 months old pond-cultured male broodstock African catfish.

Materials and Methods

A total of twenty-five (25) apparently healthy male cultured broodstock African sharptoothed catfish (*Clarias fariatus*) were used for the study. The fish were stocked in concrete ponds according to age at a stocking density of 750 per $12 \times 12 \times 1 \text{ m}^2$ and were point-fed fish feed (Vital, GCOML, Jos, Nigeria). Oguta is located within the tropical rain forest zone on 5.71° North and 6.81° East. The area lies about 121 meters above sea level, with average temperature of 34°C and 75% humidity for the month of March (FMNAR, 2005). The mean weight \pm SEM of the fishes were as follows: 6 months ($0.967 \pm 0.06 \text{ kg}$); 8 months ($1.288 \pm 0.051 \text{ kg}$); 9 months ($1.767 \pm 0.033 \text{ kg}$); 10 months ($1.933 \pm 0.033 \text{ kg}$) and 12 months ($2.025 \pm 0.025 \text{ kg}$). Five fishes were randomly selected from each of 6, 8, 9, 10 and 12 months age groups, thus having a final number of 25 male catfish for the investigation.

Sample collection and analyses

Five milliliters (5 ml) of blood were collected via the caudal abdominal vein from which serum samples were harvested by centrifugation at $10,000 \times g$ for 10 minutes; after, clotting had been allowed for one hour. The serum samples were used immediately for all the analyses following standard procedures. The serum samples were not stored. The serum biochemistry determinations were done using Quimica Clinica Aplicada (QCA) test kits (QCA, Spain) and a digital colorimeter (Labtech, India). The serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined by the Reitman-Frankel method (Reitman and Frankel, 1957). The serum alkaline phosphatase (ALP) activity was determined by the phenolphthalein monophosphate method (Klein *et al.*, 1960; Babson *et al.*, 1966). The serum total protein was determined by the direct Biuret method (Lubran, 1978). The serum globulin was determined by subtracting the value of the serum albumin from the total protein value

(Collville, 2002). The serum albumin was determined by the Bromocresol green method (Dumas *et al.*, 1971) and total bilirubin, direct and indirect bilirubins were determined by the Jendrassik-Grof method (Dumas *et al.*, 1973) respectively. The serum creatinine was determined by the modified Jaffe method (Blass *et al.*, 1974), while the blood urea nitrogen was determined by the modified Berthelot-Searcy method (Fawcett and Scott, 1960). Determination of serum cholesterol was done following the cholesterol oxidase - peroxidase (CHOD-POD) method (Allain *et al.*, 1974). Determination of serum high density lipoprotein-cholesterol (HDL-C) was done by the dextran sulphate-magnesium (II) method (Albers *et al.*, 1978), while the serum very low density lipoprotein cholesterol (VLDL-C) was calculated by dividing the serum triglyceride by 5 (Friedewald *et al.*, 1972). The serum low density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula (Friedewald *et al.*, 1972; Warnick *et al.*, 1990). The serum triglyceride was determined by glycerol phosphate oxidase enzymatic method (Bucolo and David, 1973).

Ethics

The fishes were handled in accordance with the Ethics and Regulation Guiding the Use of Research Animals as approved by the Faculty of Veterinary Medicine, University of Nigeria, Nsukka.

Statistical analyses

Data for all the determinations were presented as mean \pm standard deviation (SD). The resultant data for the different age groups were subjected to one-way analysis of variance (ANOVA). The statistical analyses were done using SPSS 16.0 (for Windows SPSS 16.0 Inc., Chicago, IL, USA). The variant means were separated using Duncan's multiple range test and significant differences were accepted at probability level less than 0.05 ($P < 0.05$).

