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SERUM BIOCHEMICAL PARAMETERS OF DONKEYS (*EQUUS ASINUS*) AS AFFECTED BY AGE, LOCATION AND SEX IN NORTHWESTERN NIGERIA

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ABSTRACT

The aim of the study was to determine how some serum biochemical parameters of donkeys vary with age, sex and location under tropical condition. Serum biochemical parameters were collected randomly from blood samples of 125 donkeys across three states in Northwestern Nigeria. Blood samples were drawn from the jugular vein using syringe and needle, stored in ice parked container, centrifuged and analyzed at pathology laboratory of Ahmadu Bello University Teaching Hospital, Shika, Zaria. Results obtained indicate that glucose, total serum protein, potassium, creatinine and uric acid differed significantly ($P < 0.05$) between male and female donkeys. There were significant difference ($P < 0.05$) between young, adult and old donkeys for glucose, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), albumin, sodium, potassium, hydrocarbonate, urea, creatinine and uric acid. Location also had effect on ALT, ALP, total serum protein, albumin, globulin, sodium, hydrocarbonate, urea, creatinine, calcium and uric acid. From the results of this study it can be concluded that serum biochemical parameters varied according to age, sex and location. Further researches should be conducted across geopolitical zones of Nigeria.

Keywords: Serum, Biochemical, Age, Sex, Location

INTRODUCTION

In Nigeria, like anywhere else, donkey (*Equus asinus*) has been used as a work animal mainly for transportation; conveying farm produce to the market or pulling of carts and other farm tillage equipment (Starkey and Starkey, 2004; Blench *et al.*, 2004; Hassan *et al.*, 2013). However, donkey production is constraint by many production factors such as poor nutrition, disease, poor genetic potential, management and harsh environmental factors (Ademosum, 1994; Yilma *et al.*, 1997; Simenew*et al.*, 2011). Of all the factors, poor nutrition and disease conditions are rampant with significant impact on the performance of animals (Ademosum, 1994; Crane, 1997). Donkeys are known to survive with little management. Their body conditions may fluctuate during the year as feed supply fluctuates resulting in poor body condition, weight loss and delay in resumption of ovarian cycles after parturition (Pearson *et al.*, 1999).The low productivity of livestock on pasture-based extensive grazing systems needs to be improved, and this entails an evaluation of their nutritional status. The assessment of nutritional and health status of animal can be made by determining the concentrations of certain blood metabolite such as non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHB), glucose and cholesterol (Ndlovu *et al.*, 2007). Their concentrations indicate the extent of the metabolism of energy, proteins and other nutrients in the animals (Pambu-Gollah *et al.*, 2000; Agenas *et al.*, 2006). Changes in the concentration of circulating nutrient-sensitive metabolites are important signals of the metabolic status of the

animal and the organs function (Lindsay *et al.*, 1993; Wettemann *et al.*, 2003). Other blood metabolites, such as total protein, albumin, globulin, creatinine and urea, are indicators of the protein status. Factors including the physiological status of an animal, health status, breed, nutrition, season and age may affect the concentration of these metabolites in the blood (Ndlovu *et al.*, 2007). However, unlike other livestock species, there is very scanty of information on the baseline serum biochemical parameters of the donkey in Nigeria. Therefore a study to determine serum biochemical profile of donkeys in Northwestern Nigerian and how these vary with age, sex and location is necessary.

MATERIALS AND METHODS

Area of the study

The field research was conducted in three selected northwestern states of Nigeria; Katsina, Zamfara and Jigawa. Within each state three (3) local governments areas with sizable donkey population were selected. The local governments covered in Katsina were; Kurfi, Daura and Dandume, in Zamfara were; Tsafe, KauranNamoda and Bakura and in Jigawa were; Roni, Kiyawa and Maigatari.

