Reference values for certain serum biochemical markers of liver damage in apparently healthy Red Sokoto goats

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Abstract

The Red Sokoto goat (RSG) is the most preponderant of all the breeds of goats in Nigeria. There is dearth of information regarding the reference values for serum biochemical markers of liver damage in this breed. This study demonstrated the reference values in apparently healthy RSG and in RSG that were truly negative for liver disorders. The study was a cross sectional survey. Research visits were made to Ikpa Market Abattoir, Nsukka, for 12 weeks. Apparently healthy goats and those truly negative for liver disorders were deliberately selected for the study. Blood samples were collected in sample bottles devoid of anticoagulants. The sera harvested were used for determination of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT) activities and levels of albumin and cholesterol. The overall mean ± standard error of mean (SEM) of the parameters assayed in apparently healthy goats were: AST, 59.54 ± 2.43; ALT, 12.72 ± 1.18; ALP, 38.31 ± 6.63; GGT, 20.83 ± 1.56; albumin, 3.31 ± 0.04; cholesterol, 82.76 ± 1.70 while the overall for the goats that were truly negative for liver disorders were: AST, 53.49 ± 2.23; ALT, 10.91 ± 0.62; ALP, 29.88 ± 2.35; GGT, 16.45 ± 0.91; albumin, 3.37 ± 0.04 and cholesterol, 81.36 ± 2.33. Serum ALP was significantly higher in the young rather than the adult goats and in the females more than in the males of apparently healthy RSG while AST was significantly higher in the adults of RSG that showed true negative for liver disorders.

Introduction

The major breeds of goats in Nigeria are Red Sokoto, Sahelian and West African Dwarf goats (Ngere *et al.* 1984). Red Sokoto goats (RSG) are the most preponderant (above 80%) of these three breeds and their distribution is more cosmopolitan (Malau-Aduli *et al.* 2003; Makun *et al.* 2008). It is estimated that the population of goats in Nigeria is about 34.5 million (Bourn *et al.* 1994). Goats are good sources of meat and milk. In Nigeria, small ruminants account for about 17% of the meat consumed (Lebbie 2004). They are good sources of income to a large majority of the populations in developing countries such as Nigeria (Kocho *et al.* 2011).

Liver diseases of goats are occasioned by some infectious and non-infectious agents. Some of the notable infectious agents that may contribute ultimately to liver disease in ruminants include but are not limited to fasciolosis, helminthiasis and some viruses (Fakae *et al.* 1999; Okoli *et al.* 2000). The larvae of some helminthes. such as Cysticercus, have been incriminated for causing liver disorders due to their migration (Radfar *et al.* 2014). Some viruses known to cause some level of hepatitis in ruminants as revealed during post mortem examinations include: Rift valley fever viruses, contagious Echythema viruses and *Peste des petits* viruses (Khan *et al.,* 2018)

Damage to the liver can be assessed biochemically by determining the serum activities of some enzymes and the levels of certain analytes that are commonly known as markers of liver disorders (Friedman *et al.* 2003). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) can detect hepatocellular injuries while alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) can assess the hepatobiliary system (Sherlock 1997). The synthetic ability of the liver can be evaluated by assaying for serum cholesterol and albumin (Thapa and Walia 2007).

There is a severe dearth of information concerning the reference values of serum biochemical markers of liver damage in goats generally and specifically in RSG. Reference values are essential data on which diagnosis can be based. This study was therefore designed to determine the reference values of certain serum biochemical markers of liver damage in apparently healthy RSG and in RSG that showed true negativity for liver disorders.

Materials and Methods

Animals: The Nigerian Red Sokoto breed of trade goats billed for sale and slaughter in Ikpa Market Lairage, Nsukka, Enugu state, Nigeria was used for the study.