Results

The overall mean serum alanine aminotransferase (ALT) activity of the fishes studied was $17.49 \pm 8.30 \text{ U/L}$, with the minimum and maximum values of 6.44 and 36.43 U/L, respectively (Table 1). The 12 months age group had significantly ($P < 0.05$) lower serum ALT than that of all the other age groups (Table 2). There was no significant ($P > 0.05$) variation in the mean serum ALT activity of the 6, 8 and 10 months age groups, but that of the 9 months age group was significantly ($P < 0.05$) higher than all the other age groups (Table 2). The overall mean serum aspartate aminotransferase (AST) activity of all ages was $125.76 \pm 28.59 \text{ U/L}$ (61.85 - 159.48) (Table 1), with the 6 months age group having significantly ($P < 0.05$) higher serum AST than that of the other age groups. There was no significant ($P > 0.05$) difference in the mean serum AST activity of the 8, 9 and 10 months age groups, but that of the 12 months age group was significantly ($P < 0.05$) lower than that of all the other age groups (Table 2). The overall mean serum alkaline phosphatase (ALP) activity was $12.42 \pm 2.56 \text{ U/L}$ (8.33-20.00). There was no significant ($P > 0.05$) variation in the mean serum ALP activity across the different age groups (Table 2).

The overall mean serum total protein and globulin levels were $3.70 \pm 1.49 \text{ g/dl}$ (0.99-6.94) and $2.10 \pm 1.38 \text{ g/dl}$ (0.16 -

5.41) respectively (Table 1). The mean serum total protein and globulin levels of the 8 months age group were significantly ($P < 0.05$) lower than that of the other age groups, but there was no significant ($P > 0.05$) variation between that of the 6 and 9 months age groups (Table 2). However, the mean serum total proteins and globulin levels of the 6 and 9 months age groups were significantly ($P < 0.05$) lower than those of the 10 and 12 months age groups, but that of the 12 months age group was significantly ($P < 0.05$) higher than that of the 10 months age group (Table 2).

The overall mean serum albumin level was 1.60 ± 0.30 g/dl, with the minimum and maximum values of 0.83 and 2.08 g/dl respectively (Table 1). There was no significant ($P > 0.05$) variation in the mean serum albumin levels of the 6 and 8 months age groups, but they were significantly ($P < 0.05$) lower than that of the other age groups (Table 2). However, there was no significant ($P > 0.05$) variation in the mean serum albumin levels of the 9, 10 and 12 months age groups (Table 2). The overall mean value of the serum albumin: globulin ratio (A/G) was 1.53 ± 1.71 (0.28 - 5.39). The mean serum A/G of the 6 months age group did not vary significantly ($P > 0.05$) when compared to that of the 9 and 10 months age groups, whereas that of the 9 months age group was significantly ($P < 0.05$) higher than that of the 10 months age group (Table 2). The mean value of the serum A/G of the 8 months age group was significantly ($P < 0.05$) higher than that of all the other groups, while that of the 12 months age group was significantly ($P < 0.05$) lower than that of all the other groups (Table 2).

The overall mean total bilirubin level was 1.71 ± 0.51 mg/dl (0.86 - 2.59) (Table 1), direct bilirubin level was 0.36 ± 0.43 mg/dl (0.07 - 1.58) and indirect bilirubin level was 1.35 ± 0.52 (0.28 - 2.09). The mean serum total bilirubin level of the 6, 8 and 10 months age groups did not vary ($P > 0.05$) significantly, but they were significantly ($P < 0.05$) higher than that of the 9 and 12 months age groups (Table 2). There were no significant ($P > 0.05$) variations in the mean serum direct bilirubin of the 6 and 12 months age groups, but these were significantly ($P < 0.05$) lower than that of the 8, 9 and 10 months age groups and there was no ($P > 0.05$) significant variation amongst these. The mean serum indirect bilirubin of the 6, 8 and 12 months age groups was significantly ($P < 0.05$) higher than that of the 9 and 10 months age groups, but there was no significant ($P > 0.05$) variation in the mean serum indirect bilirubin of the 6, 8 and 12 months age groups (Table 2).

