



Available Online at EScience Press

Journal of Zoo Biology

ISSN: 2706-9761 (Online), 2706-9753 (Print)
<https://esciencepress.net/journals/JZB>

BLOOD BIOCHEMICAL OF NILE CROCODILE (*CROCODYLUS NILOTICUS*) IN KANO ZOOLOGICAL GARDEN, NIGERIA

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ABSTRACT

The potential application of blood reference range for crocodile is a basis that can provide important clinical information about health and physiological condition of the animal. This study investigates serum biochemistry of Nile crocodile from Kano Zoological Garden, Kano, Nigeria. Six (6) adult Nile crocodile (*Crocodylus niloticus*) were captured from crocodile pond in the zoo. Blood was collected from post-occipital sinus of the physically restrain crocodile and used for serum biochemical parameters. The results revealed the Total Serum Protein (TSP) concentration of 9.2g/l, albumin concentration which is a common plasma protein is 43g/l while globulin concentration is 54g/l. Cholesterol concentration measure is registered at 5.2mmol/l with High-Density Lipoprotein (HDL) and Low-Density Lipoprotein (LDL) of 1mmol/l and 1.35mmol/l respectively. Creatinine: a breakdown product of creatinine which is an important part of muscle tissue is 44umol/l. Uric acid which is a primary catabolic end product of protein is 0.18mmol/l while glucose and triglyceride are 4.94mmol/l and 2.24mmol/l respectively while enzymes which include Alanine aminotransferase (ALT) concentration is 6U/l, Aspartate aminotransferase concentration is 5U/l while Alkaline Phosphatase is 20U/l. The biochemical values recorded were compared with available data on farm Nile crocodile. Clearly, nutritional status, age, gender, season, physiology and environment should be considered if clinical pathology is to be employed as a diagnostic tool.

Keywords: Biochemical; clinical information; plasma enzymes; physiological.

INTRODUCTION

Blood profile studies in wildlife or captive crocodiles are being carried out for scientific, educational or commercial reasons; they are being applied to conservation or reproduction projects, such as to skin and meat exploitation (Oliveira, 2004). Blood analysis is a relatively noninvasive method that can provide important clinical information about the health and physiological condition of animals (Stein, 1996).

Success in animal disease control and prevention depend greatly on a precise and rapid diagnosis. This is a key to improve the crocodile production industry. Among several parameters, blood biochemical is definitely necessary for assisting clinicians to proximate the status of the animal body (Kanchanapangka *et al.*, 1999) and because of the difficulty in obtaining meaningful reference interval for each species of reptile (Campbell, 2006), decision level are often use where assessing

reptilians.

Conditions detected through evaluation of blood biochemistry and haematology of reptile includes anaemia, inflammatory disease, parasitemias, hematopoietic disorders and haemostatic alterations (Campbell, 2006). According to Campbell (2006), normal hematologic values for reptiles (including crocodilians), as determined by different laboratories, vary significantly due to differences in blood sampling, handling, analytic techniques, difference in environmental condition of the reptiles habitat, physiologic status of the reptile, its age gender and nutrition and the use of anaesthetics.

Development in the diagnosis of the crocodile diseases has not gone far enough to a satisfying level. Additional information concerning the morphological and physiological characteristics of crocodilian blood profile is needed to make a successful differential diagnosis and disease monitoring (Kanchanapangka *et al.*, 1999).

Knowledge of blood biochemical and their physiological variations are incomplete and controversial in captive Nile crocodile. Lack of uniformity on biochemical denominations, such as confusion caused by the different species affinity is frequently verified in publications.

MATERIALS AND METHODS

Study area: Established in 1972, Kano Zoological Garden, Kano State covers a total area of 40.47 ha. It is located 3km away from the old city of Kano nationally renowned for its tourist attraction and lies between latitudes 11°58'4"N and longitude 8°31'32"E. The Zoo currently had 58 different species, consisting of 300 individual animals which includes; four Lions, eight Hyena (striped and spotted), two adult Ostriches, Elands, Elephant, Giraffes, Hippopotamus, Horney badger, Duikers, Bushbuck, Baboons, Jackal, Civet cat, Buffalo, Chimpanzee, Monkeys, porcupines, Gazelles Zebra, Warthogs, Horse, Pythons, Crocodiles, Crane crown, Marabus stock, Geese, Peacock *etc.*. It was designed to conserve animals for people to see, render recreational services, serve as a research centre and provide practical educational instructions. Kano Zoo was the second of its kind in West Africa after Cote d'Ivoire and was accredited by the Pan-African Association of Zoos and Aquaria (PAAZA) (Lawan, 2011).

