ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF VETERINARY MEDICINE

STUDY ON PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS IN APPARENTLY HEALTHY SHEEP IN AND AROUND SHEBEDINO WEREDA IN SIDAMA ZONE

BY

AYNALEM GEMECHU

JUNE, 2010

DEBRE ZEIT, ETHIOPIA

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A thesis Submitted to the School of Veterinary Medicine, Addis Ababa University in partial fulfillment of the requirements for the Degree of Master of Science in Animal Physiology

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LIST OF ABBREVIATIONS

ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
B/min	Breath per minute
B/min	Beat per minute
CK	Creatine Kinase
CSA	Central Statistical Authority
EDTA	Ethylene diamine tetracetic acid
FAO	Food and Agricultural Organization
g/dl	Gram per deciliter
GDP	Gross Domestic Product
GGT	Gamma glutamyl transferase
GOT	glutamic oxaloacetic transaminase
GPT	glutamic pyruvic transaminase
ha	Hectare
Hb	Hemoglobina
LAP	leucine amino peptidase
LDH	Lactic dehydrogenase
m.a. s .l	above sea level
mg/dl	Milligram per deciliter
mmol/l	Milimole per liter
nm	nano meter
N	number of sheep
^0C	Degree Celsius
Pa	Peasant Association
PCV	Packed Cell Volume
rpm	Revolution per Minute
SNNP	Southern nation nationality people
SPSS	Statistical Programme for Social Sciences
U/l	Unit per liter
µl	micro liter

ABSTRACT

This study was performed to evaluate the effect of age, sex and altitude on normal physiological and blood biochemical values in apparently healthy sheep in Shebedino wereda of southern region of Ethiopia. Blood samples were taken for biochemical analyses, which includes serum Aspartate aminotransferase(AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), total protein, urea, creatinine, glucose and others electrolytes (sodium and potassium) were analyzed. This research field study was conducted in Shebedino woreda of Sidama zone at two Peasant Associations (PAs) from high and mid land agro-ecology. A total of four hundred sheep's from which one hundred forty males and two hundred eight six females with an age of one up to six years were sampled on the study. Physiological and biochemical parameters where taken for an assessment of the effect of these parameters. The study result shows that physiological and biochemical parameters value varies only in different age, sex and altitude. The physiological and biochemical parameters of sheep were determined in four hundred sheep consisting of by age groups (one up to three and more than three years), sex (male and female) and altitude. The mean of physiological value temperature in age categories (one up to three and more than three years) was significant ($P<0.05$) where as respiratory and pulse rate were not significance ($P>0.05$) and were observed rectal temperature, respiration and pulse rate for the sex groups. The values obtained for serum Alkaline phosphatase (ALP), Glucose and urea were out of normal range where by serum Alkaline phosphatase (ALP) and urea are higher and serum Glucose was lower when compared to the normal biochemical parameters where as the rest of parameters such as Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Total Protein, Creatinine, Sodium and Potassium in this study were found in normal range. In the serum biochemical value, 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 Alanine aminotransferase(ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), total Protein, Glucose, Creatinine, Sodium and Potassium were not significant difference ($p>0.05$). This study has indicated that physiological and serum biochemical values and could serve as a baseline information for comparison in conditions of nutrient deficiency, physiological and health status of sheep.

KeyWord:Physiological,Biochemical,Parameters,Sheep

1. INTRODUCTION

Ethiopia's economy is based on agriculture that accounts for 85% of the total employment and 75% of export (Zewdu, 1995). 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 (Belachew, 2004). 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 and 33.3 million goats (CSA, 2006). The highlands support about 75% of sheep and 27% of goats, while the lowlands (mostly pastoral areas) are inhabited by about 25% of sheep and 73% of goats. It has the third largest number of sheep and goats among African nations and ranks eighth in the world (Alemu and Merkel, 2008). Sheep and Goat contribute a quarter of the domestic meat consumption; about half of the domestic wool requirements; about 40% of fresh skin and 92% of the value semi processed skin and hides export trade. It is estimated that 1.078,000 sheep and 1,128,000 goat are used in Ethiopia for domestic consumption annually (Alemu and Merkel, 2008).

Sheep and goat require small investment; have shorter production cycles, faster growth rate and greater environmental adaptability. Goat and 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 deites of the poor of help to provide extra income and support survival for many farmers in the tropics of sub tropics (FAO, 2005). 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 (Aklilu *et al.*, 2005) 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 (Aklilu,2002) 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 (Waruiru *et al.*, 2005). 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 (Githigia *et al.*, 2005). 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 animal. But these values are that of exotic breeds which are some how different from that of local breeds and there are quantifiable variation in blood parameters particularly in hemoglobin concentration and PCV values. Meanwhile these normal values of exotic breeds are not available for our indigenous animals' breeds. Variations of blood parameters value in animals have been reported due to several external and internal factors such as altitude, management system, feeding level, age, sex, breed, health status, method of blood collection, laboratory techniques used, diurnal and seasonal variation, ambient temperature, and physiological status of the animal (Sherman and Mary, 1994). Pregnancy and lactation are physiological statuses considered to modify metabolism in animals (Krajnicakova *et al.*, 2003; Iriadam 2007). Blood biochemical parameters including total protein and urea are important indicators of the metabolic activity in lactating animals (Karapehlivan *et al.*, 2007). 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 (Swanson et al., 2004; Yokus *et al.*, 2006).

Moreover, information is lacking about the effect of physiological and environmental factors on the normal physiological and biochemical values (Bekele, 1987; Fufa, 1999; Yasmin, 2000). As a result it is necessary and worth to establish the physiological and serum biochemical value of our indigenous breeds of animals has enables to use them as a reference in clinical diagnosis. Blood biochemical tests have been widely used for the diagnosis of various animal diseases (Bani Ismail *et al.*, 2008). The information gained from blood

parameters value would substantiate the physical examination and coupled with clinical history provide excellent basis for medical interventions (Gootwine, *et al.*, 1995; Gootwine and Pollott, 2000; Kridli *et al.*, 2006).

This paper reports the observations made on clinical, physiological and biochemical parameters of sheep in selected district of Sidama zone, Southern Nations Nationalities and Peoples Regions State of Ethiopia.

Therefore, the objectives of the current study were
:

- To determine the normal physiological and serum biochemical values of apparently healthy sheep in Shebedino district, Sidama zone, Southern Ethiopia.

