SERUM BIOCHEMISTRY PROFILE OF NIGERIAN HORSES (*EQUUS CABALLUS*, LINNAEUS 1758)

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ABSTRACT

This study evaluated the serum biochemistry profile of apparently healthy Nigerian horses and determined the influence of age, sex and season on the serum biochemistry parameters. A total of 61 apparently healthy horses of varied ages and either sex were studied during a six-month period [three months of dry season (February - April) and three months of rainy season (June - August)] at the Obollor Afor Horse Depot, Enugu State, Nigeria. Blood for the study was collected by jugular venipuncture and all the serum biochemistry determinations followed standard procedures. The overall mean for the serum biochemistry parameters were: aspartate aminotransferase (AST) – 95.09 ± 1.14 IU/L, alanine aminotransferase (ALT) – 9.44 ± 0.69 IU/L, alkaline phosphatase (ALP) – 70.49 ± 2.55 IU/L, total protein – 6.83 ± 0.07 g/dl, albumin – 3.35 ± 0.05 g/dl, globulin – 3.48 ± 0.08 g/dl, bilirubin – 3.43 ± 0.13 mg/dl, cholesterol – 108.43 ± 3.41 mg/dl, creatinine – 1.31 ± 0.04 mg/dl and urea – 32.33 ± 0.88 mg/dl. The serum cholesterol level of the females were significantly ($p < 0.05$) higher than that of the males. Serum urea level was significantly ($p < 0.05$) higher in older horses while serum ALP activity was significantly ($p < 0.05$) higher in young horses. Serum AST, ALT and ALP activities were significantly ($p < 0.05$) higher during the rainy season while serum urea and cholesterol levels were significantly ($p < 0.05$) higher during the dry season.

Keywords: Nigerian horses, *Equus caballus,* Serum biochemistry, Aspartate aminotransferase, Alanine aminotransferase, Alkaline phosphatase, Total protein, Albumin, Globulin, Bilirubin, Cholesterol, Creatinine, Urea

INTRODUCTION

The horse, *Equus caballus,* is a domesticated mammal used worldwide for draft, transport, sports, ceremonial exhibitions, research purposes, warfare, crowd control, food (meat) and as source of variety of products and medicines (Edwards, 1994; Bush and Marczak, 2005; Lee *et al*., 2007; Wikipedia, 2011). Nigerian horses are made up of the mixed Arewa breeds and their crosses with Arabian, Dongola and Sudanese breeds which are found mainly in the Northern part of Nigeria, where stallions of the Arewa breed are commonly kept and maintained mainly for the purpose of ceremonial durbar rides (Yahuza, 2005; Hendricks and Dent, 2007; Garba *et al*., 2011).

In the clinical assessment of animals, the evaluation of the serum biochemistry profile is of utmost importance because the blood is the major transport system of the body, and both the input and output substances of almost all the body's metabolic processes and any deviations from normal caused by the invasion of the body by pathogens, other forms of injury, deprivation and/or stress are commonly

reflected by changes in the blood chemistry (Coles, 1986; Ihedioha, 2004; Stockham and Scott, 2008). An evaluation of the serum biochemistry is important because of the predictive value of serum biochemical alterations in the assessment of pathological changes in vital internal organs of the body such as liver, kidney, pancreas, heart and muscles, thus serum biochemistry assays are indispensably important in arriving at a diagnosis, assessing the efficacy of therapy and the toxicity of drugs and chemical substances (Tyson and Sawhney, 1985; Coles, 1986; Stockham and Scott, 2008). Specifically in horses, serum biochemistry assessments are used as an aid to the clinical diagnosis of a variety of diseases, in monitoring recovery during treatment and to assess the health status of a single animal or an entire herd (Ricketts, 1987; Messer, 1995).

