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Influence of gender on some biochemical parameters of apparently healthy local breed sheep in Teamo village, Shebedino district, Sidam zone, Ethiopia

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Abstract

The aim objective of the present study was to determine some biochemical values of sheep with 1-3 years old from the study area Telamo peasant association, Sidama zone and to investigate the effect of gender on these biochemical parameters. Blood samples were collected in plain vacutainer from 140 males and 260 females and their serum biochemical parameters were analyzed to determine total protein, urea, glucose, creatinine, serum enzymes and electrolytes. The effect of gender on all plasma biochemical parameters examined (total proteins, creatinine, alkaline phosphatase (ALP), aspartate aminotransaminase (AST), alanine aminotransaminase (ALT), sodium (Na), potassium (K), Glucose and Urea) was not statistically significant ($P > 0.05$) but absorbed only difference in the mean value of ALP in female (372.62 ± 304.50) higher than male (338.10 ± 259.83); slightly difference in the mean value of ALT in female (15.08 ± 8.85) higher than male (13.51 ± 7.28). These parameters were remained within the normal ranges for healthy sheep. This will help to establish appropriate baseline values for various local livestock.

Keywords: Biochemical parameters, gender, sheep

Introduction

Ethiopia's economy is based on agriculture that accounts for 85% of the total employment and 75% of export [42]. In 2003, agriculture accounted for 42% of the GDP and the livestock sub sector contributed 12-16 % of the total and the 30-35% of agricultural GDP respectively [9]. Livestock is second major source of foreign currency through export of live animals, skins and hides. Ethiopia has huge population of small ruminants with an estimated 23.6 million sheep. The highlands support about 75% of sheep, while the lowlands (mostly pastoral areas) are inhabited by about 25% of sheep [11].

Sheep are play a significant role in Ethiopia's agricultural economy particularly in lowlands, where most are kept by pastoral, agro-pastorals and smallholder production systems. There are important protein sources in the deities of the poor of help to provide extra income and support survival for many farmers in the tropics of sub tropics [16]. In Ethiopia sheep and goat provide 25% of the domestic meat consumption with production surplus, which is exported mainly as live animal about three quarters of the sheep inhabit the cool highland require of Ethiopia. Thought a recent report [1] sheep provides skins, manure and wool sheep production in Ethiopia is based on indigenous breeds except for less than 1% exotic sheep group of mainly Awassi Menz cross breed the livestock sub sector accounts for about 40% of the agricultural GDP of 20% of the total GDP [2] without consideration the contribution of livestock in terms of draught power, manure and transport service.

Small ruminants are major sources of food protein, income saving, skin and manure. The full exploitation of this huge resource is hindered in the tropical environment and particularly in Africa due to a combination of factors such as drought, poor genetic potential of the animal, traditional husbandry system and the presence of numerous diseases [43]. The diseases that affect small ruminants imposes sever economic impact on sheep and goats production; they cause production losses that are manifested by reduced weight gain, lowered meat and milk production and even death especially in the young [18]. Diagnosis of these diseases and assessment of the normal status of the animal mainly relay on understanding the physiological, clinical and serum biochemical parameters based on the established reference values.

Considerable information is available on the normal blood parameter values of domestic

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animal but these values are that of exotic breeds which are somehow different from that of local breeds and there are quantifiable variations in blood biochemical parameters. Meanwhile these normal values of exotic breeds are not available for our indigenous animals' breeds. Blood biochemical parameters including total protein and urea are important indicators of the metabolic activity in lactating animals [25]. Moreover, information is lacking about the effect gender on some biochemical parameters of local breed sheep of Telamo village. As a result it is necessary and worth to establish the serum biochemical value of our indigenous sheep to serve it as a reference in clinical diagnosis.

Variations in blood parameters of animals are due to several factors such as gender, age, feeding level, altitude, breed, diurnal and seasonal variation, temperature and physiological status of animals [32]. Serum biochemical tests are widely used for the diagnosis of serious animal diseases which can lead to economic losses in animals like reduced fur, wool and milk production [6].

The information gained from blood parameters value would substantiate the physical examination and coupled with clinical history provide excellent basis for medical interventions [19, 20, 30]. Information regarding the normal blood biochemical values were lacking in the studied indigenous sheep breed in Ethiopia. Therefore, the objectives of the present study were to provide information on some biochemical parameters of sheep from Telamo village, Sidama zone in Ethiopia and to investigate the effect of gender on these parameters in apparently healthy, non-pregnant indigenous sheep breed on natural grazing land in Telamo village, Sidama region of Ethiopia.