The overall mean serum creatinine and blood urea nitrogen values, with their minimum and maximum values in brackets

were 1.13 ± 0.64 mg/dl (0.51 - 3.08) and 2.18 ± 2.47 mg/dl (1.04 - 8.30) respectively (Table 1). There was no significant ($P > 0.05$) variation in the mean serum creatinine level of the 6 and 9 months age groups, while their values were significantly ($P < 0.05$) higher than those of the other groups (Table 2). The serum creatinine level of 9 months age group was not significantly ($P > 0.05$) different from that of the 8 months age group, but that of the 10 and 12 months age groups were significantly ($P < 0.05$) lower than those of the other age groups (Table 2). The mean blood urea nitrogen value of the 12 months age groups was significantly ($P < 0.05$) higher than that of the 8 and 10 months age groups, but there were no significant ($P > 0.05$) variations in the mean urea nitrogen of the 6, 8, 9 and 10 months age groups (Table 2).

The overall mean values, with the minimum and maximum values for the serum lipid profile were as follows: total cholesterol – 128.18 ± 40.22 mg/dl (54.55 - 200.00); high density lipoprotein-C – 26.36 ± 3.73 (9.09 - 72.73); low density lipoprotein – 75.44 ± 9.30 mg/dl (2.42 - 155.15); very low density lipoprotein – 32.00 ± 2.03 mg/dl (13.33 - 40.00) and triglyceride – 160.00 ± 10.15 (66.67 - 200.00) respectively (Table 3). There were no significant ($P > 0.05$) variations in the mean serum total cholesterol value of the 6, 8 and 10 months age groups, but the mean serum total cholesterol value of the 6 and 10 months age groups were significantly ($P < 0.05$) lower than those of the other age groups (Table 4). There was no significant ($P > 0.05$) variation in the mean serum total cholesterol values of the 8, 9 and 12 months age groups (Table 4).

The mean serum high density lipoprotein cholesterol value of the 6, 9, 10 and 12 months age groups were significantly ($P < 0.05$) lower than that of the 8 months, but that of 6 months was significantly ($P < 0.05$) higher than that of 9 months; however, there were no significant variations ($P > 0.05$) in the values of the mean serum high density lipoprotein cholesterol of the 6, 10 and 12 months (Table 4). The mean serum low density lipoprotein cholesterol value of the 6 months age group was significantly lower ($P < 0.05$) than the other groups, but there were no significant variations ($P > 0.05$) in those of 8, 9 and 10 month age groups; however, that of 12 months age group was significantly higher ($P < 0.05$) than that of the 10 months age groups (Table 4). There was no significant variation ($P > 0.05$) in the means of the serum very low density lipoprotein cholesterol and triglyceride of the 6, 8 and 10 months age groups; however, that of 8 and 10 months age groups were significantly lower ($P < 0.05$) than that of 9 and 12

Table 1. The serum biochemistry profile of the broodstock African catfish (*Clarias gariepinus*)

Serum biochemistry parameters	Means \pm SD (n = 25)	Minimum and maximum values
Alanine aminotransferase (U/L)	17.49 ± 8.30	6.44 – 36.43
Aspartate aminotransferase (U/L)	125.76 ± 28.59	61.85 – 159.48
Alkaline phosphatase (U/L)	12.42 ± 2.56	8.33 – 20.00
Total proteins (g/dl)	3.70 ± 1.49	0.99 – 6.94
Albumin (g/dl)	1.60 ± 0.30	0.83 - 2.08
Globulin (g/dl)	2.10 ± 1.38	0.16 – 5.41
Albumin/Globulin	1.53 ± 1.71	0.28 – 5.39
Total bilirubin (mg/dl)	1.71 ± 0.51	0.86 – 2.59
Direct bilirubin (mg/dl)	0.36 ± 0.43	0.07 – 1.58
Indirect bilirubin (mg/dl)	1.35 ± 0.52	0.28 – 2.09
Creatinine (mg/dl)	1.13 ± 0.64	0.51 – 3.08
Blood urea nitrogen (mg/dl)	2.18 ± 2.47	1.04 – 8.30