Study design: The study design was a cross sectional survey. Research visits to the Abattoir were made 2 times a week for a period of 12 weeks (January-March, 2019). Before slaughter, the goats were physically and clinically examined for health status and the age determined. Goats aged below 2 years were classified as young while those aged 2 years and above were classified as adult. Each of the goats was tagged for identification purposes before slaughter. Upon slaughter, blood samples were collected from the jugular vein into clean test tubes devoid of anticoagulant. Sera were harvested for assay of serum biochemical markers of liver disorders. Upon evisceration, the goats were appropriately followed up for gross pathology evaluation and photography of the liver. A piece of the liver was dissected for pathological examination. Information from the gross pathology examination, histopathology evaluation and determination of serum biochemical markers of liver damage, were used to classify the goats in question as true positive, true negative, false positive and false negative.

- ‣ True positive (TP) = Presence of biochemically confirmed liver injury + gross and histopathologic hepatic lesions
- ‣ False positive (FP) = Presence of biochemically confirmed liver injury + absence of gross and histopathologic hepatic lesions
- ‣ True negative (TN) = Absence of biochemically confirmed liver injury + absence of gross and histopathologic hepatic lesions
- ‣ False negative (FN) = Absence of biochemically confirmed liver injury + gross and histopathologic hepatic lesions (Gordis 2009).

Ageing of goat: The dentition method (Millard 2002) was used to estimate the age of the goats. At birth, kids have eight milk teeth or temporary incisors, arranged in four pairs on the lower jaw. The central pair of temporary incisor teeth is shed and replaced by permanent teeth at approximately 1 year of age. At 2 years, the second pair of milk teeth is replaced by a pair of permanent incisors. At 3 and 4 years, the third and fourth pairs of permanent teeth appear. At 4 years of age the goat has a "full mouth." When a doe loses some of her incisor teeth, she is called a "broken mouth." When she loses all of her teeth she is called a "gummer". Both "broken mouth" and "gummer" goats are aged between 8-12 years (Millard 2002).

Selection of Reference individuals (inclusion/exclusion criteria): Based on the American Society for Veterinary Clinical Pathology (ASVCP) criteria for determination of reference intervals (Friedrichs *et al.* 2012), the following criteria were considered for inclusion and or exclusion of the individuals in the study:

Apparent health status: All individuals adjudged to be apparently healthy were included in the study. This was determined by thorough physical and clinical examinations. Goats showing clinical signs of disease or disorders were excluded from the study.

Physiological status: Lactating and pregnant does were excluded from the study.
Sex: Both males and females were included in the study. However, castrated males were excluded.

Age: Goats aged 2 years and above were considered adult while those less than 2 years of age were categorized as young.

Breed: Only Red Sokoto goats were included in the study. These goats came from the northwestern part of Nigeria.

Determination and elimination of outliers: Dixon's range test was used to test and eliminate the outliers. The criteria for rejection involve values higher or lower than interquartile (IQ) fences set at Q1-1.5*IQR and Q3+1.5*IQR where IQR (interquartile range) = IQ3-IQ1. IQ3 and IQ1 represent 75th and 25th percentiles respectively (Friedrichs *et al.* 2012).

Pre-analytical procedures: The pre-analytical procedures followed were consistent with the guidelines presented by ASVCP (Friedrichs *et al.* 2012). The animals presented for slaughter were well rested prior to slaughter. Following physical and clinical examinations, the animals were gently slaughtered by slitting through the jugular vein with a knife. Blood samples were collected from the jugular veins of the goats at the point of slaughter into clean test tubes devoid of anticoagulant. This was allowed to stand at room temperature (25°C) for 30 mins. Sera were harvested by centrifugation at 3, 000 g for 10 mins. Thereafter, the sera were decanted into a clean sample bottle.

Following, evisceration, the liver was photographed and a piece removed and preserved in 40% formol saline for histopathological evaluation. The experimental protocol used in this study was approved by the Ethics committee of the University of Nigeria, Nsukka and conforms with the guide to the care and use of animals for research and teaching of the University of Nigeria (Nsukka, Enugu state, Nigeria).

Analytical procedures: The test kits used to assay the serum biochemical analytes in this study were obtained from Randox, (UK) while all the standard assay procedures were observed.