The effects of age, sex, breed, exercise and environment/geographical location on the serum biochemistry of horses have been extensively studied and reported in many parts of the world (Lumsden *et al*., 1980; Krumrych, 2006; Lacerda *et al*., 2006; Altinsaat, 2008). Due to the variability from one geographical location to the other in the reference serum biochemistry values of horses (as with other animals), every laboratory or clinic in an area need to establish reference values for the horse population in its environment (Coles, 1986; Ricketts, 1987; Stockham and Scott, 2008; Krimer, 2011).

There are reasonable amount of published reference serum biochemistry values for horses, determined by laboratories, clinics and research institutes in countries in the Western world (Lumsden *et al*., 1980; Krumrych, 2006; Lacerda *et al*., 2006; Altinsaat, 2008; Krimer, 2011) and only little basic preliminary reports on the serum biochemistry profile of Nigerian horses (Egbe-Nwiyi *et al*., 2012). There is thus paucity of information on the comprehensive serum biochemistry profile of Nigeria horses, yet these horses are bought, kept and used in all parts of Nigeria. The objectives of this study were therefore to evaluate the serum biochemistry profile of apparently healthy Nigerian horses and determine the influence of age, sex and season on their serum biochemistry parameters.

MATERIALS AND METHODS

The horses used for the study were Nigerian local horses brought down from Northern Nigeria for sale at the Obollor Afor Horse Depot. These Nigerian horses are a collection of mixed Arewa breeds and their crosses with the Arabian, Dongola, Barb-Arab and Sudanese breeds which are not distinguishable from one another based on any specific breed characteristics. The Obollor Afor Horse Depot is strategically located at the border between Northern and South-Eastern Nigeria, at latitude 6º54'56" north and longitude 7º30'55" east. It is an important horse market for most of the states of South-Eastern Nigeria.

The study period was six months, made up of three months of dry season (February to April, 2012) and three months of rainy season (June to August, 2012). Research visits to the horse depot was made once a week during the study period. All horses sold on the days of research visit were subjected to physical and clinical examination (Ugochukwu, 2001). Among those examined, only clinically healthy horses (Ugochukwu, 2001) were included in the study. Those that showed any form of abnormality were excluded. Their ages were determined based on tooth eruption and wear and categorised as foals (0 – 11 months), young (1 – 4 years), adult (5 – 12 years), and old (> 12 years) (Ensminger, 1990; Evans *et al.,* 2007). Details of the age, sex and season of blood sample collection was documented for each of the 61 horses. Specific breed distinctions were not definitive as the study population were a collection of the mixed Arewa breeds and their crossbreeds with other African breeds (generally referred to as Nigerian local horses). Horses used for the study were handled humanely.

Five millilitres of blood was collected from each of the horses by jugular venipuncture. The blood was dispensed into a plain glass test tube and allowed to clot at room temperature. The serum for biochemical determinations was separated from the clot within one hour of blood collection by

centrifugation at 3,000 revolutions per minute for 10 minutes using a table centrifuge (TDL4®, B. Bran Scientific and Instruments Co., England).

The serum biochemistry determinations followed standard procedures. Quimica Clinica Aplicada (QCA) test kits (QCA, Spain) were used for all the serum biochemistry determinations. The serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined by the Reitman-Frankel method (Reitman and Frankel, 1957), while the serum alkaline phosphatase (ALP) activity was determined by the phenolphthalein monophosphate method (Klein *et al*., 1960; Babson *et al*., 1966). The total bilirubin was determined by the modified Jendrassik-Grof method (Doumas *et al*., 1973). The serum total protein were determined by the direct Biuret method (Lubran, 1978), while the serum albumin was determined by the bromocresol green method (Doumas *et al*., 1971) and serum globulin was calculated as the difference between the serum total proteins and serum albumin (Johnson, 2008). The serum urea was determined by the modified Berthelot-Searcy method (Fawcett and Scott, 1960), while the serum creatinine was determined by the modified Jaffe method (Blass *et al*., 1974). The serum cholesterol was determined by the enzymatic colorimetric method (Allain *et al*., 1974).