Table 2. Age differences in the serum biochemistry profile of broodstock African catfish (*Clarias gariepinus*)

Serum biochemistry parameters	Means \pm SD, with minimum and maximum values in bracket				
	6 months (n = 5)	8 months (n = 5)	9 months (n = 5)	10 months (n = 5)	12 months (n = 5)
ALT (U/L)	17.69 \pm 4.79 ^a (11.44–21.44)	15.19 \pm 4.79 ^a (11.44–21.44)	28.94 \pm 8.65 ^b (21.44–36.43)	16.44 \pm 4.08 ^a (11.44–21.44)	9.18 \pm 4.86 ^c (6.44–16.44)
AST (U/L)	154.55 \pm 3.34 ^a (152.5–159.48)	133.33 \pm 17.44 ^b (110.66–152.51)	129.61 \pm 10.62 ^b (123.69–145.53)	133.33 \pm 11.91 ^b (117.64–145.53)	78.00 \pm 16.98 ^c (61.85–96.72)
ALP (U/L)	12.92 \pm 2.10 ^a (10.00–15.00)	11.67 \pm 2.72 ^a (8.33–15.00)	13.75 \pm 4.17 ^a (11.67–20.00)	11.25 \pm 1.59 ^a (10.00–13.33)	12.50 \pm 2.15 ^a (10.00–15.00)
Total proteins (g/dl)	2.98 \pm 0.81 ^a (1.98–3.97)	1.98 \pm 0.81 ^b (0.99–2.98)	3.48 \pm 0.57 ^a (2.98–3.97)	4.47 \pm 0.57 ^c (3.97–4.96)	5.58 \pm 1.36 ^d (3.97–6.94)
Albumin (g/dl)	1.36 \pm 0.13 ^a (1.25–1.53)	1.39 \pm 0.40 ^a (0.83–1.67)	1.77 \pm 0.20 ^b (1.53–1.94)	1.77 \pm 0.23 ^b (1.53–2.08)	1.74 \pm 0.28 ^b (1.39–1.94)
Globulin (g/dl)	1.62 \pm 0.88 ^a (0.59–2.72)	0.59 \pm 0.67 ^b (0.16–1.59)	1.71 \pm 0.64 ^a (1.04–2.44)	2.69 \pm 0.65 ^c (1.89–3.29)	3.88 \pm 1.31 ^d (2.58–5.41)
A/G	1.15 \pm 0.84 ^{ac} (0.46–2.36)	4.21 \pm 2.23 ^b (0.87–5.39)	1.13 \pm 0.57 ^a (0.63–1.87)	0.70 \pm 0.27 ^c (0.51–1.10)	0.47 \pm 0.15 ^d (0.28–0.64)
Total bilirubin (mg/dl)	1.73 \pm 0.50 ^a (1.30–2.16)	2.05 \pm 0.41 ^a (1.73–2.59)	1.19 \pm 0.42 ^b (0.86–1.73)	1.95 \pm 0.56 ^a (1.30–2.59)	1.02 \pm 0.41 ^b (1.30–2.16)
Direct bilirubin (mg/dl)	0.09 \pm 0.04 ^a (0.07–0.14)	0.34 \pm 0.45 ^b (0.07–1.01)	0.47 \pm 0.14 ^b (0.29–0.58)	0.77 \pm 0.71 ^b (0.07–1.58)	0.14 \pm 0.10 ^a (0.07–0.29)
Indirect bilirubin (mg/dl)	1.64 \pm 0.48 ^a (1.23–2.09)	1.71 \pm 0.21 ^a (1.58–2.02)	0.72 \pm 0.52 ^b (0.28–1.30)	1.17 \pm 0.41 ^b (0.58–1.44)	1.48 \pm 0.31 ^a (1.23–1.87)
Creatinine (mg/dl)	1.67 \pm 0.49 ^a (1.03–2.05)	1.03 \pm 0.00 ^b (1.03–1.03)	1.54 \pm 1.03 ^{ab} (1.03–3.08)	0.77 \pm 0.30 ^c (0.51–1.03)	0.64 \pm 0.26 ^c (0.51–1.03)
BUN (mg/dl)	2.34 \pm 2.60 ^{ab} (1.04–6.23)	1.04 \pm 0.00 ^a (1.04–1.04)	1.82 \pm 1.56 ^{ab} (1.04–4.15)	1.04 \pm 0.00 ^a (1.04–1.04)	4.67 \pm 4.19 ^b (1.04–8.30)