The AST activity was determined by the Reitman-Frankel spectropho to metric method (Reitman and Frankel 1957; Colville 2002). The AST is a pyridoxal phosphate (PLP)-dependent enzyme that catalyzes the reaction: Aspartate + α -Ketoglutarate = Oxaloacetate + Glutamate. The transfer of amino group from aspartate to α-ketoglutarate results in the production of a colorimetric (450 nm) product proportional to the AST enzymatic activity present. AST levels in the blood are commonly used as a marker for liver function.

The serum ALT activity was determined by the Reitman-Frankel spectropho to metric method (Reitman and Frankel 1957; Colville 2002). ALT catalyzes the reversible transamination of L-alanine and α-ketoglutarate to pyruvate and L-glutamate. The pyruvate is then reduced to lactate in the presence of lactate dehydrogenase (LDH) with the concurrent oxidation of NADH to NAD. The system monitors the rate of change in absorbance at 340 nm over a fixed time interval. The rate of change in absorbance is directly proportional to the ALT activity in the sample.

The ALP activity was determined by the phenolphthalein monophosphate method (Klein *et al.* 1960; Colville 2002). ALP reacts with p-nitrophenyl phosphate to form p-nitrophenol. The rate of formation of this p-nitrophenol is directly proportional to the level of ALP activity.

The GGT activity was done following a Spectrophotometric method according to Theodorsen and Strømme 1976; Colville 2002). GGT transfers the γ-glutamyl group from the substrate L-γ-Glutamyl-p-nitroanilide, liberating the chromogen p-nitroanilide (pNA, 418 nm) proportional to the GGT activity present.

The serum albumin was determined by the bromocresol green method (Doumas *et al.* 1971; Colville 2002). The assay is based on the selective interaction between Bromocresol Green (BCG) and Albumin forming a chromophore (a green BCG complex) that can be detected at 620 nm. The signal is directly proportional to the amount of Albumin present in the serum. BCG does not react with other abundant plasma proteins such as IgG.

The serum cholesterol was determined by cholesterol oxidase-perioxidase method (Allain *et al.* 1974; Colville 2002). Cholesterol esters are hydrolyzed via cholesterol esterase into cholesterol, which is then oxidized by cholesterol oxidase into the ketone cholest-4-en-3-one plus hydrogen peroxide. The hydrogen peroxide is then detected with a highly specific colorimetric probe. The total cholesterol present is proportional to the amount of hydrogen peroxide detected.

The AST:ALT ratio was calculated by dividing the AST activity with the corresponding ALT activity.

Histopathological examination: This was done following standard procedures as described elsewhere (Drury *et al.* 1967; Comanescu *et al.* 2012).

Statistical analysis: Prior to analysis, Dixon's range test was used for detection of outliers. Data was analyzed using Student's T-test to test for differences between males and females; young and adult with regards to all the serum biochemical parameters assayed. Probability levels were set at p≤0.05. Descriptive statistics were used to analyze data on the overall reference values. Results were presented in tables and charts as means± standard error of mean (SEM).

Results

Demographic characteristics of apparently healthy RSG and RSG that show true negativity for liver disorder: The results of the demographic characteristics of apparently healthy RSG and RSG that show true negativity for liver disorder show that a total of 107 RSG were examined, out of which 103 were adjudged apparently healthy. The 103 RSG were made up of 20 males and 83 females; 8 young and 95 adults. Eighty out of the 103 were truly negative for liver disorders. Eighteen out of this 80 were males and 62 females; 8 young and 72 adults.

The overall reference values of serum biochemical markers of liver disorders in apparently healthy RSG: Results of the reference values for serum markers of liver disorders in apparently healthy RSG indicate that the mean ± SEM for AST is 59.54 ± 2.43; ALT, 12.72 ±

1.18; AST:ALT ratio, 5.44 ± 0.19; ALP, 38.31 ± 6.63; GGT, 20.83 ± 1.56; albumin, 3.31 ± 0.04 and cholesterol, 82.76 ± 1.70 (Table 1).

The overall reference values of serum biochemical markers of liver disorders in truly negative RSG: The results of the overall reference values for serum markers of liver disorders in RSG that are truly negative for liver disorders indicate that the Mean ± SEM for AST is 53.49 ± 2.23; ALT, 10.91 ± 0.62; ALP,

29.88 ± 2.35; GGT, 16.45 ± 0.91; albumin, 3.37 ± 0.04 and cholesterol, 81.36 ± 2.33 (Table 2).