Crocodilians House: The enclosure is approximately 100 x 40 (triangular) square meters designed with a circular concrete pond of approximately 1.2m height. The fence has a combination of material chain link fencing of the enclosure is properly constructed to prevent escape. This is achieved with an extension concrete above and below ground, as crocodiles can be excellent triggers and climbers. Solid cement wall is used for the circular enclosure while the chain is used to link extension below to provide bush viewing and improved safety.

Fence height is 0.5m with a turn back at the top to force a climbing animal to fall.

A Shallow concrete pool, with drain system and pump for changing water is within. The water area covers 20% of the total enclosure, leaving enough dry land area for all specimens to basking.

Materials: The material used for the study includes experimental animal (Nile crocodile), Rope or line, Hand tower (10 pieces), Weighing Balance, 21G needle, 5ml syringe, 14 ordinary sample bottle, thermometer, flexible measuring tape, writing material (field notebook, pen, biro and marker).

Methodology: All crocodile was starved for two days

before sample collection.

All crocodiles caught were physically restrained without the use of narcotics and were released within 15 minutes of being caught.

Once captured and restrained, blood samples ranging between 3 and 5 ml each were collected from the post-occipital venous sinus, dorsal midline and just caudal to the base of the head using a 21 gauge needle and a 5ml syringe as described by Guillette *et al.* (1996) and Millan *et al.* (1997). The volume of blood collected depended on the size of the animal. All blood collected was immediately transferred to blood tubes and kept cool with ice packs. Samples were centrifuged at the end of each evening and plasma samples placed in Cryovials and frozen in liquid nitrogen until analyzed in the Clinical Pathology Laboratory of General Hospital, Kano. Blood samples were only collected from living healthy animals and all animals found with a wound or look unhealthy in the study area were intentionally disregarded and did not form any part of the study.

Blood samples were analyzed for Total Serum Protein (TSP), Albumin, Globulin, Glucose, Cholesterol, Creatinine, Uric Acid, Triglycerides, High density lipoprotein (HDL), Lower density lipoprotein (LDL), and plasma enzymes: Alanine transaminase (ALT), Alkaline phosphatase (ALP), Aspartate aminotransferase (AST),

Blood biochemistry analysis: Biochemical analyses were done using a Next/Vertex Alfa Wassermann Analyzer (Alfa Wassermann B.V., Woerden, The Netherlands). Total protein was determined using a modified Weichselbaum biuret method (Weichselbaum, 1946). Albumin was measured using the bromocresol green method (Cheesbrough, 2005) globulin and the albumin-globulin ratio were calculated (Johnson *et al.*, 2002) while cholesterol was determined by enzymatic methods (Abell *et al.*, 1952; Bergmeyer and Grassl, 1983), creatinine was determined by the picrate method (Cheesbrough, 2005) and uric acid by the uricase method (Bauer, 1982). Alanine aminotransferase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST) were determined by using standard IFCC methods (Bergmeyer *et al.*, 1977; Tietz *et al.*, 1983; Bergmeyer *et al.*, 1986). The glucose oxidase method (Marks, 1996) was used to determine glucose in the samples.

Statistical analysis: All values were presented descriptively. A comparison of blood biochemistry range with report previously for Nile crocodiles in the literature was drawn.

RESULTS

Blood biochemical profile of Nile crocodile in Kano Zoological Garden: The results of the biochemical analysis of the blood sample collected from six (6) Nile crocodiles in Kano Zoological garden are presented in table 1.

Total Serum Protein (TSP) concentration is 97g/l, albumin concentration which is a common plasma protein is 43g/l while globulin concentration is 54g/l. Cholesterol concentration measure is registered at 5.2mmol/l with High-Density Lipoprotein (HDL) and Low-Density Lipoprotein (LDL) of 1mmol/l and 1.35mmol/l respectively.

Creatinine: a breakdown product of creatine which is an important part of muscle tissue was 44.2umol/l. Uric acid

which is a primary catabolic end product of protein is 0.18mmol/l while glucose and triglyceride are 4.94mmol/l and 2.24mmol/l respectively.

Blood plasma enzymes of Nile crocodile in Kano Zoological Garden.

Table 2 revealed the biochemical enzymes of six (6) Nile crocodiles from the study area.

The biochemical enzymes are important tools in diagnosing the state of the animal external organs and bones.

Alanine aminotransferase (ALT) concentration is 6U/l, Aspartate aminotransferase concentration is 5U/l while Alkaline Phosphatase is 20U/l.

Table 1. Blood biochemical profile of Nile crocodile in Kano Zoological Garden.