a,b,c,ab,ac: Different superscripts in a row indicate significant differences across the groups ($P < 0.05$).

months age groups, while that of the 12 months was significantly higher ($P < 0.05$) than that of 6 months, but there were no significant variations ($P < 0.05$) in that of the 9 and 12 months age groups (Table 4).

Discussion

The significant reduction in the serum aspartate aminotransferase (AST) activity from 6 months through 8, 9 and 10 months, with the 6 and 12 months age group having the highest and lowest serum AST activity respectively, and 9 and 12 months age group having the highest and lowest ALT activity respectively, was not in agreement with Akrami *et al.* (2013) and Kuzminova *et al.* (2014) who reported an age-related increase in both ALT and AST activities in the *Huso huso* and *Trachurus mediterraneus*. The ALT and AST activities observed in the current study were both lower than that reported by Bello *et al.* (2014) and Okorie-Kanu and Unakalamba (2014) for *C. gariepinus* and *Heterobranchius longifilis* in Nigeria. The lack of significant variation in the activity of serum ALP across the age groups was not clearly understood, but it may be due to how close the ages of the fishes were. Also, it may be attributed to the similarity in the rate of breakdown of energy reserves which is used for growth (Ghosh *et al.*, 2008) or similarity in biliary canal or osteoblastic

activity (Akrami *et al.*, 2013). This is in contrast to that reported in domestic animals (Stockham and Scott, 2008; Ihedioha and Agina, 2013) as young ones are known to have higher serum ALP activity attributed to high osteoblastic activity.

From 9 months of age, there was an age-related increase in the serum total proteins of male *Clarias gariepinus* broodstock. This finding is supported by the reports of increase in the total protein levels of Indian carp (Das, 1964) and hybrid striped bass (Hrubecek *et al.*, 2001) with age. Increase in the serum total protein level is primarily due to the influx of colloidal proteins from extravascular sources into the blood (Kori-Siakpere *et al.*, 2011) or a reduction in deamination capacity of the liver, as a result of reduced aminotransferase activity, consequent upon structural alterations (Burtis and Ashwood, 1996). The observed increase in the total protein is in tandem with a decrease in the AST and ALT, especially at 12 months of age. Total protein level determination is of great diagnostic importance in evaluating the physiological status and condition of fish (Svetina *et al.*, 2002), as these proteins (albumin and globulin) play significant role in immune response (Wiegertjes *et al.*, 1996). Also, the increase in total protein and globulin particularly are associated with better innate response in fish (Acharya and Mohanty, 2014).

Table 3. The serum lipid profile of broodstock African catfish (*Clarias gariepinus*)

Parameters	Means \pm SD	Minimum and maximum values
Total Cholesterol (mg/dl)	128.18 \pm 40.22	54.55 – 200.00
HDL-C (mg/dl)	26.36 \pm 3.73	9.09 – 72.73
LDL-C (mg/dl)	75.44 \pm 9.30	2.42 – 155.15
VLDL-C (mg/dl)	32.00 \pm 2.03	13.33 – 40.00
Triglyceride (mg/dl)	160.00 \pm 10.15	66.67 – 200

Table 4. Age differences in the serum lipid profile of broodstock African catfish (*Clarias gariepinus*)