Age-related differences in the reference values for serum biochemistry parameters of apparently healthy RSG: Age-related differences in the reference values for serum biochemistry parameters of apparently healthy RSG were evaluated and the results are presented in table 3. There were no significant differences ($p > 0.05$) between the young and adult with regards to all the parameters assayed (Table 3).

Table 1 The overall reference values of serum biochemical markers of liver disorders in apparently healthy RSG

Parameters	Mean ± SEM	Minimum	Maximum
Aspartate aminotransferase (IU/L)	59.54 ± 2.3	28.60	152.04
Alanine aminotransferase (IU/L)	12.72 ± 1.18	2.59	113.41
AST:ALT Ratio	5. ± 0.19	1.34	12.74
Alkaline phosphatase (IU/L)	38.31 ± 6.63	2.99	157.00
Gamma glutamyltransferase (IU/L)	20.83 ± 1.56	3.47	104.22
Albumin (g/dl)	3.31 ± 0.04	2.18	4.18
Cholesterol (mg/dl)	82.76 ± 1.70	33.84	132.42

Table 2 The overall reference values of serum biochemical markers of liver disorders in truly negative RSG

Parameters	Mean ± SEM	Minimum	Maximum
Aspartate aminotransferase (IU/L)	53.49 ± 2.23	28.60	92.0
Alanine aminotransferase (IU/L)	10.91 ± 0.62	2.59	29.65
AST:ALT Ratio	5.37 ± 0.25	2.80	12.74
Alkaline phosphatase (IU/L)	29.88 ± 2.35	2.99	74.74
Gamma glutamyltransferase (IU/L)	16.45 ± 0.91	3.47	42.85
Albumin (g/dl)	3.37 ± 0.04	2.75	3.88
Cholesterol (mg/dl)	81.36 ± 2.33	33.84	132.42

Table 3 Age-related differences in the reference values for serum biochemistry parameters of apparently healthy RSG

Parameter	Age	Mean ± SEM	Minimum	Maximum	P value*
AST (IU/L)	Young	67.12 ± 16.07	29.89	121.67	0.648
	Adult	59.14 ± 2.43	28.60	152.04	
ALT (U/L)	Young	10.79 ± 0.91	7.51	13.12	0.198
	Adult	12.82	2.59	113.41	
AST:ALT	Young	6.06 ± 1.24	3.82	10.48	0.629
	Adult	5.41 ± 0.19	1.34	12.74	
ALP (U/L)	Young	78.03 ± 27.73	18.21	78.03	0.518
	Adult	37.82 ± 7.72	2.99	157	
GGT(U/L)	Young	15.74 ± 2.36	10.42	24.32	0.097
	Adult	21.09 ± 1.64	3.47	104.22	
ALB (g/dl)	Young	3.55 ± 0.04	3.17	3.91	0.096
	Adult	3.29 ± 0.04	2.18	4.18	
CHOL (mg/dl)	Young	89.61 ± 6.30	73.65	106.92	0.325
	Adult	82.40 ± 1.76	33.84	132.42	

AST=Aspartate aminotransferase; ALT= Alanine aminotransferase; AST:ALT= Ratio of AST to ALT; ALP=Alkaline phosphatase; GGT=Gamma glutamyl transferase; ALB=Albumin; CHOL=Cholesterol

Sex-related differences in the reference values for serum biochemical markers of liver damage in apparently healthy RSG: The results of sex-related differences in the reference values for serum biochemical markers of liver damage in apparently healthy RSG are presented in table 4. From the results, the mean ALP activity of females was significantly ($p < 0.05$) higher than that of the male counterpart (Table 4).

Age-related differences in the reference values for serum biochemistry parameters of RSG that showed true negativity for liver injury: Results of the age-related differences in the reference values for serum biochemistry parameters of RSG that showed true negativity for liver injury are presented in table 5. The results show that the mean AST activity of the adult

goats was significantly ($p < 0.05$) higher than that of the young while ALP activity was significantly ($p < 0.05$) higher in the young than in the adults. The AST:ALT ratio was also significantly ($p < 0.05$) higher in adults when compared to the young (Table 5).

