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## **Influence of age and altitude on some biochemical values in apparently healthy local breed sheep in Shebedino district of Sidama Zone, Ethiopia**

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**Abstract**

This study was done to determine the impact of age and altitude on some biochemical parameters in apparent healthy local breed sheep at Shebedino district Sidama zone of Ethiopia. Total 400 sheep aging 1 -3 and 3-6 years old are used. A cross sectional study was conducted from September 2009 to June 2010 in sheep in Shebedino district Sidama zone in order to obtain base line information concerning the normal serum biochemical parameters, to identify the influences of age and altitude on serum biochemical parameters in apparently healthy local breed sheep. Biochemical parameters of local breed's sheep were determined consisting of 400 apparently healthy indigenous sheep breed in Shebedino district with different age groups (1-3 years and 3-6 years aged) from two different altitudes (high and midland). The study showed that only urea was a significant difference ( $P < 0.05$ ) between the two age groups in the study area but the rest of biochemical parameters such as ALT, AST, ALP, Protein, Glucose,  $\text{Na}^+$ ,  $\text{K}^+$  and Creatinine were not significant difference ( $P > 0.05$ ). For the two different altitudes effects (highland and midland) in serum biochemical parameters only ALP and  $\text{Na}^+$  were highly significant associated ( $P < 0.05$ ) whereas the level of  $\text{K}^+$  was significant ( $P < 0.05$ ). Therefore, this study has indicated serum biochemical values that could serve as baseline information for comparison in conditions of nutrient deficiency, physiological and health status of sheep kept under native husbandry system in Sidama zone of Southern Nations, Nationalities and Peoples Region (SNNPRs) of Ethiopia.

**Keywords:** Age, Altitude, Biochemical parameters, Sheep

**Introduction**

Ethiopia's economy is based on agriculture that accounts for 85% of the total employment and 75% of export [46]. 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 [8]. 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 [15].

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 [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 without consideration the contribution of livestock in terms of draught power, manure and transport service [2].

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 [44]. 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 [19].

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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]. Variations in blood parameters of animals are due to several factors such as altitude, feeding level, age, sex, breed, diurnal and seasonal variation, temperature and physiological status of animals [32].

Establishment biochemical reference intervals are an important tool to assess health of animals and understand the impact of disease on individual and population levels [17, 18]. Nutrition, stress, reproductive status, age, sex, genetics, management, housing, and other environmental factors (temperature, relative humidity etc.) are known to have a profound effect on the biochemical profiles of small ruminants [4, 35].

The metabolic profiles of the sheep, containing serum mineral, hormones and biochemical parameters were important to determine both health conditions, feed status and metabolic conditions, which are led to improve the production and reproduction of animals [3, 24]. On the other hand, the physiologic and pathologic trails can be better when values of blood parameters were around within natural rang [34]. Blood parameters are changed by many factors, including management, feeding system, age, sex, breed, environment conditions, etc. [26, 33]. Many studies were reported that the biochemical parameters values vary between young and adult sheep and these values are changing with age [3, 27, 36]. Based on our information, there are lack studies about the impact of age and altitude in local breed sheep, therefore, the aim of this study was to shown the effect of age and altitude on the levels of some biochemical parameters of sheep in Shebedino area, Ethiopia.

Considerable information is available on the normal blood parameter values of domestic 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 [28]. During pregnancy, maternal tissues are involved in providing energy for reproduction processes, which may affect blood serum chemistry values, and also affected by several other factors such as breed, age, malnutrition, fetal growth, or season [40, 45]. Moreover, information is lacking about the effect age and altitude on the biochemical values of local Shebedino breed sheep. 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 altitude, feeding level, age, sex, 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]. Blood biochemical tests have been widely used for the diagnosis of various animal diseases [5]. The information gained from blood parameters value would substantiate the physical examination and coupled with clinical history provide excellent basis for medical interventions [20, 21, 31].

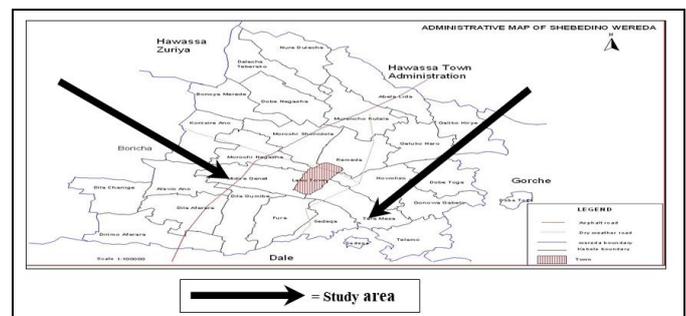
Information regarding the normal blood biochemical values

were lacking in the studied indigenous sheep breed in Ethiopia. Therefore, the main objectives of this study were to determine the values of some biochemical parameters in apparently healthy indigenous sheep breed on natural grazing land in highland (Telamo) and midland (Morocho Negasha) of shebedino district, Sidama zone and to analyze the influence of age and altitude on those values.