Parameters	Value (n= 6)
Total Serum Protein TSP (g/l)	97
Albumin (g/l)	43
Globulin (g/l)	54
Cholesterol (mmol/l)	5.2
Creatinine (μ mol/l)	44.2
Uric Acid (mmol/l)	0.18
Glucose (mmol/l)	4.94
Triglycerides (mmol/l)	2.24
High-density lipoprotein (HDL) (mmol/l)	1
Lower density lipoprotein (LDL) (mmol/l)	1.35

Table 2. Blood plasma enzymes of Nile crocodile in Kano Zoological Garden.

Enzyme(s) (u/l)	Value (n=6)
Aspartate aminotransferase (AST)	5
Alanine aminotransferase (ALT)	6
Alkaline phosphatase (ALP)	20

Comparison of biochemical parameters of captive Nile crocodile in the current study and previous studies: Table 3 shows the comparison of blood biochemical with previously reported values for Nile crocodile in captivity.

Total Serum Protein (TSP), albumin and globulin concentrations of 97g/l, 43g/l and 54g/l respectively are much higher than the reported concentration for the Nile crocodile by Osagiobare (2013) in FCWM zoo of Nigeria and Foggin *et al.* (1987) in Zimbabwe farm.

Cholesterol concentration of 5.2mmol/l is also higher than 4.5mmol/l for Nile crocodile in FCWM zoo while

creatinine concentration (44.2umol/l) recorded in the current study is lower to 66umol/l by Osagiobare (2013) in FCWM Zoo. Uric acid of 0.18mmol/l is also lower to 0.43mmol/l for FCWM zoo Nile crocodile and 0.24mmol/l from the same animal in Zimbabwe Farm (Foggin, 1987). Glucose concentration (4.9mmol/l) in the study is much closer to 5.0mmol/l recorded in FCWM (Osagiobare, 2013) and 4.57mmol/l at Zimbabwe Farm (Foggin, 1987).

Triglyceride of 2.2mmol/l is substantially higher than 0.4mmol/l recorded for Nile crocodile in FCWM Zoo (Osagiobare, 2013).

Table 3. Comparison of biochemical parameters of captive Nile crocodile in the current study and previous studies.

Parameters	Current study (n=6)	Federal College of Wildlife Mgt. (FCWM) New Bussa, Nigeria (Osagiobare, 2013)	Zimbabwe farm (Foggin <i>et al.</i> , 1987)
Total Serum Protein TSP (g/l)	97	33	53
Albumin (g/l)	43	16	19
Globulin (g/l)	54	17	31
Cholesterol (mmol/l)	5.2	4.5	-
Creatinine (umol/l)	44.2	66	-
Uric Acid (mmol/l)	0.18	0.43	0.24
Glucose (mmol/l)	4.9	5.0	4.57
Triglycerides (mmol/l)	2.2	0.45	-
High density lipoprotein (HDL) (mmol/l)	0.4	--	-
Lower density lipoprotein (LDL) (mmol/l)	0.6	--	-

Comparison of plasma enzymes Nile crocodile in the current study and previous studies: Table 4.4 shows that Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) concentration of 5U/l and 6U/l respectively measured in the plasma of the Nile crocodiles in the study area were much lower than the

value recorded in the FCWM Zoo (Osagiobare, 2013). Plasma Alkaline Phosphatase (ALP) concentration of 20U/l in the study is relatively lower to the value recorded (46U/l) in FCWM zoo but higher to that of Zimbabwe Farm (16.6U/l) (Foggin, 1987).

Table 4. Comparison of plasma enzymes captive Nile crocodile in the current study and previous studies.

Enzyme(s) (u/l)	Current study(n=6)	Federal College of Wildlife Mgt. (FCWM), New Bussa, Nigeria (Osagiobare, 2013)	Zimbabwe Farm (Foggin <i>et al.</i> , 1987)
Aspartate aminotransferase (AST)	5	27	13.1
Alanine aminotransferase (ALT)	6	39	--
Alkaline phosphatase (ALP)	20	46	16.6

DISCUSSION

Blood biochemical interpretation in captive crocodile is very challenging due to the influence of variability species, nutritional status, age, gender, seasons, physiology (Thrall *et al.*, 2004; Lawrence, 1987) and environment. Though, there is paucity in publication in terms of establishing a reference range for captive Nile crocodile however, biochemical parameters seem variable when compared with previous work even in the same species within a geographical location.

High Total Serum Protein and Albumin concentration in the current study could indicate possible inflammation or other parasitism (Campbell, 1996). Elevated Globulin concentration is indicative in reptiles of altered immune activity and the presence of infections in the population

(Campbell, 2006; Thrall *et al.*, 2004).