Data generated from the study were subjected to appropriate statistics using the statistical package for social sciences (SPSS) version 15.0 (SPSS Inc., USA). The differences between the serum biochemistry parameters of the male and female horses and the influence of the seasons (dry and rainy) were analyzed using students t-test. Analysis of age-related variations in the parameters evaluated was done using one way analysis of variance (ANOVA), and variant means were separated by the least significant difference method (Okafor, 1992). For all the analyses, significance was accepted at $p < 0.05$.

RESULTS

The overall means for the serum AST, ALT and ALP activities (IU/L) of the horses were 95.09 ± 1.14, 9.44 ± 0.69 and 70.49 ± 2.55, respectively (Table 1). There were no significant sex-related differences in the serum AST, ALT and ALP activities of the horses (Table 2), but the serum ALP of the young and adult horses were significantly ($p < 0.05$) higher than that of the old horses (Table 3). The serum AST, ALT and ALP activity of the horses were significantly ($p < 0.05$) higher during the rainy season than the dry season (Table 4).

For the serum proteins (g/dl), the overall means were as follows: total proteins – 6.83 ± 0.07, albumin – 3.35 ± 0.05 and globulin – 3.48 ± 0.08 (Table 1). The serum albumin levels of the male horses were significantly ($p < 0.05$) higher than that of the females, but there were no significant ($p > 0.05$) differences between the serum total proteins and globulin levels of the males and females (Table 2). The mean serum total protein of the adult horses was significantly ($p < 0.05$) lower than that of the old horses, but there were no significant ($p > 0.05$) age-related differences in the serum albumin and globulin levels (Table 3). There were no significant ($p > 0.05$) seasonal differences in the serum total proteins, albumin and globulin levels of the horses (Table 4).

The overall means of the serum total cholesterol and bilirubin were 108.43 ± 3.41mg/dl and 3.43 ± 0.13 mg/dl, respectively (Table 1). The mean serum total cholesterol of the males was significantly ($p < 0.05$) lower than that of the females, but there was no significant difference ($p > 0.05$) between the mean serum total bilirubin of the males and females (Table 2). There were no significant age-related variations in the serum total cholesterol and bilirubin (Table 3). Serum total cholesterol was significantly ($p < 0.05$) higher during dry season than during the rainy season, but there was no significant ($p > 0.05$) season-related differences in the serum total bilirubin levels of the horses (Table 4).

Table 1: The serum biochemistry profile of Nigerian horses (n = 61)

Serum biochemistry parameters	Means	Ranges
Aspartate aminotransferase (IU/L)	95.09 ± 1.14	74.38 – 115.06
Alanine aminotransferase (IU/L)	9.44 ± 0.69	3.01 – 29.43
Alkaline phosphatase (IU/L)	70.49 ± 2.55	34.24 – 109.76
Total protein (g/dl)	6.83 ± 0.07	5.57 – 7.74
Albumin (g/dl)	3.35 ± 0.05	2.77 – 4.06
Globulin (g/dl)	3.48 ± 0.08	2.16 – 4.50
Total cholesterol (mg/dl)	108.43 ± 3.41	66.67 – 185.36
Total bilirubin (mg/dl)	3.43 ± 0.13	2.16 – 4.75
Creatinine (mg/dl)	1.31 ± 0.04	0.80 – 2.00
Urea (mg/dl)	32.33 ± 0.88	15.16 – 45.19

Table 2: Comparison of the serum biochemistry profile of the male and female Nigerian horses