Experimental Animals

A total of 125 donkeys owned by subsistence farmers were sampled using simple random sampling method from all the states. They consisted of forty (40) from the three Local Governments areas in Katsina {Kurfi (15), Daura (15) and Dandume (10)}, forty three (45) from the three local government areas in Zamfara {Tsafe (13), KauranNamoda (18) and Bakura (14)} and thirty nine (39) from three Local Government Areas in Jigawa {Roni (12), Kiyawa (14) and Maigatari (13)}. The animals were grouped according to their locations, sex and age. There were a total of 66 males and 59 females sampled across the three states. The animals were divided into three age groups namely; Young (≤3 years, N = 33), Adult (3 - 10 years, N = 53) and Old (> 10 years, N = 39). The ages of the animals were determined using dentition (Crane, 1997).

Collection of blood

Blood samples (8ml) were drawn from the jugular vein using a 10 ml using syringe and needle. Blood samples were placed into plane vacuum tubes. Immediately after collection blood samples were placed in an ice packed "polio vaccine" box containing ice (Charles, 2007; Weiser 2002a).

Serum Biochemical Methods

Blood plasma was separated by centrifugation at 1800 x*g* for 15 min at 4ºC. The plasma was stored at –20ºC and transported to Ahmadu Bello University Teaching Hospital Zaria for the assay of the nutrient sensitive metabolites. The concentrations of the nutrient-sensitive metabolites were measured using the Vitros 950 Chemistry Systems auto-analyser (Ortho Clinical Diagnostics, UK). Blood glucose was measured based on the method of Trinder (1969), while cholesterol was determined based on the enzymatic method of Allain *et al.* (1974). The total blood protein concentration was determined based on the biuret reaction (Doumas *et al*., 1981) and albumin determination based on the method of Doumas and Biggs (1972), while globulin concentration in plasma was computed as the difference between the total protein and albumin concentrations (Mapekula *et al.*, 2011). Urea determinations were based on the method by Sampson *et al.* (1980), while creatinine determination was done according to the method by Ambrose *et al.* (1983). The concentration of Total bilirubin, triglyceride and activities of alkaline phosphate, alamine aminotransferase (ALT) and Aspartate aminotransferace (AST) were determined using automated system photometer 5010 (Robert Riece GmbH and coKG, Berlin, Germany). Serum electrolytes: sodium, potassium, chloride and calcium were analyzed using Roche AVL 9180 Snap pack Electrolyte analyzer (Roche Diagnostic Corporation 9115 Hague road, USA, 2002). The

results was automatically analyzed and printed out with the sample identification number. The accuracy and reliability of the procedures, instruments and the reagents were ensured though quality control and careful handling of the sample.

Data Analysis

The Data obtained were analyzed using the General Linear Model (GLM) procedure of SAS (2008) and the significant means in the treatments were separated using Duncan's Multiple Range Test.

RESULTS

Table 1 shows the results of biochemical parameters of donkeys found in Northwestern Nigeria. Glucose level of donkeys of Northwestern Nigeria ranges from 1.40 mmol/L to 4.60 mmol/L with mean value of 3.03mmol/L and Standard error of 0.05. The AST values ranges from 4.00 IU/L to 48.00 IU/L with mean value of 19.80 IU/L and Standard error of 0.81. The ALT values ranges from 4.00 IU/L to 51.00 IU/L with mean value of 16.30 IU/L and Standard error of 0.72. The ALP values of donkeys of Northwestern Nigeria ranges from 54.00 IU/L to 191.00 IU/L with mean value of 115.10 IU/L and Standard error of 2.39. The Total serum protein values of donkey ranges from 58.00 g/L to 89.00 g/L with mean value of 72.9 g/L and Standard error of 0.85. The albumin values of donkey ranges from 25.00 g/L to 53.00 g/L with mean value of 36.8 g/L and Standard error of 0.70. The globulin values of donkey ranges from 23.00 g/L to 56.00 g/L with mean value of 36.10 g/L and Standard error of 0.73. The sodium levels of donkey ranges from 112.4 mmol/L to 156.00 mmol/L with mean value of 131.10 mmol/L and Standard error of 0.87. The potassium levels of donkey found in Northwestern Nigeria ranges from 3.70 mmol/L to 10.00 mmol/L with mean value of 5.21 mmol/L and Standard error of 0.10. The chloride levels of donkey ranges from 83.00 mmol/L to 110.00 mmol/L with mean value of 101.40 mmol/L and Standard error of 0.39. The bicarbonate levels of donkey ranges from 5.40 mmol/L to 58.00 mmol/L with mean value of 27.30 mmol/L and Standard error of 0.87. The urea levels of donkey found in Northwestern Nigeria ranges from 2.30 mmol/L to 18.60 mmol/L with mean value of 7.87 mmol/L and Standard error of 0.32. The creatinine levels of donkey found in Northwestern Nigeria ranges from 47.00 mmol/L to 172.00 mmol/Lwithmean value of 127.20 mmol/L and Standard error of 2.68. The calcium levels of donkey found in Northwestern Nigeria ranges from 1.48 mmol/L to 5.86 mmol/Lwithmean value of 3.03 mmol/L and Standard error of 0.08. The serum pH of donkey ranges from 7.00 to 9.00 withmean value of 7.76 and Standard error of 0.04. The uric acid levels of donkey found in Northwestern Nigeria ranges from 80.00 mmol/L to 180.00 mmol/Lwithmean value of 133.67 mmol/L and Standard error of 3.08.