Materials and methods

Study Area

This study was carried out in the Telamo area, Shebedino district, Sidama zone of Southern Nations, Nationalities and Peoples Region (SNNPRs) of Ethiopia. The zone is located in the northern part of SNNPRs, with its capital town at Hawassa, which lies about 275 km south of Addis Ababa. Geographically, the study area located between between 6°10'-7°12' North latitude and 38° -39°11' East longitude 302km from Addis Ababa. It consists of 19 districts of which Shebedino district is one of the Sidama region with a total land area of 26,990 hectare. Out of this, 5,229 hectare is covered by annual crop such as maize, wheat, pea, beans, potatoes and 16,497 hectare is covered by perennial crops such as 'inset', 'chat', coffee, the remaining 5,264 hectare of land is covered with bushes, hillside and un productive land. The Altitude range is from 1500-2500meter above sea level and the annual rain fall range is from 900-1500mm per year. The average daily ranges of maximum and minimum temperature are 16 °C and 25 °C, respectively. The study area Telamo is one of the peasant associations (PAs) 10 km far away from Shebedino district that located at an altitude 2000meter above sea level. The dry season from November to February while the rainfall occurring between March and April and from late May to October [38].

Study animals

Apparently healthy sheep (1-3 years age) owned by the farmers were considered from Telamo village and their surroundings of Sidama zone, Ethiopia.

All sheep were reared in extensive farming system (free to

graze on pasture with rare provision of other supplements like straw and grains mostly after work). The criteria considered being animals apparently healthy the following conditions were included: study animal is normal physiological parameters (temperature, pulse rate respiratory rate), normal body condition and normal feeding habit ate and stand on all of its feet. The blood samples were screened for the presence of haemoparasites using standard laboratory techniques [13, 36]. Faecal sample from each animal was collected and examined for the presence of helminth ova using floatation method [8, 41]. All sheep are routinely vaccinated for FMD, PPR and Anthrax. Animals with lesions, gastrointestinal or haemoparasites were excluded from the study.

The study animals were grouped into males and females group with 1-3 years age. All sampling units were tagged and sampling carried on after two weeks of deworming with broad spectrum Albendazole 300mg (Ashish Life Science Ltd, Mumbai, India) at a dose of 7.5mg/kg of body weight. Physiological parameters of 400 local breeds of sheep were recorded (rectal temperature, heart rate, respiratory and pulse rate). From all 400 apparently healthy sheep (140 male and 260 females' sheep) were taken 7 ml blood samples from jugular vein into plain tubes for serum biochemistry analyses.

Sample size determination

Local breed in the study area was used as a sampling population 400 apparently healthy sheep were selected using purposive sampling method.

Study design and methodology

A cross sectional study has been conducted from September 2009 to June 2010 to obtain base line information concerning the serum biochemical parameters in apparently healthy local breed sheep from Telamo village of Sidama zone.

Collection of blood samples

Blood samples 7ml were collected from jugular vein without EDTA for serum separation.

Assessment of physiology parameters

Assessment of important physiological parameters like body temperature, respiratory rate, heart and pulse rate were taken two times per day (morning 8.30am and afternoon 5.00pm) to observe and check the variations in the bodies of the study animals. For proper measurement and to minimize errors in sampling a well recording format and animal identification with temporary paints was used. Body temperature was taken by digital thermometer, and is achieved by first lubricating the bulb end of the thermometer to being gently inserted with a rotatory action through the anal sphincter into the rectum and held there until keeping sound is heard. Care has been taken to ensure that the bulb of the thermometer makes contact with the mucous membrane of the rectum. After each reading the thermometer has been cleaned with gauze and placed into its cover. Pulse rate has been taken at the external maxillary artery on the medial aspect of the ventral border of the mandible by counting the number of pulses per minute according to [26]. Respiratory rate was taken by placing stethoscope on the trachea for one minute to get the number of breaths per minute. Similarly heart rate was taken with stethoscope under the animal's forelimb in the heart area on the left side of the animal and beats within one minute were counted and recorded. Gut sounds were measured using stethoscope.

Serum biochemical profiles

In the current study blood samples were collected from the study sheep while they are at rest and under conditions of least excitement. Seven ml of blood was collected from the jugular vein in plastic tube without anticoagulants from all study animals before grazing for biochemical analyses. Serum from the sampled blood in plain vacutainer tube is kept by centrifugation (3000 rpm for 15 minutes) and then only the serum part was decanted into another plain tube. While transporting the serum to the laboratory all samples were kept in icebox with icepack. The serum samples were stored at -20°C until analyses [7, 15, 21, 22, 39]. The serum sample is analyzed at Addis Ababa University, College of Veterinary Medicine and Agriculture, Physiology laboratory.