Parameters	Means \pm SD, with minimum and maximum values in bracket				
	6 months (n = 5)	8 months (n = 5)	9 months (n = 5)	10 months (n = 5)	12 months (n = 5)
T chol. (mg/dl)	90.91 \pm 25.71 ^a (54.55 – 109.09)	127.27 \pm 51.43 ^{ab} (90.90-200.00)	150.00 \pm 27.27 ^b (127.27-181.82)	118.18 \pm 23.47 ^a (90.90 -145.45)	154.54 \pm 45.76 ^b (90.90-200.00)
HDL-C (mg/dl)	27.27 \pm 10.50 ^a (18.18 – 36.36)	50.00 \pm 17.41 ^b (36.36 – 72.73)	15.91 \pm 4.55 ^c (9.09 – 18.18)	18.18 \pm 12.86 ^{ac} (9.09-36.36)	20.45 \pm 11.43 ^{ac} (9.09-36.36)
LDL-C (mg/dl)	14.01 \pm 11.23 ^a (2.42 – 24.24)	84.69 \pm 49.45 ^{bc} (46.05 -155.15)	92.88 \pm 27.52 ^{bc} (69.09 – 118.79)	73.33 \pm 13.45 ^b (59.39 – 91.51)	112.30 \pm 8.71 ^c (105.46-123.64)
VLDL-C (mg/dl)	30.00 \pm 12.77 ^{ab} (13.33 – 40.00)	26.67 \pm 0.00 ^a (26.67 – 26.67)	36.67 \pm 6.67 ^{bc} (26.67 – 40.00)	26.67 \pm 10.89 ^a (13.33 - 40.00)	40.00 \pm 0.00 ^c (40.00 – 40.00)
Triglyceride (mg/dl)	150.00 \pm 63.83 ^{ab} (66.67 – 200.00)	133.33 \pm 0.00 ^a (133.33-133.33)	183.33 \pm 33.34 ^{bc} (133.33 – 200.00)	133.33 \pm 54.43 ^a (66.67-200.00)	200.00 \pm 0.00 ^c (200.00 -200.00)

a,b,c,ab,ac
Different superscripts in a row indicate significant differences across the groups (P < 0.05).

The increase in the albumin levels of the 9, 10 and 12 months of age as compared to the 6 and 8 months of age suggest some age-related increase, unlike that of globulin, which mirrored the total protein level across the groups. The globulin level observed in the hereby study closely approximates that reported by Bello *et al.* (2014) for *C. gariepinus*, but the albumin obtained in the current study was higher than theirs. Albumin and globulin are two important proteins, and changes in these parameters affect the level of total proteins (Shahsavani *et al.*, 2010). Fedonenko *et al.* (2016) reported that albumin is the main fraction of total protein, accounting for up to 55%. Albumin in fish blood transports lipids (Acharya and Mohanty, 2014) and steroid hormones (Shahsavani *et al.*, 2010). The A/G ratio reported by Omitoyin (2007) and Bello *et al.* (2014) for *C. gariepinus* were lower than that observed in this study. Alterations in the albumin-globulin (A/G) ratio is an indication of poorer liver function or a sign of increased protein loss due to impaired gill function (Omitoyin, 2007); thus lower A/G ratio might be an indication of better immune response. Therefore, the 10 and 12 months old fish may have better immune potentials compared to other ages, especially the 8 months old. This result also collaborates the finding of the serum globulin. Total bilirubin, direct and indirect bilirubin is helpful when evaluating liver function or hemolysis caused by chemical intoxication in aquatic bodies. The finding of a significantly lower total bilirubin and direct bilirubin in the 12 month age group was not clearly understood.