- .To investigates the effect of sex, age and altitude factors on the physiological and serum bio chemical parameters of the sheep in shebedino district.

2. LITERATURE REVIEW

Sheep (Ovis aries) are believed to have been among the first animals to be domesticated preceded by the goat. The domestication of both sheep and goats probably dates back to the resettled agricultural period. It is also believed that most domestication took place in western Asia where the majority of the present day small ruminant breeds likely originated. Existence of some ancestral stock of wild sheep extends from Western Europe to China.

In Ethiopia, there exists a great variation in climate and topography harboring diversified livestock species, which have variability among themselves. The sheep types found in Ethiopia falls into different breeds and types, whose habitat ranges from tropical to temperate environments. The present fat-tailed sheep of Ethiopia that are believed to have replaced the original African Long-thin tailed sheep came from Asia through the strait of Bab Elmandeb. Although sheep were domesticated as dual purpose animals to produce wool and meat, early people would have valued sheep milk (Alemu and Merkel, 2008).

2.1 Blood and importance of determining biochemical parameters

Blood is a specialized connective tissue within the vascular system of animal and one of the most important and frequently examined indexes of physiological and pathological alterations in animals and human (Andrews *et al.*, 1990). The blood in an animal serves as a transport medium. Whole blood comprises of fluid and cells. The fluid component is plasma, which comprises about 90% water and 10% dissolved constituents such as proteins, carbohydrates, vitamins, hormones, enzymes, lipids, salts, waste materials, antibodies, other electrolytes and molecule (Colville, 2003). Being specialized fluid connective tissue within the vascular system blood circulates carrying the necessities of life for all cells of the body, transport waste products of metabolism to the organs of excretion. Blood cellular parameters as well as serum biochemical parameters are usually subjected to adverse effects of diseases, which decreased or increased blood parameters in compared to the normal physiological values.

2.2. Reference physiological parameters

2.2.1 Body temperature

Rectal temperatures were measured using a digital thermometer inserted into the anus and left for 5 minutes. Heart rates were measured using a stethoscope; Respiratory rates was measured by observation of the movement of the thoracic cage per a minute (Kelly, 1979).

Heat production in the body results from intracellular oxidative and other processes, but is added to form the exterior by radiation, conduction and convection. The liver and heart produce heat constantly at a fairly uniform rate, but the muscles when active, are the site of greatest heat production, contributing some 80% of the total. Insurance against overheating is provided by the physical phenomena of radiation, conduction and convection, as well as vaporization of water from the skin and respiratory system and the excretion of feces and urine (Kelly, 1979). Sheep normal body temperature ranges from 38.7-39.1^{o}c(Swenson and Reece, 1996).

2.2.2. Heart and pulse rate

Heart rate can be taken from the left thoracic wall of the sheep on the heart area using stethoscope (Aroch *et al.*, 2005). The normal range of heart rate in sheep is 70-80 Beat/min (Swenson and Reece, 1996).In sheep the pulse is taken at the femoral artery, high up in the inguinal region on the medial aspect of the thigh (Kerr, 1989).

Insertion the ball of one or more fingers on the skin over the selected area and applying gentle pressure until the pulse wave can be detected and can take pulse rate. It is stated that species variation; size; age; sex; physical condition; physiological state (pregnancy, parturition and

lactation); excitement; exercise and environmental temperature of the animal are known to affect the pulse rate of an animal (Kelly, 1979).

2.2.3. Respiratory rate

It is a chemical and mechanical process, which enables an organism to exchange gases with its environment. It is not possible to diagnose any given disease with certainty by observations on the physical aspects of respiration alone but it is sometimes tentatively help to recognize specific entities by noting the character of respiratory movements. The respiratory rate has been determined by observing nostril movements; or more efficiently by auscultation over the thorax or trachea (Kelly, 1979). The animals' respiration rate can increase with hot or humid weather, exercise, fever or pain. The normal range of respiratory rate in sheep is 16-34 Breath/minute (Swenson and Reece, 1996).

2.3. Plasma enzymes and other biochemical components

Enzymes are protein catalysts synthesized by all living things on the earth. As catalysts their only biologic activity is to alter the rate at which equilibrium is established between reactants and their products. Plasma (serum) enzymes can be placed into two distinct classes. The first consists of the plasma-specific enzymes, which are those that have a definite and specific function in plasma. Their normal site of action is in the plasma, and they are present in higher level in plasma than in most tissue cells. The second class consists of the non plasma specific enzymes that are present in concentrations much lower than their concentration in certain tissues. This group is divided into two sub classes: The first class consists of enzymes associated with cellular metabolism, and the second class consists of enzymes of secretion. Enzymes of cellular metabolism are located within tissue cells and are present there in high concentrations as long as the cell remains healthy and the membrane is intact. The level of these enzymes in extra cellular fluid and plasma is low. Secretory enzymes are rapidly disposed through excretory channels such as the intestinal tract, urine and bile, and by inactivation and degradation their normal plasma levels are relatively low and are constant (Coles, 1986).

Appears in extremely high concentrations in muscle, both skeletal and cardiac, it is of value in confirming a diagnosis of muscular degeneration. Serum AST levels may be increased with liver disease in all species but cannot be considered to be a specific test for liver damage. However, in animal species other than the dog, cat and primate, AST levels may be dramatically increased with hepatocellular destruction (Coles, 1986). The normal AST value of sheep in Ethiopia 88.1- 94.4 U/L (Tesefaye, 2008).

2.3.3. Gamma glutamyl transferase (GGT)

It is s an enzyme found in the cytosol associated with cell membranes, particularly those near the bile canaliculi. The enzyme is present in several organs but serum activity of this enzyme almost exclusively results from the GGT of hepatic origin. Gamma glutamyl transferase increases in longer-term liver damage; it is particularly useful in horses and ruminants (Malikides *et al.*, 2001). The normal GGT value of sheep in Ethiopia 19.6 - 44.1 U/L (Khan, 2005).

2.3.4. Alkaline phosphatases (ALP)

Phosphatases are agents that hydrolyze phosphoric Easters with the liberation of inorganic phosphate. Two principal types of phosphatases are found in the blood. An alkaline phosphatase has its optimum enzymatic activity at a pH optimum between 9 and 10, whereas an acid phosphatase has its optimum enzymatic activity at a pH of approximately 5 (Coles, 1986). This enzyme appears in large amounts in liver, spleen, bone, gut-lining and kidneys, and its serum level will increase in tissue damage of the spleen, gut, lungs, liver and particularly the biliary system (gall bladder and bile duct from liver to gut) (Harris *et al.*, 1999). The alkaline phosphatase will be the first and foremost enzyme to be elevated, unlike aminotransferase elevation that indicates hepatocyte damage. In bone, elevated level signifies bone cancer or rickets (Colville, 2003).