Sex-related differences in the reference values for serum biochemistry parameters of RSG that showed true negativity for liver injury: The results of sex-related differences in the reference values for serum biochemistry parameters of RSG that showed true negativity for liver injury are presented in table 6. The results indicate that there were no significant differences ($p > 0.05$) between the males and the females for all the assayed parameters (Table 6).

The gross and histomorphologic pictures of *RSG* ***showing true negativity for liver disorder:*** The liver gross pictures (labeled A&B) of the RSG that show true negativity for liver disorder present livers with sharp edges, normal colour and texture of normal livers while the histomorphology (labeled C&D) show normal central veins (CV), portal triads (PT) and hepatocytes (arrows) arranged in cords (Figure 1).

Table 4 Sex-related differences in the reference values for serum biochemical markers of liver damage in apparently healthy RSG

Parameter	Sex	Mean ± SEM	Minimum	Maximum	P value*
AST(U/L)	Male	51.07 ± 5.06	32.74	121.67	0.088
	Female	61.25 ± 2.71	28.60	152.04	
ALT (U/L)	Male	9.97 ± 0.92	5.37	18.49	0.052
	Female	13.28 ± 1.40	2.59	113.41	
AST:ALT	Male	5.49 ± 0.49	2.84	10.48	0.914
	Female	5.43 ± 0.20	1.34	12.74	
ALP (U/L)	Male	33.60 ± 6.91	15.37	135.68	0.010
	Female	43.84 ± 8.05*	2.99	157.00	
GGT(U/L)	Male	17.58 ± 2.24	6.95	42.85	0.185
	Female	21.48 ± 1.82	3.47	104.22	
ALB (g/dl)	Male	3.43 ± 0.06	3.04	3.91	0.058
	Female	3.28 ± 0.04	2.18	4.18	
CHOL (mg/dl)	Male	87.83 ± 4.76	53.95	132.42	0.244
	Female	81.73 ± 1.80	33.84	130.24	

*= Significantly higher than the counterpart at P ≤ 0.05
AST=Aspartate aminotransferase; ALT= Alanine aminotransferase; AST:ALT= Ratio of AST to ALT; ALP=Alkaline phosphatase; GGT=Gamma glutamyl transferase; ALB=Albumin; CHOL=Cholesterol

Table 5 Age-related differences in the reference values for serum biochemistry parameters of RSG that showed true negativity for liver injury

Parameter	Age	Mean ± SEM	Minimum	Maximum	P value*
AST (IU/L)	Young	43.26 ± 1.16	40.80	48.91	0.000
	Adult	54.45 ± 2.40*	28.60	92.04	
ALT (U/L)	Young	10.53 ± 0.95	7.93	13.46	0.726
	Adult	10.95 ± 0.67	2.59	29.65	
AST:ALT	Young	4.22 ± 0.34	3.61	5.35	0.017
	Adult	5.47 ± 0.27*	2.80	12.74	
ALP (U/L)	Young	61.50 ± 2.96*	54.86	71.22	0.000
	Adult	26.89 ± 2.14	2.99	74.74	
GGT(U/L)	Young	18.06 ± 1.99	12.74	24.32	0.456
	Adult	16.29 ± 0.97	3.47	29.4	
ALB (g/dl)	Young	3.35 ± 0.13	3.05	3.69	0.833
	Adult	3.37 ± 0.04	2.75	3.88	
CHOL (mg/dl)	Young	80.02 ± 7.07	63.83	98.36	0.853
	Adult	81.49 ± 2.48	33.84	132.42	

*= Significantly higher than the counterpart at P ≤ 0.05
AST=Aspartate aminotransferase; ALT= Alanine aminotransferase; AST:ALT= Ratio of AST to ALT; ALP=Alkaline phosphatase; GGT=Gamma glutamyl transferase; ALB=Albumin; CHOL=Cholesterol