Table 1: Biochemical values of donkey in Northwestern Nigeria

Parameters	Number	Mean ± S.E	Minimum	Maximum	C.V
GLU (mmol/L)	125	3.03±0.05	1.40	4.60	18.10
AST (IU/L)	125	19.8±0.81	4.00	48.0	40.62
ALT (IU/L)	125	16.3±0.72	4.00	51.0	44.04
ALP (IU/L)	125	115.1±2.39	54.0	191.0	20.73
TSP (g/L)	125	72.9±0.85	58.0	89.0	11.62
ALB (g/L)	125	36.8±0.70	25.0	53.0	19.06
GLO (g/L)	125	36.1±0.73	23.0	56.0	20.14
Na (mmol/L)	125	131.1±0.87	112.4	156	6.67
K (mmol/L)	125	5.21±0.10	3.70	10.0	19.30
Cl (mmol/L)	125	101.4±0.39	83.0	110.0	3.80
HCO_3 (mmol/L)	125	27.3±1.19	5.0	58.0	43.79
Urea (mmol/L)	125	7.87±0.32	2.30	18.6	40.92
Cr (umol/L)	125	127.2±2.68	47.0	172.0	21.06
Ca (mmol/L)	125	3.03±0.08	1.48	5.86	26.71
pH	125	7.76±0.04	7.00	9.00	5.11
UA (umol/L)	125	133.67±3.08	80.0	180.0	23.08

S.E: Standard error, C.V: Coefficient of variation, GLU: Glucose, AST: Aspartate transaminase, ALT: Alanine transaminase,ALP: Alkaline phosphatase, TSP: Total serum protein,ALB: Albumin, GLO: Globulin, Na: Sodium, K: Potassium, Cl: Chloride, HCO_3: Bicarbonate , Cr: Creatinine, Ca: Calcium, pH: serum pH, UA: Uric acid.

Table 2 shows the variations in biochemical parameters with sex in donkeys of Northwestern Nigeria. Glucose, total serum protein, potassium, creatinine and uric acid levels were significant higher (P<0.05) in female than male donkeys.

Table 2: Effect of sex on biochemical parameters of donkey

Parameters	N	Male	Female	S.E.M	LOS
GLU (mmol/L)	125	2.86±0.07[b]	3.27±0.08[a]	0.05	*
AST (IU/L)	125	20.36±1.13	19.02±1.11	0.98	NS
ALT (IU/L)	125	16.61±1.05	15.81±0.89	1.03	NS
ALP (IU/L)	125	113.39±3.21	117.63±3.56	3.50	NS
TSP (g/L)	125	71.64±1.07[b]	74.81±1.34[a]	1.09	*
ALB (g/L)	125	36.58±0.90	37.22±1.13	1.00	NS
GLO (g/L)	125	35.07±0.99	37.59±1.03	1.10	NS
Na (mmol/L)	125	130.53±1.23	131.90±1.19	1.21	NS
K (mmol/L)	125	5.03±0.09[b]	5.47±0.20[a]	0.08	*
Cl (mmol/L)	125	101.61±0.54	101.05±0.53	0.50	NS
HCO_3 (mmol/L)	125	27.46±1.57	26.96±1.85	1.22	NS
Urea (mmol/L)	125	8.14±0.44	7.47±0.46	0.40	NS
Cr (umol/L)	125	125.33±3.99[b]	129.93±3.11[a]	0.17	*
Ca (mmol/L)	125	2.99±0.11	3.07±0.11	0.10	NS
pH	125	7.74±0.06	7.78±0.05	0.14	NS
UA (umol/L)	125	126.27±4.09[b]	144.29±4.20[a]	4.08	*