When ALP level is supported by other clinical signs, it may help to confirm a diagnosis, especially when bile duct obstruction is suspected. It is also essential in bone remodeling, the process by which bone tissue is continually responding to the stresses of exercise. Increased

serum levels are a general indication of osteoblast activity, the cells involved in building new bone tissue (Simprag *et al.*, 2004). Therefore, ALP levels will normally be higher in young animals during rapid growth. A follow up examination is highly recommended for mature animals with high levels of GGT and/or bilirubin accompanying serum increases of ALP. Previous study on the normal value of ALP in sheep in Ethiopia 44.4-50.4U/L (Tesefaye, 2008).

2.3.5. Creatinine

It is formed in the liver and pancreas, and enters into the blood stream and is distributed to many cells, especially to muscle cells. With in cells and in the presence of the enzyme creatinine kinase, creatinine is phosphorylated to form creatinine phosphate, which is available to muscles as an immediate energy source. The body content of creatinine is proportional to muscle mass. It is useful in measuring major kidney damage. It increases due to renal disfunction, blocked urethra and ruptured bladder, and decreases due to sample deterioration. Creatinine is important in localizing problems and monitoring responses to changes in training or treatments (Ehnene *et al.*, 1990). The normal value of Creatinine in sheep 1.1-1.2 mg/dl (Tesefaye, 2008).

2.3.6. Urea

It is formed in the liver and represents the principal end product of protein catabolism. This substance normally has no useful function in the body other than a possible mild diuretic action and is excreted almost entirely by the kidneys. The glomerulus's filters urea from the plasma and under normal conditions approximately 25 to 40 percent of filtered urea is reabsorbed as it passes through the tubules (Coles, 1986).

Urea increases due to excess dietary protein, dehydration, congestive heart failure, renal failure, blocked urethra, and ruptured bladder (Ehnene *et al.*, 1990). The normal value of Urea in sheep in Ethiopia 10 - 26 mg/dl (Tesefaye, 2008).

2.3.7. Total protein

A protein is a compound of high molecular weight consisting primarily of chains of amino acids united in a peptide linkage. Until late in the nineteenth century it was assumed that plasma was composed of a single protein. With various salt precipitation methods two proteins were recovered from serum and identified according to their solubility characteristic as albumin and globulin. The globulin in turn is made up of α1, α2, β, and γ globulins. Further study utilizing the principle of electrophoresis has ultimately resulted in the identification of at least 22 distinct plasma proteins.

The total protein test is a faster and cheaper test that estimates the total of all fractions together. The traditional method for measuring total protein uses the biuret reagent, which is the simplest chemical quantitative analytical method. Total serum protein analysis is usually performed on automated analyzers along with other laboratory tests (Coles, 1986).

Plasma protein alterations are not usually specific for a particular disease condition. However, certain alterations in the total concentration or a variation in the components comprising the total plasma protein may be of significance both diagnostically and prognostically. Any abnormality in plasma proteins indicates that some pathologic, physiologic, or other induced factor is responsible. The total protein analysis is important in evaluating the water balance status of an animal along with the determination of PCV or hemoglobin or both; in the estimation of the nutritional state of an animal and reflect alterations in metabolism where it is linked with the functional state of liver and kidney. Apart from this fact estimation of total protein value following shock, dehydration or hemorrhage is of value as a guide in the administration of fluids during an emergency (Coles, 1986).

Alteration in the total plasma protein is most often due to a decrease in the quantity of albumin. A decrease in the total albumin is often accompanied by a relative hyperglobinemia. However, such a hyperglobinemia is not usually sufficient to maintain the total plasma protein concentration and hypoproteinemia results. Serum total protein increases due to dehydration and chronic inflammation. It decreases due to overhydration, severe congestive heart failure (with edema), protein-losing nephropathy, protein-losing enteropathy, hemorrhage, burns, dietary protein deficiency, mal-absorption and some viral conditions (Magdesian *et al.*, 1992). The normal value of Protein of sheep in Ethiopia 4.9-5.2g/dl (Tesefaye, 2008).

2.3.8. Glucose

Blood glucose determination may be included as part of a routine physical examination of patients, particularly aged animals.Determination of concentration of blood glucose is important in sheep and cattle in which ketosis is suspected and in young pigs suspecting of having hypoglycemia associated with starvation in aged animals, as it will often reveal the existence of a disease entity not previously suspected glucose estimation is included in biochemical profiles (Coles, 1986). The normal value of glucose of sheep in Ethiopia 40.4-43.6 mg/dl (Tesefaye, 2008).

2.4. Serum electrolytes parameters

Electrolytes are important to realize that the body has no mechanism for storing ''extra reservation'' of electrolytes. Therefore, while the body closely regulates electrolytes, much is lost in the sweat, urine and feaces during exercise and hence is an important in monitoring an animal (Simprag *et al.*, 2004).The serum electrolytes concentrations are affected by body fluid problems, water deprivation, nutritional deficiency and other factors. Such factors cause disturbance of the electrolytes particularly sodium, chloride, calcium, potassium, phosphorous, iron and magnesium in the body of the animal.

The role of electrolytes in the animal body is diverse, as there are almost no metabolic processes that are not affected by electrolytes. A number of instruments and techniques are available that permit accurate, reproducible analytical measurements of electrolytes. With increasing knowledge about electrolyte balances in various animal diseases, the use of electrolyte replacement therapy has become a routine practice in veterinary medicine. In order that therapy can be most efficacious, it is essential for the clinician to have an understanding of the basic mechanisms involved in electrolyte alterations and an ability to interpret results of laboratory estimations for electrolyte balance (Coles, 1986).