The levels of plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatinine, total protein and glucose were analyzed using photometer 5010 (Robert Riele GmbH & Co KG, Germany, 2002) and commercially available kits. The level of plasma enzymes was expressed in U/L. The level of plasma AST (GOT) and ALP was determined using kits (Biocon Diagnostik, Germany), whereas the level of plasma ALT (GPT) was obtained using commercial (Human, Germany) kits. Kinetic method was employed for the determination of the level of AST, ALP and ALT. The absorbance of ALP was read at 400nm wavelength, while that of AST and ALT were read at 340nm wavelengths. Plasma creatinine concentration (mg/dl) was determined by kinetic colorimetric assay using a kit (Biocon Diagnostik, Germany). The absorbance was read at 492nm wavelength. The level of total protein (g/dl) in the plasma read at wavelength of 546nm was determined by colorimetric assay using commercially available kit (Biocon Diagnostik, Germany). Serum electrolytes like sodium and potassium were analyzed using Roche AVL 9180 Snap pack Electrolyte Analyzer (Roche Diagnostic Corporation 9115 Hague road, USA, 2002). To ensure the accuracy of the test

results biochemical analyzers and reagents were checked daily with quality control kits of known values for the different parameters. The accuracy and reliability of the procedures, instruments were ensured by quality controls. The quality controls were performed before analyzing the samples, after replacement of reagents, at maintenance and whether there is any doubt about the accuracy of the analyses.

Data analysis

Data were entered in to Microsoft excel spread sheet from which dependent variables of the serum biochemical values; whereas the independent ones were gender (sex) and age. Before applying a statistical analysis, data were checked for normality. The statistical analysis was performed using the SPSS 15.0 for windows package (2003). Descriptive statistic and Independent sample T-test was applied to determine the means (\pm sd) range and the 95% confidence interval (CI). Comparisons of gender differences were analyzed after setting the level of significance using independent t-test and one-way ANOVA. Level of significance was taken at $p < 0.05$.

Results

The results of some serum biochemical parameters were expressed as means \pm standard deviations (\pm sd), 95% confidence interval and range (minimum - maximum values).

Analysis of serum biochemical parameters

Table 1 showed that the result of biochemical parameters of sheep affecting by gender. Even though, in this study the effects of gender on biochemical values of sheep were not observed statistically significance ($p > 0.05$) but only absorbed difference in the mean value of ALP in female higher (372.62 ± 304.50) than male (338.10 ± 259.83); slightly difference in the mean value of ALT in female higher (15.08 ± 8.85) than male (13.51 ± 7.28) (Table 1).

Table 1: Effect of gender on serum biochemical parameters in Sheep.

Parameters	Sex	N	Mean \pm SD	95% Confidence Interval for Mean	Range	P-value
AST (U/l)	Male	114	67.61 \pm 25.92	62.195-73.38	10-216	0.906
	Female	286	67.97 \pm 28.16	65.006-72.036	11-301	0.902
ALT (U/l)	Male	114	13.51 \pm 7.28	11.548-14.735	7-56	0.093
	Female	286	15.08 \pm 8.85	13.901-396.23	6-75	0.068
ALP (U/l)	Male	114	338.10 \pm 259.83	287.782-396.23	19-1401	0.287
	Female	286	372.62 \pm 304.50	339.148-407.32	22-1993	0.255
Protein (g/dl)	Male	114	5.04 \pm 1.18	4.8045-267-5.26	1-7	0.377
	Female	286	4.91 \pm 1.27	4.767-5.058	1-8	0.363
Glucose (mg/dl)	Male	114	28.39 \pm 12.31	25.144-29.90	7-76	0.878
	Female	286	28.19 \pm 12.24	25.900-28.89	8-75	0.878
Sodium (mmol/l)	Male	114	144.28 \pm 9.82	142.858-145.67	82-180	0.091
	Female	286	145.71 \pm 6.51	144.822-146.59	129-180	0.155
Potassium (mmol/l)	Male	114	5.592 \pm 0.81	5.432-5.72	3.0-8.1	0.739
	Female	286	5.563 \pm 0.79	5.470-5.65	3.0-9.3	0.743
Creatinine (mg/dl)	Male	114	0.976 \pm 0.27	0.912-0.4	1-1.8	0.883
	Female	286	0.954 \pm 0.25	0.911-0.3	1-1.6	0.823
Urea (mg/dl)	Male	58	46.07 \pm 37.21	35.645-57.58	8-206	0.628
	Female	208	49.07 \pm 42.88	47.121-59.26	2-321	0.601

Discussion

The present study showed that the reference range of some serum biochemical parameters of sheep in Telamo village, Shebedino district of Sidama Zone. The overall result indicated that most of the biochemical parameters are within the normal reference values.

The increase in urea level in three factors age, sex and altitude might be due to the feed type and protein metabolism. Comparison of the present result with mean value and reference ranges for sheep not shows big variations may be described to differences in laboratory equipments [26]. In this study the total protein values were higher in the male than in

the female Sheep. These results were in contrast with findings of Awah and Nottidge, H.O (1998) in Dogs [5].