S.E.M: Standard error of mean, LOS: Level of Significance, GLU: Glucose, AST: Aspartate transaminase, ALT: Alanine transaminase, ALP: Alkaline phosphatase,TSP: Total serum protein, ALB: Albumin, GLO: Globulin, Na: Sodium, K: Potassium, Cl: Chlorine, HCO_3: Bicarbonate, Cr: Creatinine, Ca: Calcium,pH: serum pH, UA: Uric acid.
Means within rows with different superscript are significantly different.
*= P<0.05

Table 3 shows the variations in biochemical parameters with ages of donkeys in the Northwestern Nigeria. Glucose levels were significant higher (P<0.05) inyoung donkeys than in Adult and old. Aspartate transaminase (AST) enzymes were significantlyhigher (P<0.05) in Young and adult than old donkeys. Alanine transaminase (ALT) enzymes were significantly higher (P<0.05) in old than adult and young donkeys. Alkaline phosphatase (ALP) levels were significantly higher (P<0.05) in young than in adult and old donkeys. Albumins, potassium and uric acid content of donkeys' serum were significantly higher (P<0.05) in young and adult than old donkeys. Sodium concentrations were significantly higher (P<0.05) in Young and Old than in Adult donkeys of Northwestern Nigeria. Bicarbonate concentrations were significantly higher (P<0.05) in adult and old than in young donkeys.

Urea concentrations were significantly higher (P<0.05) in adult donkeys than in young and old donkeys found in Northwestern Nigeria. Creatinine levels were significantly higher (P<0.05) in young donkeys than adults and old donkeys.

Table 3: Effect of age on biochemical parameters of donkey

Parameters	Young	Adult	Old	SEM	LOS
GLU (mmol/L)	3.25±0.07[a]	2.96±0.09[b]	2.86±0.09[b]	0.07	*
AST (IU/L)	21.21±1.57[a]	19.39±1.28[a]	18.63±1.19[b]	1.11	*
ALT (IU/L)	15.79±0.65[b]	17.86±1.19[a]	17.71±1.92[a]	0.64	*
ALP (IU/L)	120.82±3.47[a]	113.98±3.79[b]	109.38±5.34[c]	1.34	*
TSP (g/L)	73.69±1.64	73.93±1.26	70.13±1.44	1.68	NS
ALB (g/L)	37.21±1.16[a]	37.30±1.18[a]	35.50±1.25[b]	0.85	*
GLO (g/L)	36.49±1.16	36.62±1.12	34.63±1.63	1.59	NS
Na (mmol/L)	131.80±1.63[a]	128.94±1.09[b]	133.96±1.98[a]	1.30	*
K (mmol/L)	5.31±0.22[a]	5.29±0.15[a]	4.92±0.09[b]	0.38	*
Cl (mmol/L)	100.20±0.53	101.79±0.66	102.25±0.76	1.52	NS
HCO_3 (mmol/L)	22.14±1.55[b]	29.63±1.83[a]	30.04±2.77[a]	2.00	**
Urea (mmol/L)	6.61±0.41[b]	9.72±0.48[a]	6.27±0.57[b]	0.90	**
Cr (umol/L)	133.52±6.08[a]	122.65±2.8[b]	122.13±5.08[b]	2.06	**
Ca (mmol/L)	2.97±0.13	2.98±0.12	3.19±0.19	1.87	NS
pH	7.81±0.06	7.69±0.07	7.78±0.07	0.17	NS
UA (umol/L)	134.24±4.83[a]	136.89±5.37[a]	127.08±5.39[b]	2.09	*