Table 6 Sex-related differences in the reference values for serum biochemistry parameters of RSG that showed true negativity for liver injury

Parameter	Sex	Mean ± SEM	Minimum	Maximum	P value*
AST(U/L)	Male	48.06 ± 3.76	32.74	84.71	0.132
	Female	55.22 ± 2.66	28.60	92.04	
ALT (U/L)	Male	10.71 ± 1.14	5.37	18.49	0.851
	Female	10.97 ± 0.73	2.59	29.65	
AST:ALT	Male	4.88 ± 0.42	2.84	7.48	0.225
	Female	5.52 ± 0.31	2.80	12.74	
ALP (U/L)	Male	27.96 ± 3.19	15.37	54.86	0.563
	Female	30.49 ± 2.93	2.99	74.74	
GGT(U/L)	Male	17.32 ± 2.35	6.95	42.85	0.655
	Female	16.17 ± 0.95	3.47	28.95	
ALB (g/dl)	Male	3.41 ± 0.07	3.00	3.83	0.609
	Female	3.36 ± 0.05	2.75	3.88	
CHOL (mg/dl)	Male	85.52 ± 5.75	53.95	132.142	0.393
	Female	80.04 ± 2.48	33.84	120.97	

AST=Aspartate aminotransferase; ALT= Alanine aminotransferase; AST:ALT= Ratio of AST to ALT; ALP=Alkaline phosphatase; GGT=Gamma glutamyl transferase; ALB=Albumin; CHOL=Cholesterol
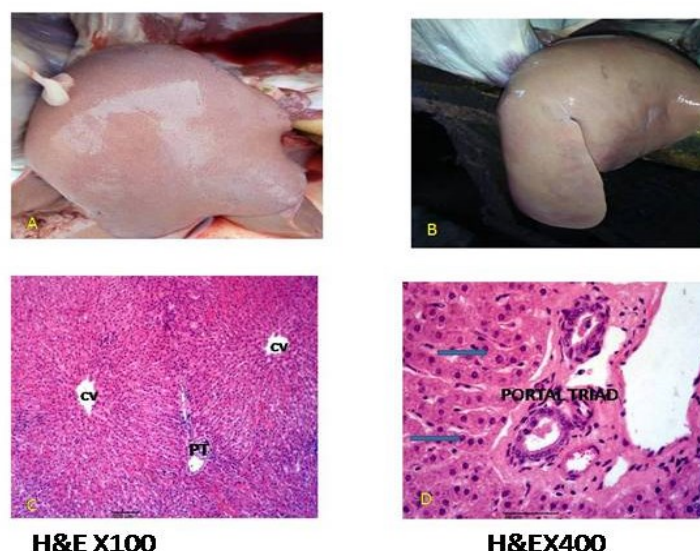
H&E X100   **H&EX400**

Figure 1   The gross and histomorphological pictures of RSG showing true negativity for liver disorder
The liver gross pictures (labelled A&B) of the RSG that show true negativity for liver disorder present livers with sharp edges and the normal colour and texture of normal liver while the histomorphology (labelled C&D) show normal central veins (CV), portal triads (PT) and hepatocytes (arrows) arranged in cords (Figure 1).

Discussion

The results of demographic characteristics of apparently healthy RSG and RSG that show true negativity for liver disorder showed that adult female goats were preponderant when compared to the number of the males and the young ones. This observation may be related to the preference of the buyers, availability or cost effectiveness of the healthy female goats compared to their counterparts. Similar studies have equally corroborated the finding of female adult goats dominating the sample population (Berhanu *et al.* 2012).