To compare the result of sheep parameters there were no previously published works in the study areas. These parameters are usually affected by the level of nutrition and closely associated with metabolic activities of individual animals. Sheep were within the normal range reported for the ovine specie [17]. Although, there were mean differences in some of blood biochemical values in this study but the data were within the normal ranges for apparently healthy sheep.

Total protein is an important factor for blood viscosity, acid-base balance, and supplying necessary enzymes [27]. The mean total protein values obtained from sheep males and females were within the range of 5.5-10.0 g/dl reported for various ruminant species [23]. Plasma protein level was slightly higher in male (5.04±1.18) than female (4.91±1.27) but the difference was not statistically significant. This finding is in agreement with the results obtained by Khan (2013) [28] who also reported higher total protein values in male (8.32 ± 0.38) than in female (8.18 ± 0.37) sheep.

Urea is an important metabolite synthesized from ammonia in the liver during protein metabolism [27]. In this study plasma urea level was higher in females (49.07±42.88) than males (46.07±37.21) but the difference was not statistically significant. These result were not similar in observation was reported by Borjesson *et al.* (2000) [10] in their study on desert bighorn sheep.

Plasma creatinine concentration was slightly higher in males (0.976±0.27) than females (0.954±0.25) but the difference was no statistically significant. The amount of creatinine secreted daily is a function of the muscle mass and is not affected by age, sex, altitude. Female excrete less creatinine than males because of their smaller muscle mass [3].

The results of the present study showed that the effect of gender on both plasma enzymes and electrolytes is not statistically significant. Enzymes are protein catalysts synthesized by all living organisms. They are constantly and rapidly degraded but the supply is renewed by new synthesis [12]. ALT is an enzyme found in the highest amount in liver and typically used to detect liver injury [37]. ALT values were slightly lower in males (13.51±7.28) than females (15.08±8.85) but not similar in comparing with the mean values of ALT (10±1.1 U/L) that reported by Milostav *et al.* [33] in West African sheep.

AST is found in practically every tissue of the body, and the measurement of the AST levels is helpful for the diagnosis and following case of myocardial infarction, hepatocellular disease and skeletal muscle disorders [35]. AST is an enzyme abundantly found in liver and heart muscles and plays an important role in amino acid metabolism [42]. AST values for males were lower (67.61±25.92) than those of females (67.97±28.16) which was in agreement with result reported by Mabruka Saleh (2014) [31]. The level of ALP (alkaline phosphatase) obtained in this study was higher in females (372.62±304.50) than males (338.10±259.83) but earlier report in desert bighorn sheep by Borjesson *et al.* (2000) [10] showed higher alkaline phosphatase activity in young male with less than one year old sheep. Duncan *et al.* (1994) [14] and Kock *et al.* (1987a) [29] suggested that the multiple isoenzymes of ALP and its wide reference range in domestic ruminants rendering it an insensitive clinical indicator of disease. The activity of ALT, AST, and ALP reported in the present study was not significantly influenced by gender. This

finding is similar with the results reported by Mostaghni *et al.* (2005) [34] and Khan (2013) [28].

Maintaining electrolytes in appropriate amounts is essential for normal biochemical and physiological functions of the body. Electrolytes are distributed in body fluids and play a key role in all parts of animal life [24]. The mean plasma potassium value was higher in males (5.592±0.81) than females (5.563±0.79) and the mean sodium level was also higher in females (145.71±6.51) than males (144.28±9.82) in agreement with result reported by Mabruka Saleh (2014) [31] in sheep but the difference for all electrolytes between both genders was not statistically significant.

AL-Hadithy *et al.* (2012) [4] reported in a study on Iraqi Awassi sheep higher blood sodium and potassium levels than the values reported in the present study. The gender effect on blood electrolytes level in that study was significant with levels higher in males than females. Similar observation to the findings reported in the present study for gender effect on blood electrolytes in sheep has been reported by Sowande *et al.* (2008) [40] who found no significant difference between males and females for blood electrolytes.

Conclusion and recommendations

In the present study biochemical values determined for sheep were slightly different from previously known reference values. Most of the biochemical parameters there was no significant influence by gender. In the result should be given attention in utilizing the values of these parameters for assessing the biochemical parameters of animals for diagnostic purpose. Based on the results of this study the following points are recommended:

- Reference values establishment for indigenous sheep for mandatory for diagnosis a disease conditions. Therefore, further studies should be carried out to establish the serum biochemical values of the indigenous sheep species in different regions of Ethiopia.
- The results obtained in the present study likely represent most of healthy sheep in Ethiopia and constitute potential reference values that can be used for clinical purposes against the previous known values. Because of the possible individual or combined influence of different factors other than gender, and the subsequent interpretation of normal ranges in sheep for pathological condition should be carefully considered.
- The findings of this study may serve as references values to determine the healthy status of animals.

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Competing Interest

The authors declare no conflicts of interest.

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