## Materials and Methods

### Study Area

This study was carried out in the Sidama zone of Southern Nations, Nationalities and Peoples Region (SNNPRs). 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  $6^{\circ}10' - 7^{\circ}12'$  North latitude and  $38^{\circ} - 39^{\circ}11'$  East longitude 302km from Addis Ababa. It consists of 19 districts of which Shebedino district is one of the Sidama region district. Shebedino is a district 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  $16^{\circ}\text{C}$  and  $25^{\circ}\text{C}$ , respectively. The study area comprises of two Peasant Associations (PAs) representing highland (Telamo) and midland (Morocho Negasha). Telamo one of the PAs 10 km far away from Shebedino that located an altitude 2000meter above sea level and Morocho Negash also one of the Peasant Associations 4 Km far away from Shebedino that located an altitude 1500 meter 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].



Source: SZPEDO (2009)

Fig 1: The Map of the study area.

### Study animals

Apparently healthy sheep owned by the farmers were considered from two ecological zones of highland (Telamo) and midland (Morocho Negasha), and their surroundings of Sidama zone. 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 habitate and stand on all of its feet. The blood samples were screened for the presence of haemoparasites using standard laboratory techniques [9, 36]. Faecal sample from each animal was

collected and examined for the presence of helminth ova using floatation method [8, 42].

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 two age groups (1-3 years and 3-6 years old); and highland and midland based on altitude. 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 were taken 7 ml blood samples from jugular vein into plain tubes for serum biochemistry analyses.

#### Sample size determination

Local breeds of sheep in the study area were 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 of Shebedino.

#### Collection of blood samples

Blood samples 7ml were collected from jugular vein into plain tubes 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 Kellay W.R. [28]. 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

Blood samples were collect 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 animals before grazing for biochemical analyses. Serum from the sampled blood in plain vacationer 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. All serum samples were stored at -20°C until analyses [7, 11, 22, 24, 38]. 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.

#### Questionnaire survey

Before the commencement of sampling work, a pilot survey was done with the collaboration of peasant associations (PAs) elders and district agricultural officers to know the distributions and conditions of sheep within the district of the study area. The target community was selected from 100 household's level. Fifty of the households were included in the questioner survey. A questionnaire survey was conducted in order to gain problem solving information about the production and the management, disease type, feedings and economic contribution of sheep.

#### Data analysis

Data were entered in to Microsoft excel spread sheet from which dependent variables of the clinically important and serum biochemical values; whereas the independent ones were age and altitude. 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 age and altitude 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, 95% confidence interval and range (minimum - maximum values).

### Analysis of serum biochemical parameters

Tables 1 and 2 showed that the result of biochemical parameters of sheep affecting by age and altitude, respectively.

In the serum biochemical profile, only urea was a significant difference ( $P < 0.05$ ) between the age group 1-3 ( $52.54 \pm 44.57$ ) and above 3 age group ( $38.24 \pm 31.58$ ) of Sheep in the study area. This result was in contrast with the finding of Rabee A.S. *et al.*, (2014) [35] but the rest of biochemical parameters were not significant difference ( $p > 0.05$ ) (Table 1).

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

Parameters	Age	N	Mean $\pm$ SD	95% Confidence Interval for Mean	Range	P-value
AST/GOT (U/l)	1-3	300	68.23 $\pm$ 28.40	65.00-71.45	11-301	0.814
	>3	100	67.48 $\pm$ 24.20	62.68-72.28	10-186	0.799
ALT/GPT (U/l)	1-3	300	16.52 $\pm$ 8.44	15.56-17.48	6-75	0.516
	>3	100	15.91 $\pm$ 7.11	14.50-17.32	7-56	0.480
ALP (U/l)	1-3	300	356.20 $\pm$ 295.98	322.57-389.83	19-1993	0.437
	>3	100	382.52 $\pm$ 282.59	326.45-438.59	28-1236	0.427
Protein (g/dl)	1-3	300	4.92 $\pm$ 10.27	4.78-5.06	1-8	0.447
	>3	100	5.03 $\pm$ 10.17	4.80-5.26	2-8	0.429
Glucose (mg/dl)	1-3	300	27.69 $\pm$ 12.30	26.29-29.08	7-75	0.114
	>3	100	29.92 $\pm$ 11.99	27.54-32.30	10-76	0.111
Sodium (mmol/l)	1-3	300	144.96 $\pm$ 7.69	144.09-145.84	82-180	0.126
	>3	100	146.31 $\pm$ 7.31	144.86-147.76	135-180	0.118
Potassium (mmol/l)	1-3	300	5.602 $\pm$ 0.82	5.508-5.695	3.0-9.3	0.184
	>3	100	5.479 $\pm$ 0.71	5.338-5.620	3.0-7.3	0.154
Creatinine (mg/dl)	1-3	300	0.981 $\pm$ 0.28	0.949-1.014	1-2.4	0.936
	>3	100	0.978 $\pm$ 0.51	0.876-1.080	1-5.5	0.951
Urea (mg/dl)	1-3	187	52.54 $\pm$ 44.57	46.13-58.96	2-321	0.010*
	>3	79	38.24 $\pm$ 31.58	31.17-45.32	1-206	0.003*

\* Significance difference at  $P < 0.05$

For the two different altitudes effects (highland and midland) in serum biochemical parameters, ALP and sodium were

highly significant associated ( $P < 0.05$ ). And the level of potassium was significant in the two altitudes (Table 2).