S.E.M: Standard error of mean, LOS: Level of Significance, GLU: Glucose, AST: Aspartate transaminase, ALT: Alanine transaminase, ALP: Alkaline phosphatase,TSP: Total serum protein, ALB: Albumin, GLO: Globulin, Na: Sodium, K: Potassium, Cl: Chlorine, HCO_3:Bicarbonate , Cr: Creatinine, Ca: Calcium,pH: serum pH, UA: Uric acid.
Means within rows with different superscript are significantly different.
*= P<0.05

Table 4 shows the variations in biochemical parameters due to locations of donkeys. Alanine transaminase (ALT) enzymes were significantly higher (P<0.05) in donkeys found in Jigawa and Zamfara than that of Katsina. Alkaline phosphatase (ALP) were significantly higher (P<0.05) in donkeys found in Katsina than Zamfara and Jigawa. Total serum protein and Albumin levels were significantly higher (P<0.05) in donkeys of Katsina than Jigawa and Zamfara. Globulins were significant higher (P<0.05) in donkeys found in Zamfara than Jigawa and Katsina. Sodium concentrations were significantly higher (P<0.05) in donkeys of Jigawa than Katsina and Zamfara. Bicarbonate concentration were significantly higher (P<0.05) in donkeys found in Zamfara followed by Jigawa and Katsina. Urea levels were

significantly higher (P<0.05) in donkeys of Zamfara followed by Jigawa and Katsina. Creatinine concentrations were significantly higher (P<0.05) in donkeys found in Katsina than Jigawa and Zamfara. Calcium concentrations were significantly higher (P<0.05) in donkeys of Jigawa followed by Katsina and Zamfara. Uric acid concentration were significantly higher (P<0.05) in donkeys found in Katsina followed by Jigawa and Zamfara.

Table 4: Effect of location on biochemical parameters of donkey

Parameters	Jigawa	Katsina	Zamfara	SEM	LOS
GLU (mmol/L)	3.07±0.07	3.20±0.09	2.91±0.10	1.88	NS
AST (IU/L)	18.38±0.85	20.83±2.37	20.83±2.34	1.54	NS
ALT (IU/L)	16.25±0.66^{a}	13.11±1.01^{b}	17.67±1.49^{a}	1.22	**
ALP (IU/L)	106.03±2.64^{c}	128.50±4.86^{a}	118.07±4.27^{b}	2.09	**
TSP (g/L)	71.35±1.32^{b}	77.56±2.01^{a}	72.48±1.24^{b}	1.62	*
ALB (g/L)	36.68±1.00^{b}	43.17±1.79^{a}	34.29±0.88^{b}	2.18	**
GLO (g/L)	34.68±0.92^{b}	34.39±1.12^{b}	38.19±1.36^{a}	1.30	*
Na (mmol/L)	136.47±1.42^{a}	128.56±1.82^{b}	127.06±0.92^{b}	1.69	*
K (mmol/L)	5.53±0.22	5.16±0.17	4.92±0.08	0.98	NS
Cl (mmol/L)	101.49±0.46	97.89±1.22	102.76±0.47	2.80	NS
HCO_3 (mmol/L)	20.12±0.78^{b}	15.39±1.30^{c}	39.13±1.12^{a}	1.27	**
Urea (mmol/L)	7.07±0.52^{b}	5.00±0.24^{c}	9.85±0.38^{a}	0.06	**
Cr (umol/L)	121.15±4.27^{b}	154.94±2.31^{a}	121.10±3.74^{b}	3.88	**
Ca (mmol/L)	3.74±0.09^{a}	3.08±0.10^{b}	2.32±0.06^{c}	0.15	*
pH	7.86±0.05	8.11±0.06	7.51±0.06	1.20	NS
UA (umol/L)	137.40±4.41^{b}	142.78±7.91^{a}	126.19±4.84^{c}	1.18	*