Serum biochemistry parameters	Sexual differences	
	Males (n = 39)	Females (n = 22)
Aspartate aminotransferase (IU/L)	95.55 ± 1.43	94.29 ± 1.94
Alanine aminotransferase (IU/L)	10.25 ± 0.95	8.00 ± 0.82
Alkaline phosphatase (IU/L)	69.37 ± 3.17	72.47 ± 4.36
Total protein (g/dl)	6.83 ± 0.08	6.82 ± 0.12
Albumin (g/dl)*	3.46 ± 0.06	3.15 ± 0.05
Globulin (g/dl)	3.37 ± 0.11	3.66 ± 0.12
Total cholesterol (mg/dl)*	102.34 ± 3.46	119.21 ± 6.70
Total bilirubin (mg/dl)	3.53 ± 0.16	3.23 ± 0.22
Creatinine (mg/dl)	1.34 ± 0.05	1.25 ± 0.08
Urea (mg/dl)	32.89 ± 1.09	31.16 ± 1.48

**Asterisk superscript letter(s) on any parameter indicate significant difference between males and females, (p < 0.05)*

For the serum creatinine and urea levels, the overall means for the horses were 1.31 ± 0.04 mg/dl and 32.33 ± 0.88 mg/dl, respectively (Table 1). There were no significant (p > 0.05) sex or season-related differences in the serum creatinine and urea levels (Table 2 and 4). The serum urea levels of the young horses were significantly (p < 0.05) lower than that of the adult and old horses, while that of the adult horses were significantly (p < 0.05) higher than that of the young but significantly (p < 0.05) lower than that of the old horses (Table 3). There were no significant (p > 0.05) age-related variations in the serum creatinine levels of the horses (Table 3).

DISCUSSION

Serum AST and ALP activity values as recorded for horses in this present study was lower than that reported for horses by Coles (1986), Radostits *et al*. (2007) and Krimer (2011). The serum AST activity recorded in this study was however comparable to but slightly higher than that reported by Egbe-Nwiyi *et al*. (2012) for horses in Maiduguri, Northern Nigeria. It is thought that the lower AST activity recorded for Nigerian horses may be due to their relatively smaller muscle mass when compared to horses in the Western world, as one of the major cellular sources of serum AST activity is the skeletal myocytes (Stockham and Scott, 2008). The significantly higher serum ALP activity recorded for the young horses relative to the adults and the old ones may be attributed to reported high bone-ALP in the young caused by the high osteoblastic activity and mineralization associated with physiological bone growth (Coles, 1986; Sarac and Saygili, 2007; Stockham and Scott, 2008). The values for the serum ALT activity of the horses in the present study were comparable to and not different from that reported for horses by Coles (1986), Radostits *et al*. (2007) and Egbe-Nwiyi *et al*. (2012). The significantly higher serum AST, ALT and ALP activity recorded during the rainy season in comparison to the dry season is worthy of note and is in agreement with earlier reports of seasonal variations in the serum activity of these enzymes in goats (Tibbo *et al*., 2008a;

Table 3: Comparison of the serum biochemistry profile of the Nigerian horses of different ages

Serum biochemistry parameters	Age differences		
	Young (1 – 3 yrs) (n = 5)	Adult (4 – 10 yrs) (n = 45)	Old (> 11 yrs) (n = 11)
Aspartate aminotransferase (IU/L)	96.14 ± 4.57 [a]	95.73 ± 1.16 [a]	90.73 ± 4.53 [a]
Alanine aminotransferase (IU/L)	10.13 ± 4.05[a]	9.40 ± 0.68 [a]	9.43 ± 2.91 [a]
Alkaline phosphatase (IU/L)	80.23 ± 15.43 [a]	72.38 ± 2.78 [a]	55.06 ± 4.55 [b]
Total protein (g/dl)	7.13 ± 0.21 [ab]	6.76 ± 0.08 [b]	7.16 ± 0.12 [a]
Albumin (g/dl)	3.36 ± 0.30 [a]	3.33 ± 0.05 [a]	3.49 ± 0.13 [a]
Globulin (g/dl)	3.78 ± 0.23 [a]	3.43 ± 0.10 [a]	3.67 ± 0.18 [a]
Total cholesterol (mg/dl)	108.69 ± 10.89 [b]	110.20 ± 3.98 [b]	97.24 ± 5.85 [a]
Total bilirubin (mg/dl)	3.68 ± 0.22 [a]	3.39 ± 0.14 [a]	3.61 ± 0.49 [a]
Creatinine (mg/dl)	1.00 ± 0.06 [a]	1.32 ± 0.05 [b]	1.33 ± 0.15 [b]
Urea (mg/dl)	23.54 ± 3.55 [a]	31.96 ± 0.91 [b]	37.66 ± 1.74 [c]