The overall results for the reference values of serum biochemical markers of liver disorders for apparently healthy RSG indicate that the value for AST is 59.54 ± 2.43; ALT, 12.72 ± 1.18; AST:ALT ratio, 5.44 ± 0.19; ALP, 38.31 ± 6.63; GGT, 20.83 ± 1.56; albumin, 3.31 ± 0.04 and cholesterol, 82.76 ± 1.70 while those obtained for RSG that were truly negative for liver disorder show that AST is 53.49 ± 2.23; ALT, 10.91 ± 0.62; ALP, 29.88 ± 2.35; GGT, 16.45 ± 0.91; albumin, 3.37 ± 0.04 and cholesterol, 81.36 ± 2.33. These results are within the reference interval for goats as reported by Radostitis *et al.* (2000) and are also consistent with the findings of Matthew (1999). It is observed that the activities of AST, ALT, ALP and GGT for apparently healthy RSG were higher than the corresponding values obtained from the counterpart that showed true negative for liver disorders. This could suggest that the apparently healthy goats may harbour subclinical liver injuries while true negative goats are devoid of both pathological and biochemical lesions of liver injury (Gordis 2009).

On the basis of health status, reference values which were generated from apparently healthy RSG were not found to be significantly ($p > 0.05$) different between the young and the adult. Similarly, the males and the females did not show significant differences in the tested parameters except for ALP activities where the female goats had significantly ($p < 0.05$) higher activities of ALP than their male counterparts. This may probably be as a result of discrepancies in other sources of serum ALP such as from intestine, kidney, pancreas and bone but not from placenta since pregnant does were excluded from the study. Researchers have demonstrated that these organs (intestine, kidney, pancreas and bone) are also potent sources of serum ALP (Sharma *et al.* 2014). These discrepancies from other sources may have contributed to the significantly ($p < 0.05$) higher activities of serum ALP in females than males of apparently healthy RSG.

Results of reference means and intervals for the serum biochemical parameters for age and sex were determined for Red Sokoto breeds of goat using values obtained for samples that were truly negative for liver disorders. True negative samples involve those samples whose biochemical analyses revealed the absence of liver disorders and gross and histopathology assessment also showed the absence of liver lesions (Gordis 2009). The parameters whose normal reference means and intervals were determined included AST, ALT, AST:ALT, ALP, GGT albumin and cholesterol.

Age-related significant differences occurred between young and adults in AST, AST:ALT ratio and in ALP while there were no significant differences in all other parameters. The AST and AST:ALT activities of adult goats were significantly ($p < 0.05$) higher than those of the young goats. The ALP activities of the young goats were significantly ($p < 0.05$) higher than those of the adult goats. Significantly ($p < 0.05$) higher activities of AST of the adult goats compared with the young goats may be associated with more prolonged exposure to minor subclinical injuries that may have accumulated in adults over time compared with the young goats (Thapa and Walia 2007). Significantly higher ALP activity in young goats compared with the adult ones may not be unrelated with the fact that young goats may have a lot of osteoblastic activities going on in their system. The bone is a major source of

ALP in the young where they perform the duty of mineralization during osteogenesis (Golub and Boesze-Battaglia 2007). The results of sex-related reference means and interval of RSG revealed that there were no significant differences between the males and females in any of the parameters considered.

The findings of chocolate brown colouration as the normal colour of the liver of RSG that showed true negativity for liver disorders and the consistent histological findings of polygonal appearance of the hepatocytes are in consonant with the reports of earlier studies by Madhan and Raju (2014). The researchers in a publication entitled "Comparative histology of human and cow, goat and sheep liver", noted that the liver of normal goats was consistently polygonal as opposed to that of the human counterpart that was hexagonal in shape (Madhan and Raju 2014).

It was concluded that the overall reference values for serum biochemical markers for liver damage of apparently healthy RSG are: AST, 59.54 ± 2.43; ALT, 12.72 ± 1.18; AST:ALT ratio, 5.44 ± 0.19; ALP, 38.31 ± 6.63; GGT, 20.83 ± 1.56; albumin, 3.31 ± 0.04 and cholesterol, 82.76 ± 1.70 while those obtained for RSG that were truly negative for liver disorder are: AST, 53.49 ± 2.23; ALT, 10.91 ± 0.62; ALP, 29.88 ± 2.35; GGT, 16.45 ± 0.91; albumin, 3.37 ± 0.04 and cholesterol, 81.36 ± 2.33. The marginally higher values in apparently healthy goats as opposed to those that were truly negative for liver disorders may be attributed to possible subclinical liver disorders in apparently healthy goats.

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