High Cholesterol concentration could be as a result of high nutritional plane and general lack of physical activity of the captive crocodile (Padilla *et al.*, 2011). Presence of both the High-Density Lipoprotein (HDL) and Low-Density Lipoprotein (LDL) has shown that there is no deficiency of lipoprotein which could have resulted in organs dysfunctional.

Creatinine value in the study is within the range for Nile crocodile in the wild (Lovely *et al.*, 2007) but lower to the value recorded in captivity (Osagiobare, 2013). Low creatinine is a breakdown product of creatine which is an important part of muscle tissue. Low creatinine is indicative of low muscle mass. However, creatinine is not considered important for assessing renal diseases in

reptiles (Campbell, 1996).

Uric acid concentration is lower than those reported by Osagiobare (2013) and Foggin *et al.* (1987) for captive Nile crocodile but higher the value reported for wild Nile crocodile (Lovely *et al.*, 2007). Uric acid is the main nitrogenous waste product in urine and faeces of reptile, with 80 - 90% of nitrogen excreted as uric acid. The values of uric acid in most reptiles range from 0 mg/dl to 10mg/dl and values above 15mg/dl are considered high (Campbell, 1996; Frye, 1991). High values of uric acid tend to occur a day after an individual has eaten (Campbell, 1996). For Glucose, the concentration were similar with the value documented in captive Nile crocodile (Osagiobare, 2013; Foggin, 1987) and fall within the range reported by Lovely *et al.*, (2007) in free-ranging wild Nile crocodile. Glucose might be related to diet, feeding before capture and time between blood sample collections and processing (Campbell, 1996). Elevated blood glucose concentration in reptiles are often related to metabolic conditions, systemic diseases and stress associated hyperglycemia resulting from glucocorticoid and epinephrine release (Campbell, 2006; Thrall *et al.*, 2004) and this may result to pancreatitis related die-off of Nile crocodile (Botha, 2011). However, the observed in this study shows that the studied animal are neither suffering from hyperglycemia nor hypoglycemia (<200mg/dl) (Botha, 2011).

Triglyceride in this study is substantially higher than the previously reported values in Captive Nile by Osagiobare (2013) but within the range reported in free-ranging wild crocodile (Botha, 2011). Low triglyceride concentrations are indicative of malnutrition in reptiles (Campbell, 2006; Thrall *et al.*, 2004), Hence the observed values is considered normal for Nile crocodile as the recorded value is above most of the values recorded for Captive crocodile at Olifant River where it was hypothesized that animal are suffering from malnutrition (Botha, 2011).

Plasma Enzymes: Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) are serum transaminase activity. Elevation of serum levels of both enzymes can occur with states of altered hepatocellular membrane permeability. The activity of these enzymes is not related to the functioning of specific organs in Nile crocodile (Campbell, 2006) though may be useful in evaluating muscle and liver damage or degeneration [23]. In this study, AST and ALT are comparatively lower to values documented for captive Nile crocodile (Osagiobare, 2013; Foggin, 1987) and free-ranging Nile crocodile (Lovely *et*

al., 2007; Botha, 2011).

Alkaline Phosphatase (ALP) concentration in Nile crocodile from Kano Zoo were just below the concentration recorded in Nile crocodile from Federal College of Wildlife Management, New Bussa, Nigeria (Osagiobare, 2013) but above the reported concentration from Zimbabwe farm (Foggin, 1987). The observed values in this study fall within the range reported by Lovely *et al.* (2007) in free-ranging Nile crocodile Okavango Delta, Botswana. Though, these enzymes are not necessarily associated with veterinary diseases rather an implication of environmental contamination (Botha, 2011), therefore it's hypothesized that crocodile pool at Kano Zoological Garden is probably free of pollutant.

CONCLUSION

Based on the results of this study, the conclusion can be drawn that, on average, the blood biochemical of the Nile crocodiles in Kano Zoological Garden fall within the range for healthy crocodiles. However, there are pertinent exceptions to this statement as reported that the elevated plasma protein (Total serum protein, albumin and globulin) is an indication of possible inflammation possibly due to malnutrition associated with general weakness and immune problems.

RECOMMENDATION

This study provides information on biochemical profiles of the large body-sized Nile crocodile, it is then important that further studies should be conducted between classes and sex of Nile crocodile in the study area if clinical pathology is to be used as predictive biomarkers of the health status of Nile crocodile in the study area.

Other variables that needed to be considered for future investigations are; the role of geographical distribution, dietary requirement, seasonal variability and age determination. Moreover, the data from this study can also be used as a reference range for future studies.

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