2.4.1. Serum sodium

Sodium ion is the major anion of extra cellular fluid whose primary function in the body is to maintain osmotic pressure and acid-base balance. Sodium ion functions at the cell membrane level by creating an electrical potential difference between cell membranes causing the transmission of nerve impulses and neuromuscular excitability to be maintained. Sodium ion is also involved in some enzyme catalyzed reactions as a cofactor Low sodium ion values in blood, hyponatremia, usually reflect a relative excess of body water rather than low total body sodium. Reduced sodium level may be associated with low sodium intake, sodium losses due to vomiting or diarrhea with adequate water and inadequate salt replacement or diuretics abuse, osmotic diverse seen in diabetes mellitus and lack of aldosteron during adrenocortical insufficiency (Addison's disease) (Burtis and Ash wood, 1994).

Elevated sodium values, hypernatremia, are associated with conditions with water loss in excess of salt loss through profuse sweating, sever vomiting or diarrhea or diabetes insipidus, increase renal sodium conservation in hyper aldosteronism, or advanced chronic renal failure with a low glomerular filtration rate. Therefore, the sodium value obtained may be used in a diagnosis or monitoring of all disturbances of the water balance, infusion therapies, vomiting, diarrhea, kidney insufficiency, central or renal diabetes insipid us, endocrine disturbance and primary or secondary cortex insufficiency of the adrenal gland or other diseases involving electrolytes imbalance (Norbert, 1990). The normal value of sodium in sheep141-159 mmol/l.

2.4.2. Serum Potassium

Potassium is the major intracellular action that has important role in normal muscular function, cardiac function, nerve impulse transmission and helps maintain acid-base balance and osmotic pressure (Colville, 2003). Hyperkalemia, an elevated blood potassium levels, occurs secondary to hypoadrenocorticism, renal failure particularly acute failure, diffuse cell necrosis or altered membrane permeability and during acidosis especially when there is concomitant kidney dysfunction. Pseudo-hyperkalemia may occur during delayed separation of serum from clotting blood with extreme leukocytes or thrombocytosis as well as during hemolytic (Meyer and Harvey, 1998).

High or low potassium levels may cause changes in muscle irritability and myocardial function. The potassium value obtain may be used to monitor electrolyte imbalance in the diagnosis and treatment of infusion therapies, heart or circulatory insufficiency, acid-base imbalance, therapy with diuretic, kidney problems, diarrhea and hyper and hypo-function of adrenal cortex and other disease involving electrolyte imbalance (Burtis and Ash Wood, 1994; Norbert, 1990). The normal value of Potassium in sheep in Ethiopia 4.3 - 6.3 mmol/l (Tesefaye, 2008).

2.5. Factor affecting serum biochemical parameters

Factors affecting serum enzyme concentration unlike many biochemical tests, serum enzyme estimation is much more method dependent (i.e. in variability in techniques) and the wide variation in animal species may render the need to establish normal values for each laboratory of various animal species.Moreover, it has been indicted that in clinically health individuals diet, sex, age, seasonal variation, breed, pregnancy and environmental factors. There are significantly elevated levels of AST and ALT in young sheep than adult but no significant sex difference was noted. It has been indicted that hemolysis should be avoided due to the high transaminase content of red cells (Harris *et al*, 1998).

2.5.1. Age

Although all cells contain the same DNA, not every gene is expressed in every cell instead those proteins proper to the cells function are synthesized while other generate suppressed, so that each cell type contains its own finger print of enzymes (Kerr, 1989). Of the different factors assumed to influence the serum enzyme concentration in animal's age is the important one. The influence of age was reported by (Coles, Otesile and Kasli, 1992) and Alonso *et al.* 1997), where neonates have high levels of many enzymes and young animals before the closure of the epiphysis growth plate do have higher levels of serum ALP than adults. Otesile and Kasali 1992) found significantly elevated levels of AST and ALT in young sheep than adults, but no significant sex difference was noted.

2.5.2. Breed

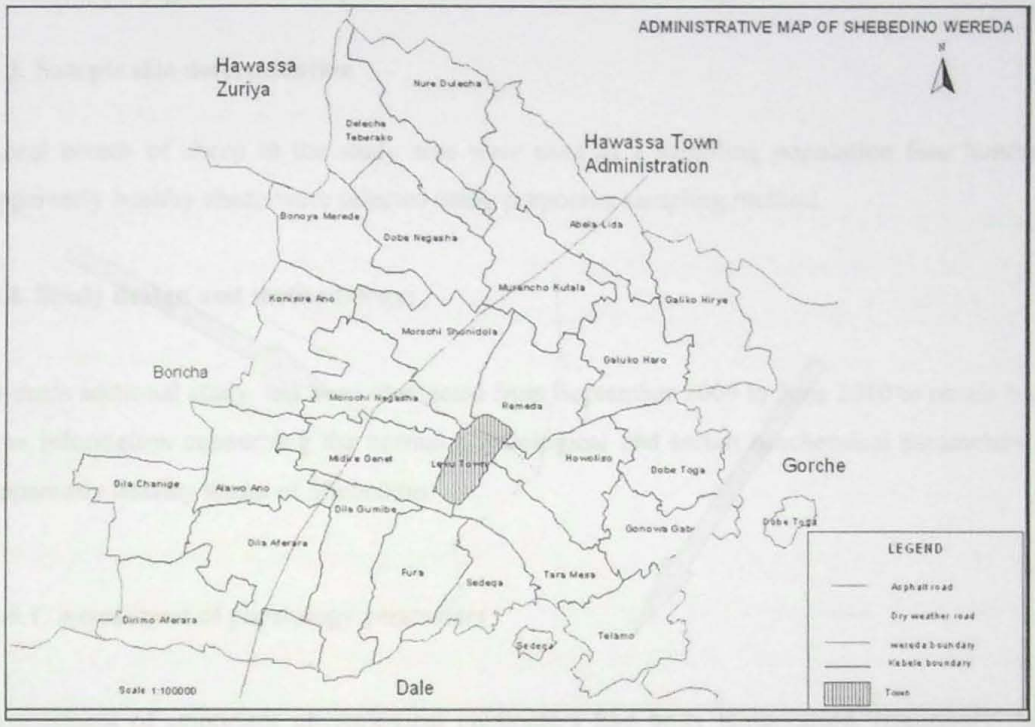
Breed different effect in this biochemical status of serum was reported by (Kerr, 1989) and indicate that the haemolysis should be avoided due to the high transaminase content of red cells. According to author, unlike many others biochemical tests, serum enzyme estimation is much more method dependent (i.e, variability is techniques) and the wide variation in animal species may render the need to establish normal values for each laboratory and various animal values species. Moreover, (anonymously Sigma diagnostics, 1990) indicated that diet, sex, age, diurnal variation of physical activity, pregnancy and environmental factors in clinically healthy individuals may influence certain biochemical measurements.