**Table 2:** Effect of altitude on serum biochemical parameters in Sheep.

Parameters	Altitude	N	Mean $\pm$ SD	95% Confidence Interval of mean	Range	P-value
AST (U/l)	Highland	199	69.62 $\pm$ 30.42	65.357-73.779	1-301	0.209
	Midland	201	66.13 $\pm$ 24.71	62.865-71.265	10-187	0.209
ALT (U/l)	Highland	199	14.42 $\pm$ 8.09	12.915-15.326	8-75	0.617
	Midland	201	14.85 $\pm$ 8.80	13.483-15.887	6-64	0.617
ALP (U/l)	Highland	199	299.21 $\pm$ 248.79	258.938-338.69	19-1993	0.000***
	Midland	201	425.72 $\pm$ 318.55	389.863-469.41	54-1631	0.000***
Protein (g/dl)	Highland	199	4.83 $\pm$ 1.40	4.655-5.00	1-8	0.060*
	Midland	201	5.06 $\pm$ 1.06	4.892-5.23	2-7	0.060*
Glucose (mg/dl)	Highland	199	28.31 $\pm$ 12.73	25.309-28.89	9-76	0.914
	Midland	201	28.18 $\pm$ 11.77	25.973-148.21	7-68	0.914
Sodium (mmol/l)	Highland	199	147.18 $\pm$ 7.13	146.149-148.21	133-180	0.000***
	Midland	201	143.44 $\pm$ 7.64	142.396-144.45	82-180	0.000***
Potassium (mmol/l)	Highland	199	5.460 $\pm$ 0.80	5.350-5.57	3.0-8.0	0.006*
	Midland	201	5.681 $\pm$ 0.77	5.564-5.78	3.8-9.3	0.006*
Creatinine (mg/dl)	Highland	199	0.976 $\pm$ 0.46	0.926-1.02	1-5.5	0.797
	Midland	201	0.985 $\pm$ 35.34	0.935-1.03	1-1.6	0.798
Urea (mg/dl)	Highland	199	48.99 $\pm$ 35.34	45.804-58.14	2-206	0.703
	Midland	201	46.75 $\pm$ 56.48	40.170-61.24	3-321	0.760

\*\*\*Highly Significance difference at  $P < 0.05$

\* Significance difference at  $P < 0.05$

### Discussion

The present study showed that the reference range of serum biochemical parameters of sheep in Shebedino district of Sidama Zone. The overall result indicated that most of the biochemical parameters are within the normal reference values. In some of biochemical analyses the study has shown that there are some variations. The values of Urea by age was statically significance ( $P < 0.05$ ) whereas AST, ALT, Protein, ALP, Creatinine and electrolytes (Sodium and Potassium) were not significance difference ( $P > 0.05$ ). In contrast to this study Red Sokoto oats reported that age has a significant

effect ( $P < 0.05$ ) on ALP [40]. Even though, age no significant associations ( $p > 0.05$ ) were observed in most of the biochemical parameter values on the study animal.

In the altitude the values of ALP and Sodium were highly significance ( $P < 0.05$ ) but for Potassium was significance. The biochemical values of total protein, Glucose, Creatinin, urea, ALT AST were not significance ( $P > 0.05$ ). Although an increase of ALP level can be influenced by pregnancy. An increased the ALP could be possible to attribute to different seasons, feed conditions and local environment [12, 21, 40, 41]. AST, ALP and Urea were higher than the range value where

as the level of Glucose was lower than the range values and then the others biochemical parameters were in normal range (ALT, Protein, Glucose, creatinin, Sodium and Potassium). 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<sup>[46]</sup>. In this study age had no effect on serum glucose concentrations in sheep. This result agrees with the results of Eshratkhan, B. *et al.* (2008)<sup>[13]</sup> and Kiran, S. (2012)<sup>[29]</sup>.

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<sup>[15]</sup>. Although, there were significant differences in some of blood biochemical values in this study but the data were within the normal ranges for apparently healthy sheep.

### Conclusion and Recommendations

In the present study biochemical values determined for sheep were slightly different from previously known reference values. The serum biochemical values were also affected by age and altitude. Generally, from all the factors considered for their effects on the biochemical parameters in this study, altitude showed prominent effect on some biochemical values than age. 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 above results of this study the following points are recommended:

- The results obtained in the present study more 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 age and altitude, and the subsequent interpretation of normal ranges in sheep for pathological condition should be carefully considered.
- 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 in different areas of Ethiopia.
- The findings of the study may serve as references values to determine the healthy status of local breed Sheep in Shebedino areas.

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### Competing Interests

The authors declare no conflicts of interest regarding the publication of this paper.

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