Different superscripts letters in a row indicate significant differences between the age groups (p < 0.05).

Table 4: Comparison of the serum biochemistry profile of Nigerian horses during the dry and rainy seasons

Serum biochemistry parameters	Seasonal differences	
	Dry season (n = 38)	Rainy season (n = 23)
Aspartate aminotransferase (IU/L) *	92.46 ± 1.27	99.45 ± 1.90
Alanine aminotransferase (IU/L) *	7.72 ± 0.56	12.27 ± 1.40
Alkaline phosphatase (IU/L) *	64.31 ± 3.13	80.71 ± 3.49
Total protein (g/dl)	6.74 ± 0.09	6.97 ± 0.10
Albumin (g/dl)	3.32 ± 0.07	3.41 ± 0.07
Globulin (g/dl)	3.42 ± 0.11	3.56 ± 0.13
Total cholesterol (mg/dl) *	113.82 ± 4.78	99.52 ± 3.84
Total bilirubin (mg/dl)	3.64 ± 0.18	3.25 ± 0.18
Creatinine (mg/dl)	1.36 ± 0.05	1.22 ± 0.08
Urea (mg/dl) *	34.89 ± 1.08	28.76 ± 1.11

[*]Asterisk superscripts on a parameter indicate significant differences between the seasons (p< 0.05)

Gwaze *et al*., 2012), rabbits (Okab *et al*., 2008), sheep (Tibbo *et al*., 2008b), humans (Miyake *et al*., 2009) and cattle (Hadzimusic and Krnic, 2010).

The values recorded for the serum total proteins, albumin and total cholesterol in the present study were higher than that reported for horses by Coles (1986), but were comparable to and not different from those reported by Radostits *et al*. (2007) and Krimer (2011). The serum total bilirubin values recorded in this present study were however relatively higher than that reported by Coles (1986), Radostits *et al*. (2007) and Krimer (2011). The significantly higher serum albumin recorded for males relative to females may be attributed to gender-related hormonal influences on hepatic synthesis of albumins (Colville, 2002). The higher serum total proteins of the old horses relative to the adults is in agreement with reports of effect of age on serum total proteins in rats (Toyama, 1919), mice (Rodgers and Gass, 1983) and goats (Alberghina *et al*. 2010).

The serum creatinine values recorded in this study were comparable to and not different from those reported by Coles (1986), Radostits *et al*. (2007), Krimer (2011) and Egbe-Nwiyi *et al.* (2012). The serum urea recorded for the horses in this present study was however slightly lower than that reported by Radostits *et al*. (2007) and Krimer (2011), but that reported by Egbe-Nwiyi *et al*. (2012) was in agreement with data obtained for old horses in this study. The significantly higher serum urea recorded for the old horses relative to the adults and young may be attributed to ageing-related impairment of renal function (Ju *et al*., 1993; Gupta *et al*., 2002). The slightly higher serum urea recorded for the horses during the dry season in comparison to the rainy season may be attributed to the hydration status of the horses during the dry season of higher environmental temperatures and greater water loss through sweating.

Based on the results of this study, it is concluded that the serum ALT activity, proteins, total cholesterol and creatinine levels of Nigerian horses were comparable to and not different from that reported in available literature for horses in the Western world, but the serum AST and ALP activity were markedly lower, urea was slightly lower and total bilirubin was higher.

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