3. MATERIALS AND METHODS

3.1. Study area

Sidama Administration Zone is found of Southern Nation Nationalities and Peoples regional state (SNNP) and it is one of the 13 zones in the region. It is located in the north eastern part of the region and bounded by Oromiya in the North, east and south east, with Gedieo Zone in the South, and Wolayta Zone in the west. Its geographic location lies between $6^0$10'- $7^0$12' North and 38^0 -$39^0$11' East. It consists of 19 woredas.From those Shebedino is one of the wereda. The study area comprises of two Pesant Association (PAs) representing highland (Telamo) and midland (Morocho Negasha).Shebedino is a district. with a total land area of 26,990 hectare. Out of this, 5,229hectar is covered by annual crop such as maize, wheat, pea, beans, potatoes and 16,497hectar is covered by perennial crops such as 'inset', 'chat', coffee, the remaining 5,264 ha of land is covered with bushes, hillside and un productive land. And located 27 Km south from Hawassa.The Altitude range is from 1500-2500meter above sea level and the annual rain fall range is from 900-1500m per year. The average daily ranges of maximum and minimum temperature are 16 ^{O}C and 25 ^{O}C, respectively. Telamo one of the PAs10.Km.to Shebedino located an altitude 2000meter above sea level. Morocho Negash also one of the pas 4 Km to Shebedino located an altitude 1500 meter above sea level. The dry season from November to February with rainfall occurring between March and April and from late May to October It has 32 peasants associations. The total population of the woreda is estimated to be 291,556 living in 29 rural kebeles and 3 the urban. The livestock resource of 129,676 cattle, 40,000 sheep, 27,808 goat, 8207 equines.

Figure 1. Map showing the study area.



Source:): Sidama zone plan and economy development office map of shebedino wereda (2009)

= Study area

=

3.2. Study population

Apparently healthy sheep owned by the farmers were sampled from the study areas (Telamo, Morocho Negasha and their surroundings). 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. It should stand on all of its feet. The study animals were grouped into males and females, different age groups (1-3 years and above 3 years age) and based on altitude (highland and midland). Physiological parameters were recorded included rectal temperature, heart rate, respiratory and pulse rate. These were taken from 400 local breeds of sheep. From the 400 sheep clinically considered 7

ml blood samples were taken from one hundred forty male and two hundred eighty six females from jugular vein into plain tubes for serum biochemistry.

3.3. Sample size determination

Local breeds of sheep in the study area were used as a sampling population four hundred apparently healthy sheep were selected using purposive sampling method.

3.4. Study design and methodology:

A cross sectional study has been conducted from September 2009 to June 2010 to obtain base line information concerning the normal physiological and serum biochemical parameters in apparently healthy sheep of Shebedino.

3.4.1. 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 (Kelly, 1979). 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

animals fore limb in the heart area on the left side of the animal and beats within one minute were counted and recorded.

3.4.2. Serum biochemical profiles

In the current study blood samples were collect from the study sheep while they are at rest and under conditions of least excitement to minimize any physiologic variations in cell counts (Jain, 1993). The levels of plasma alanine aminotransferase (ALT), aspartate aminotansferase (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 from the sampled blood in plain vacationer tube is kept by centrifugation (3000 rpm for 10 minutes) and then only the serum part was decanted into another plain tube. Since the serum sample is analyzed at Addis Ababa University, Faculty of Veterinary Medicine, Physiology laboratory it was stored at -20 ^{0}C until transporting and also even until analyses (Jain, 1993; Barell *et al.*, 2002; Hoffman *et al.*, 2005; Simenew, 2007; Eyob, 2007). While transporting the serum samples were kept in icebox with icepack. 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.

3.4.3. Questionnaire survey:

Based on physiological assessment and serum biochemical profiles to establish and compare the parameters in sheep with different age, sex, altitude under healthy condition. Before the commencement of sampling work, a pilot survey was done with the collaboration of PAs elders and district agricultural officers to know the distributions and conditions of sheep within the district (study area). The target community was selected from 100 house holds level. Fifty of the house holds were included in the questioner survey.A questionnaire survey was conducted in order to gain an information about the production and the management of sheep disease type, feedings and economic contribution of sheep.

3.5.Data analysis

Data were entered in to Microsoft excel spread sheet from which dependent variables of the clinically important physiological, and serum biochemical values where as the independent ones age,sex 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 (±sd) range and the 95% confidence interval. Comparison of the factors affecting the measured physiological and biochemical parameters after setting the level of significance at 0.05.

4. RESULTS

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

4.1. Analysis of clinical parameters

Reference values for clinical parameters of sheep based on age, sex and altitude are shown in table, 1, 2 and 3 respectively.

The rectal temperature in two age categories was significant (P<0.05) were as respiratory and pulse rate were not significance (P>0.05). (table 1)

Table 1: Value of Physiological parameters in sheep by age

Parameters	Age	N	Mean±SD	95%Confidence Interval of the Mean	Range	P-value
Temperature ^{0}C	1-3	300	39.21±0.55	39.15-39.28	37-40	0.036*
	>3	100	39.35±0.61	39.23-39.48	35-40	0.048*
Respiration	1-3	300	25.66±5.69	25.01-26.30	16-36	0.195
(br/m)	>3	100	26.49±5.11	25.48-27.50	16-38	0.172
Pulse (beat/m)	1-3	300	73.74±3.28	73.37-74.11	69-80	0.916
	>3	100	73.70±3.28	73.05-74.35	68-80	0.916

* Significante difference at P<0.05

Significant variation (P<0.05) were observed rectal temperature, respiration and pulse rate for the two sex groups. Where as Temperature and pulse rate were highly significance (P<0.05). (Table 2)

Table 2: Value of Physiological parameters in sheep by sex

Parameters	Sex	N	Mean±SD	95%Confidence Interval of the Mean	Range	P-value
Temperature ^{0}C	male	114	38.96±0.66	38.84 - 39.08	35-40	0.000***
	female	286	39.36±0.48	39.31 - 39.42	37-40	0.000***
Respiration (br/m)	male	114	24.55±5.4	23.55 - 25.56	16-34	0.003*
	female	286	26.39±5.54	25.74 - 27.03	16-38	0.003*
Pulse (beat/m)	male	114	72.83±2.93	72.29 - 73.38	68-80	0.001*
	Female	286	74.09±3.34	73.70 - 74.80	69-80	0.000 ***