S.E.M: Standard error of mean, LOS: Level of Significance, GLU: Glucose, AST: Aspartate transaminase, ALT: Alanine transaminase, ALP: Alkaline phosphatase,TSP: Total serum protein, ALB: Albumin, GLO: Globulin, Na: Sodium, K: Potassium, Cl: Chlorine, HCO_3:Bicarbonate, Cr: Creatinine, Ca: Calcium,pH: serum pH, UA: Uric acid.
Means within rows with different superscript are significantly different.
*= P<0.05
**= P<0.01

DISCUSSION

Results on biochemical analysis showed significant differences (P <0.05) between male and female donkeys for glucose, total serum protein, potassium, creatinine and uric acid, with female having the higher level than male. These findings are similar to those obtained by Zinkl *et al.* (1990) and Al-Busadah and Homeida (2005). The higher glucose level in female may be due to the less workload compared to males. Jordana

et al. (1998) recorded no effect of sex on these parameters in Catalonian donkey breeds. Possible explanations are not available in literature, also when other species have been considered.

Age had the most influence on the serum biochemical parameters studied. Eleven out of the sixteen variables showed significant differences between young and adult donkeys. Glucose, aspartate transaminase (AST), alkaline phosphatase (ALP), albumin, sodium, potassium and uric acid showed a significant decrease with advancement of age. Similar results, with some exceptions were reported by Zinkl *et al.* (1990), Sow *et al.* (2012), Girardi *et al.* (2013) and Annita *et al.* (2016). The high levels of glucose in young donkeys during the first year of age may be due to nutrition, which is mainly done by breastfeeding in the beginning, and naturally change during this period, with increasing forage intake (Annita *et al.,* 2016). The higher the forage intake, the lower the glucose level.Aspartate transaminase (AST) level is lower in old animals. This agreed with the report of Sow *et al.* (2012). The abundance of muscle and physical efforts influence significantly the values of AST (Padalino *et al.,* 2007). The nutritional status could explain the muscle abundance, thus the variation of AST values was surely due muscle abundance. The result of decreasing ALT level agreed with the finding of (Veronesi *et al.,* 2014). The ALP was significantly higher in young donkey and it decreases with age, this agreed with the findings of Zinkl *et al.* (1990), Laus *et al.* (2015) and Stanisic *et al.* (2015). Albumin level of young and adult are statistically similar and higher than the level of albumin in old, this was in agreement with the report of Keneko *et al.* (1989) that reported that there is slight decrease of albumin as age advances. Biocarbonate increases with age. The early decrease of serum urea concentrations is similar to that reported by Sgorbini *et al.* (2013), Veronesi *et al.* (2014) and Annita *et al.* (2016). Creatinine level reduced with age, this agreed with the finding of Etana *et al.* (2011). The significant differences for creatinine concentrations found, may perhaps reflect the differences in the feeding conditions between different groups of donkeys (Al Shafei *et al.* 2015). Creatinine concentration depends upon the total body content of creatinine, which in turn, depends upon dietary intake and muscle mass (Kaneko, 1989; Al-Busadah and Homeida 2005). The higher level of urea in adult donkey compared to young donkeys may be due to components of adult donkey dietary intake regard to the abundance of nitrogen-containing basic organic compound in natural pasture, especially proteins, where most of the adult donkeys grazed.

However, the investigation of French and Patrick (1995) using large sample of approximately 4000 donkeys also showed that biochemical parameters were not affected by sex or age, suggesting that the differences observed by other authors may be due to small non-normal sample data or inadequate use of parametric statistics.

CONCLUSION

This study indicated that there are considerable variations in serum biochemical values due to sex, age and location of donkeys and these should be noted during biochemical assessments of donkeys in Nigeria. The study shows that, female donkeys have higher serum glucose and protein levels; whereas age has strong influence on the serum biochemical values. Although there are pioneering data on some of these parameters, the present findings will contributed a lot to enhance understanding on physiological parameters of Nigerian Donkeys.

CONFLICT OF INTEREST

The authors have no conflict of interest

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