The significantly higher creatinine levels of the 6 and 9 months compared to the 10 and 12 months old in the present study could be attributed to age-related variations in the creatinine clearance by the kidneys (Adamu and Kori-Siakpere, 2011). The mean creatinine value obtained hereby was similar to that reported by Omitoyin (2007) for *C. gariepinus*. The blood urea nitrogen level of the 12 month old fishes was the highest and was significantly higher than that of the 8 and 10 months age groups. This could possibly be attributed to age-related variations in the synthetic capability of the liver and clearance by the kidneys. High serum urea nitrogen level in the 12 month old may be attributed to slow conversion of ammonia to urea by the liver and reduced clearance from blood, which reflects ageing-related impairment of renal function as observed in domestic animals (Ihedioha and Agina, 2013). Fluctuations in serum urea and creatinine indicate declining liver function or impairment of gill osmoregulatory capacity (Zhou *et al.*, 2009). High blood

urea nitrogen is related to liver or gill dysfunction (Nicula *et al.*, 2010). The mean blood urea nitrogen level in the current study was lower than that reported by Omitoyin (2007).

Lipid stores represent major energy reserves in fish, and during sexual maturation they are mobilized and directed from previously stored tissue to gonads, in order to sustain their development (Wallaert and Babin, 1994; Nicula *et al.*, 2010). Lipoproteins function in fish for lipid transport (Babin and Vernier, 1989). Hill (1982) reported that cholesterol concentration increases as the fish size increases. The findings of the current study showed that, apart from the 10 months age group, cholesterol levels increased with age, even though the increase was not statistically significant. However, the cholesterol levels of the 9 and 12 months age groups were significantly higher than that of 6 months age group. Total cholesterol level is associated with disease resistance in fish (Maita *et al.*, 1998), thus it is an important diagnostic tool in this specie. The elevated serum cholesterol levels may be due to mobilization of stored cholesterol from tissue or due to its decrease conversion into gonadal steroids (Singh and Singh, 1979). The absence of size-related increase in cholesterol may be due to the fact that the fish had all attained sexual maturity and also the fish were pond-cultured and so did not exhibit natural tendency towards seasonal breeding. However, there was no significant variation in the cholesterol concentrations with regards to age, season and body condition as reported by Svetina *et al.* (2002) in young carp. The mean cholesterol levels observed in this study was similar to that reported by for *C. gariepinus* (Omitoyin, 2007), lower than that reported for *C. batrachus* (Acharya and Mohanty, 2014), trout (Kocaman *et al.*, 2005) and Chub (Aras *et al.*, 2008).

High levels of serum triglycerides may be due to the elevated metabolic rate (Aras *et al.*, 2008; Nicula *et al.*, 2010) which may explain the significantly higher serum triglyceride levels of the 9 and 12 months compared to the 8 and 10 months old African catfish in the present study. The mean triglyceride levels observed hereby was higher than that reported by Omitoyin (2007) for African catfish, but within the range reported by Aras *et al.* (2008) for chub. Very low density lipoprotein cholesterol (VLDL-C) are believed to be components of the innate, non-adaptive immune defence system, thus decline in VLDL-C leads to immune suppression (Javed and Usmani, 2015). Therefore, the significantly lower VLDL-C of the 8 and 10 months old catfish in this study might suggest poorer immune status

compared to the other age groups. The mean serum VLDL-C and LDL levels observed in this study are within the range reported by Aras *et al.* (2008) for chub, but lower than the levels reported by Kocaman *et al.* (2005) for trout. The mean HDL for catfish in this study was lower than those reported by Aras *et al.* (2008) for chub and Kocaman *et al.* (2005) for trout.

Conclusions

Based on the findings of the current study, it was concluded that serum AST and ALT activities, as well as creatinine concentration decreases with age, while blood urea nitrogen increased with age. The low density lipoprotein cholesterol level of the 6 months age group was lower, while the high density lipoprotein level of the 8 months age group was higher than the other age groups. Also, no age-related variation was reported in the serum ALP activity of male broodstock African catfish. Based on the above observations, younger male broodstock fish may be better candidates for spawning than older ones and the 8 months males may be the healthiest candidates for spawning purposes. Thus, male broodstock fish for spawning should not be selected solely based on age. However, there is need to ascertain beyond just the serum biochemical and lipid profiles, the best reproductive profile/potentials of aged catfish broodstock candidates for optimal reproductive output.

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