* ** highly Significance difference at P<0.05 * Significance difference at P<0.05

The rectal temperature were highly significant (P<0.05) in the two altitudes where as the respiratory rate also significant (P<0.05) in the two altitudes Were as the plus rate in to two altitude were not significant (P>0.05) (table 3)

Table 3: Value of Physiological parameters in sheep by altitude

Parameters	Altitude	N	Mean±SD	95%Confidence Interval of the Mean	Range	P-value
Temperature (^{0}C)	Highland	199	39.48±0.43	39.42-39.54	37-35	0. 000***
	Midland	201	39.02±0.59	38.94-39.54	37-40	0. 000***
Respiration (br/m)	Highland	199	25.86±5.87	25.04-26.68	16-34	0.984
	Midland	201	25.87±5.25	25.14-26.60	16-38	0.984
Pulse (beat/m)	Highland	199	74.13±3.51	73.63-74.62	68-80	0.016*
	midland	201	73.34±2.99	72.92-73.75	69-80	0.016*

* Significance difference at P<0.05 *** Significance difference at P<0.05

4.2. Analysis of serum biochemical parameters

Tables 4, 5 and 6 show the result of biochemical parameters of sheep affecting by age, sex and altitude respectevely.

In the serum biochemical profile, only urea had a significant difference ($P< 0.05$) between the two age groups in the study area but the rest of biochemical parameters were not significantly differente($p>0.05$) (Table 4).

Table 4:.Serum biochemical result in sheep by age

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

* Significance difference at $P<0.05$

Even though, no significant associations ($p>0.05$) were observed in biochemical parameters, only significant difference ($p<0.05$) were observed in the two sex groups for ALT where as significant difference ($p<0.05$) only the mean value of ALP in female higher than male groups. (Table 5)

Table 5: Value of serum biochemical result in sheep by sex.

Parameters	Sex	N	Mean±SD	95%Confidence Interva for Mean	Range	P-value
AST (U/l)	Male	114	67.61±25.92	62.195-73.38	10-216	0.906
	Female	286	67.97±28.16	65.006-72.036	11-301	0.902
ALT (U/l)	Male	114	13.51±7.28	11.548-14.735	7-56	0.093
	Female	286	15.08±8.85	13.901-396.23	6-75	0.068
ALP (U/l)	Male	114	338.10±259.83	287.782-396.23	19-1401	0.287
	Female	286	372.62±304.50	339.148-407.32	22-1993	0.255
Protein(g/dl)	Male	114	5.04±1.18	4.8045.267-5.26	1-7	0.377
	Female	286	4.91±1.27	4.767-5.058	1-8	0.363
Glucose mg/dl)	Male	114	28.39±12.31	25.144-29.90	7-76	0.878
	Female	286	28.19±12.24	25.900-28.89	8-75	0.878
Sodium (mmol/l)	Male	114	144.28±9.82	142.858-145.67	82-180	0.091
	Female	286	145.71±6.51	144.822-146.59	129-180	0.155
Potassiummmol/l	Male	114	5.592±0.81	5.432-5.72	3.0-8.1	0.739
	female	286	5.563±0.79	5.470-5.65	3.0-9.3	0.743
Cereatinin (mg/dl)	Male	114	0 .976±0.27	0.912-0.4	.1-1.8	0.883
	female	286	0.954±0.25	0.911-0.3	1-1.6	0.823
Urea(mg/dl)	Male	58	46.07±37.21	35.645-57.58	8-206	0.628
	Female	208	49.07±42.88	47.121-59.26	2-321	0.601

For the two altitude in serum bio chemical parameters, ALP and sodium were highly significantly associated ($p<0.05$). And the level of potassium was significant in the two altitudes. (Table 6).

Table 6: Serum biochemical results in sheep by altitude

Parameters	Altitude	N	Mean±SD	95% Confidence Interval of mean	Range	P-value
AST (U/l)	Highland	199	69.62±30.42	65.357-73.779	11-301	0.209
	M/land	201	66.13±24.71	62.865-71.265	10-187	0.209
ALT (U/l)	highland	199	14.42±8.09	12.915-15.326	8-75	0.617
	midland	201	14.85±8.80	13.483-15.887	6-64	0.617
ALP (U/l)	highland	199	299.21±248.79	258.938-338.69	19-1993	0.000***
	midland	201	425.72±318.55	389.863-469.41	54-1631	0.000***
Protein g/dl)	highland	199	4.83±1.40	4.655-5.00	1-8	0.060*
	midland	201	5.06±1.06	4.892-5.23	2-7	0.060*
Glucose (mg/dl	highland	199	28.31±12.73	25.309-28.89	9-76	0.914
	midland	201	28.18±11.77	25.973-148.21	7-68	0.914
Sodium(mmol/l)	highland	199	147.18±7.13	146.149-148.21	133-180	0.000***
	midland	201	143.44±7.64	142.396-144.45	82180	0.000***
Potassium	highland	199	5.460±0.80	5.350-5.57	3.0-8.0	0.006*
(mmol/l)	midland	201	5.681±0.77	5.564-5.78	3.8-9.3	0.006*
Cereatinin	highland	199	0.976±0.46	0.926-1.02	.1-5.5	0.797
(mg/dl)	midland	201	0.985±35.34	0.935-1.03	.1-1.6	0.798
Urea (mg/dl)	highland	198	48.99±35.34	45.804-58.14	2-206	0.703
	midland	68	46.75±56.48	40.170-61.24	3-321	0.760

* Significance difference at P<0.05 *** Significance difference at P<0.05

4.3. Analysis of questioner survey

Table 7: Result of questioner survey

Questioners	Number of respondent agro ecology			
	highland	Percentage	Midland	Percentage
1. Keeping of sheep				
income	15	60	17	68
insurance	7	28	5	20
consumption	3	12	3	12
2.Others animals				
Yes	22	88	12	48
No	3	12	13	52
3.Ranking owned animal imp				
Cattle	11	44	13	52
Sheep	10	40	9	36
Poultry	4	16	3	12
4.Feeding type				
Grazing	13	52	17	68
Cut and carry	12	48	8	32
5. Disease occurrence				
Yes	25	100	25	100
No	0		0	
6.Mention the disease occurred				
coughing	10	40	11	44
Sneezing	5	20	2	8
GIT	10	40	12	48
7.Method used to treat	15	60	13	52
mode renal	10	40	12	48
Traditional				

5. DISCUSSION

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

This study shown that in physiological parameters the value of temperature was highly significance ($p<0.05$) in to altitude (highland and midland) and pulse rate was significance. Whereas the value of respiratory in the altitude was not significance ($p>0.05$). The Reason could be in mid land the animal may developed an adaptation mechanism that can dissipate heat from the body and high land the humid air can affect transpiration and loss of heat through sweating. And increased respiratory rate (Buhari *et al.*, 2007; Gweba *et al.*, 2009). It was also reported that respiratory rate increase in response to CNS depression to meet the body's requirement of oxygen (Hall and Clark, 1983).

Physiological results affected by sex highly significance temperature and pulse rate were as respiratory was significance ($p<0.05$). And also the value of temperature affected by age was significance ($p<0.05$) where as pulse and respiratory rate were not significance ($p>0.05$).

In some of biochemical analyses the study has shown that there are some variations. The values of Urea by age was significance ($P<0.05$) where as AST, ALT, T.protein, ALP, Creatinine, Sodium and Potassium were not significance difference ($P>0.05$) Age was also observed to have a significant effect on ALP in this study similar to Red Sokoto oats (Tambuwal *et al.*,2002). Even though, no significant associations ($p>0.05$) were observed in biochemical parameter.

In the altitude the value of ALP and Sodium highly significance ($P< 0.05$) the Potassium was significance where as the other biochemical values T.protein, Glucose, creatinin, urea, ALT AST were not significance ($P>0.05$). Although the increase of ALP level can be influenced by pregnancy. And when increased the ALP could be possible to attributed to different seasons, feed conditions and local environment.(Eshratkhan *et al.*,2008;Tesefay,2008, Gul *et al.*,2007;Tewoderos,2009).

The others biochemical parameters were not significance. 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 are 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 (Zinkl *et al.*, 1990) mean value and reference ranges for sheep not shows big variations may be described to differences in laboratory equipments.

To compare the result of sheep parameters there where no previously published works. 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 (Forehead *et al.*, 2002).

Although, there were significant differences in some of the physiological and blood biochemical values in this study, the data were within the normal ranges for apparently healthy sheep. Difference in clinical, biochemical parameters in sheep of different age groups altitude and sex were conducted previously from these results clear that factor play key role in conditioning normal values for a diagnostic purpose in sheep.

The questioner survey Indicated that sheep is the dominant species owned by farmers for different reasons. All animals were kept in extensive management system and all owners were mixed farmers. A questionnaire survey was conducted in order to gain information about the production and the management of sheep. Sixty 60% of the owners indicated that among the family members woman's were more responsible for the management of sheep and males were more concerned to make a decision when there is a need to sale the animal or to use it for other purposes. Sixity (60%) were said that reason for keeping of sheep was to sale and gain income,28% sheep were kept as a capital and to use it when an emergency case was faced. The remaining group said for consumption. Almost all sheep owners had others animals and they were ranked according to their importance, sheep was the second 40% next to cattel 44%,and the rest of 20%are others animals. As it was mentioned about all sheep's were in extensive system and for about 80% feeding was by grazing system.

The most frequent disease encountered on sheep were coughing and sneezing 20%,gastro intestinal parasite 40% and external parasite 40%. Eighty(80%) of the owners were

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The most frequent disease encountered on sheep were coughing and sneezing 20%,gastro intestinal parasite 40% and external parasite 40%. Eighty(80%) of the owners were

mentioned that disease burden was increase during wet season. May be these was related with increasing of gastrointestinal parasite and lung worm infestation during wet season.60% of the owners were used modern treatment by sending their sheep to animal health clinics or by using tablets bought from the market and the remaining 40% were used traditional treatment or herbal drugs. Almost all were replied that diseased sheep were not treated at a time when the diseased was first observed. This may indicate the negligence of the owners to the production. In general low attention given to the sheep production should be improved in order to make the production more profitable.

6. CONCLUSION AND RECOMMENDATIONS

In the present study physiological and biochemical values determined for sheep were slightly different from previously known reference values. Most of the clinical parameters there was a limited influence by age, sex and altitude. The serum biochemical value were also affected by age, sex and altitude .Generally from all the factors considered for their effects on the physiological parameters in these study, altitude showed prominent effect more than sex and age. In the result should be given attention in utilizing the values of these parameters for assessing the physiological status and biochemical parameters of animals for diagnostic purpose. Therefore, based on this conclusion the following points are forwarded: On the questioner may indicate the negligence of the owners to the production.

- Reference values establishment for indigenous sheep for mandatory for diagnosis a disease conditions. Therefore, further studies should be carried out to establish the clinically important physiological and serum biochemical values of the indigenous sheep species in different regions of Ethiopia.

- The result obtained in the present study likely represents most of healthy sheep in Ethiopia and constitute potential reference values that can be used for clinical purposes against known values. Because of these possible individuals or combined influence of different factors age, sex and altitude and the sub sequent interpretation of normal physiological and biochemical parameter. in sheep for pathological condition should be carefully considered.

- The findings of the study may serve as references for clinical use in which alterations in the physiology and healthy status of animals.

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8. ANNEXES

Annex.1. Questionnaire

Owners' name _____

Woreda _____ PA_____

1. Reason for keeping sheep?

Income_____

Insurance_____

Consumption_____

2. Do you have other animals?_____

Yes_____

No_____

3. Ranking of animal owend related to importance_____

Cattle_____

Sheep_____

Poultry_____

4. How do you feed your animal?_____

Grazing_____

Cut and carry_____

5. Do you know any disease in sheep?

Yes_____

No_____

6. Can you menstion them?

Coughing_____ gastro intestinal parasite_____

7. Is there seson variation in the diseas ?

Wet seson_____ Dry seson_____

8. How do you treated? _____

Moderen_____ Traditional_____

9. Do you treat diseased cases as they occur?

Yes_____ No_____

10. Do you give concentration feed ?

Yes_____ No_____

Annex 2. Procedures for serum enzymes and other metabolites analyses

Materials required

Micro pipette of different capacity for measuring reagents and samples

Micro pipette tips

Test tubes

Photometer (Automated Machine)

Constant temperature incubator set at 37 ^{0}C

Reagents for each analyte

Serum samples to be analyzed

Distilled water to wash the machine after each analysis

Control sera for each analyte to check the quality of the photometer

Serum electrolyte analysis

Materials required

Automated electrolyte analyzer

Snap pack reagent

Serum sample to be analyzed

Procedures

Perform daily maintenance, conditioning and cleaning of the analyzer

Check the quality of the machine using the control sera solution

Bring the reagents and fit into the electrolyte analyzer

Bring the sample to room temperature

Provide the serum sample to be analyzed

Read the result after 56 seconds

Urea test

Procedures

Bring the reagents and samples to room temperature

Add 1 vial of R1 (urease) into 1 bottle of R2 (phosphate buffer, EDTA, Sodium salicylate and sodium nitroprusside) and mix

Pipette 1000µl (micro liter) of the mixed R1 and R2 into labeled tubes

Pipette 10µl (micro liter) of the serum sample over the tubes containing the mixed reagents

Mix, incubate at 370c for 5 minutes

Pipette 200µl of R3 (sodium hypo chloride and sodium hydro oxide) over the tubes containing the sample and the mixed R1 and R2.

Glucose test

Procedures

Bring the reagents and samples to room temperature

Pipette 1000µl of the reagent (glucose mono reagent) into the labeled tubes

Pipette 10µl of the serum sample over the reagents in the labeled tubes

Mix and let the tubes stand for 5 minutes at 37^0C

Read the absorbance of the samples and the standard at 500nm of wave length against the reagent blank

Total protein test

Procedures

Bring the reagents and the samples to room temperature

Pipette 1000µl of reagent R1 (potassium iodide, copper sulphate, sodium hydro oxide and potassium sodium tartarate) into labeled tubes

Pipette 20µl of the serum sample into the tubes containing R1

Mix, incubate for 5 minutes at 37^0c and read the absorbance at 546nm wave length

Creatinine test

Procedures

1. Bring the reagents and the samples to room temperature
2 Mix equal volume of R1 (picric acid) and R2 (sodium hydro oxide)
3. Pipette 1000µl of the mixture in to labeled tubes
4. Pipette 100µl of serum sample to containing the mixed reagents
5. Mix, read A1 exactly after 20 seconds and A2 after 80 seconds at 492nm wave length and calculate delta absorbance

ALT/GPT test

Procedures

1. Bring the reagent and samples to room temperature
2. Mix 5 volume of R1 (tris buffer pH 7.8, L-Alanine and lactate dehydrogenase) and 1 volume of R2 ($NADH_2$ and 2-Oxoglutarate)
3. Pipette 1000µl of the mixed reagent into the labeled tubes
4. Pipette 100µl of serum sample over the labeled tubes containing the mixed reagents
5. Mix, incubate for 1 minute and read the absorbance after exactly 1, 2 and 3 minutes and then calculate delta absorbance at 340nm wave length

ALP test

Procedures

1. Bring the reagent and samples to room temperature
2. Mix 5 volume of R1 (Diethanolamine buffer, pH 9.8, Magnesium sulphate and detergents and stabilizers) and 1 volume of R2 (p-nitrophenylphosphate and stabilized liquid)
3. Pipette 1000µl of the mixed reagent into the labeled tubes
4. Pipette 20µl of the serum sample over mixed reagent into the labeled tubes
5. Mix and read the absorbance after exactly 1, 2 and 3 minutes and then calculate delta absorbance at 405nm wave length

AST test

Procedures

Bring the reagent and samples to room temperature

1.Pipette 1000µl of the reagent into the labeled tubes

Pipette 100µl of serum sample over the labeled tubes containing the reagent

Mix, incubate and read the absorbance after exactly 1, 2 and 3 minutes and then calculate delta absorbance at 340nm wave length.

Annex: 4. Laboratory procedures and materials

Serum biochemical kites and methods of processing for serum analysis

Analyte	Unit	Method of processing	Analisis	Kit used
AST/GOT	IU/L	IFCC with out pyri Odoxal phosphate activate	Kinetic	GOT(ASAT) fluid Centrionic GambH Warten berg/Germ any.
ALT/GPT	IU/L	IFCC with out pyri Odoxal phosphate activate	Kinetic	GPT fluid Centrionic GambH Warten berg/Germ any.
ALP	IU/L	DGKC method	Kinetic	Alkaline phosphate In body fluid Dgkc.
Creatinine	MG/dl	Jaffe method with Out depolarization	Kinetic	Creatinine Jaffer Kinetic fluid centr Onic Gmbh war. Germany
Glucose	MG/dl	GOD method with Out depolarization.	End point	Glucose PAP fluid Mono reagent Centrionic GambH Warten berg/Germ any.
Total Protein	G/dl	End point	End point	T.protein fluid Centrionic GambH Warten berg/Germ any.
Urea	MG/dl	End point	End point	Urea fluid Centrionic GambH Warten berg/Germ any.
Sodium	Mmol/l	End point	End point	
Potassium	Mmol/l	End point	End point	

Annex: 5.Age determination of sheep depending on teeth.

	Permanent teeth	Age of sheep
1.	Milk teeth	Less than one year
2.	Two tooth	One up two years old
3.	Four tooth	Two years
4.	Six tooth	Three years
5.	Full mouth	Three up to four years
6.	Broken mouth	greater than four years
7.	Gummy	Aged

Source:Alemu,et al (2008)

Source: Kahn (2005)

Annex 6: Serum Biochemical References Range of Sheep.

Serum biochemical components	Units /U/L/	Sheep
ALT	U/L	14.8-43.8
ALP	U/L	26.9-156.1
AST	U/L	49.0-123.3
GGT	U/L	19.6-44.1
Protein	g/dl	5.9-7.8
Sodium	mmol/L	141.6-159.6
Potassium	mmol/l	4.3-6.3
Glucose	mg/dl	44-81
Urea	mg/dl	10-26
Creatinine	mg/dl	0.9-2

9. DECLARATION

This thesis is my original work, has not been presented for a degree in any University and that all sources of material used for the thesis have been duly acknowledged.

Name Aynalem Gemechu Kekeba

Signature_____

Date of submission _____

This thesis has been submitted for examination with our approval as University advisors.

	Signature
Dr. Kibeb Legesse (DVM, PhD)	_____
Dr. Gezahegn Mamo (DVM, MSc)	_____