STUDY OF BLOOD BIOCHEMISTRY OF BALAMI SHEEP AT SOKOTO MODERN ABATTOIR BEFORE SLAUGHTER

Hassan Maina Ibrahim[1], Fatima Abubakar Yabo[1], Saidu Bashir[1], Jibreen Abdurahman[1], Abdullahi Ishaq Ja'afaru[1], Sadiq Habibullah[1], Yahaya Abubakar Yabo[1], Adamu Abubakar[2], Bashiru Garba[3], Mahmuda Aliyu[4].

Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary Medicine[1]
Department of Veterinary Surgery and Radiology, Faculty of Veterinary Medicine[2]
Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine[3]; Department of Veterinary Parasitology and entomology[4],
Usmanu Danfodiyo University Sokoto, Nigeria

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ABSTRACT
Hematological parameters of 50 sheep consisting of 26 males and 24 females at Sokoto modern abattoir were analyzed. This study was conducted to determine the reference values of hematological parameters in apparently healthy sheep. The blood sample was collected from the animals before slaughter by puncturing the jugular vein followed by immediate transferred of the blood into EDTA bottle, transport it to the Physiology and Biochemistry laboratory where they are process and analyzed using standard method. The results obtained shows significance differences at $p>0.05$ in all hematological indices between sexes. From the present study, it can be concluded that the hematological parameters for sheep studied fall within normal range with slight variation. The observed differences may be due to nutritional, environmental or unknown effect. Study of hematological parameters across the age group, breed, season and region is recommended to reaffirm the normal reference hematological value.
Key words: - EDTA, Hematology, Physiology, Biochemistry, Small ruminants

INTRODUCTION

Small ruminants such as sheep and goats play important role in the livestock subsector of the Nigerian agricultural economy with about 21,230 million sheep population (Arthur and Baidoo, 2011). Three breed of sheep Balami, Yankasa and Ouda dominate, especially in northern part of the country where they made up of up to 70% of the total population (Iyiola-Tunji et al., 2010). In most of Nigerian villages, sheep and goats production are the main source of income among the small scale farmers (Julius, 2012). One of the most important agricultural investments to rural and urban farmers is small ruminant production because it supplied human population with milk especially in new born babies, meat, hair and other products. Sheep are small ruminants that have special attributes over other livestock resources (Amankwah et al., 2012). Most importantly, sheep's adapt easily to wide ranges of environment changes, feeding as well as short generation interval, reproductive cycles and high productivity (Hassan et al., 2015).

Blood is an important vital tissue that maintained the normal physiologic and biochemical well being of organism as well as preventing patho-physiologic changes that may result from several factor such as diseases, stress, environmental changes (Tsigos et al., 2016). Variations in hematological parameters have been widely used in diagnosis of different diseases and nutritional status of animal (Coroian et al., 2017). Information obtained from blood parameters, physical examination, history, laboratory result and chief complaint from client are used for the diagnosis of diseases and health status of the animals (Mayumi et al., 2016). Ideally, references values from the blood parameters of diseases free animals that are well managed are needed for comparison in the diagnosis of unknown diseases or patho-physiological changes (Girling et al., 2015). Hematologic results such as total and differential count, haemoglobin concentration and serum biochemistry differs among animals, species, breeds, sex, environment, management system feeding level, age, health status of the animal. Method of blood collection, hematological techniques used, diurnal and seasonal variation, ambient temperature (excrement, muscular exercise, pregnancy, estrus, parturition, time of sampling, water balance and transportation and physiological status of the animal may affect overall result even if the animal is disease free (Choi et al., 2016).

Hence, this study was aim at providing normal hematological values that will serve as reference among indigenous sheep breeds found in the semiarid zones of Nigeria for clinical uses.

MATERIAL AND METHODS

Materials

Ethylene diamine tetra acetic acid bottle (EDTA), hand gloves, cotton wool, hydrogen peroxide, syringe and needle, microscope, micro-hematocrit reader, micro-hematocrit capillary tube, glass slide, cover slip, centrifuge machine, Leishman stain, Turks solution, hayerm's fluid, plasticine. Apparently healthy sheep of both sexes (25 males and 25 females) were used in this study. The blood sample was collected from the animals before slaughter by puncturing the jugular vein with 5 mL syringe, 19 gauge needles and aspirate, followed by immediate transferred of the blood into EDTA bottle. All the procedures for the analysis of blood sample were carried out at the veterinary physiology and Biochemistry laboratory, faculty of veterinary medicine Usmanu Dan Fodiyo University Sokoto. These procedures include PCV, total red blood cell count, total white blood cell count, differential white blood cell count, and determination of hemoglobin concentration. The packed cell volume is measured using micro-hematocrit method. Blood samples were filled up 2/3rd of the tube and then sealed with plasticine at one end. Samples were first centrifuged with micro-hematocrit centrifuge at 10,000 revolutions for 5minutes. The reading was then taken with the aid of hematocrit reader and the result was presented as percentage total volume of whole blood. Blood samples were drawn with red cell pipette to the level of 0.5 marks. The tip end of the pipette was wiped with cotton wool and diluted with normal saline to the level of 101 marks. The pipette was held horizontally and shakes frequently before dropping the mix blood sample into neubauer counting chamber and cover it with cover slip. Reading was taking with the aid of microscope at 10x objective.

HEMOGLOBIN DETERMINATION

5mls of Drapkin solution was placed in to a clean curvette and 0.02mls of blood was then added with the aid of hemoglobin pipette. Blood was then dispensed into the curvette, pipette was rinsed several times with diluents and the tube was stoppered and inverted two or three times. The mixture was allowed to stand for 10 minutes for maximum homogenization followed by wiping and cleaning of the curvette as well as placing it into a calorimeter for reading. Reading was taken at 450nm absorbent of test samples and standard (blank reagents).

ERYTHROCYTIC INDICES

The erythrocytic indices includes: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) was determined by using the below formulas.

MCV= PCV×10/RBCg/dl
MCH= Hb×10/RBCg/dl
MCHC=Hb×10/PCVg/dl

WHITE BLOOD CELL COUNT

Blood samples were drawn into the white cell pipette to level of 0.5 mark, the tip end was wiped with cotton wool. Prepared normal saline was also drawn to mix the blood to the level of 101 marks. The pipette was held horizontally and shakes before dropping the sample into neubauer counting chamber and cover it with cover slip. Reading was taking with the aid of microscope at 10x objective.

DIFFERENTIAL LEUKOCYTE COUNT

A drop of blood was placed on a clean grease free slide; another slide was then used to spread the drop blood till a feather tail obtained on the slide. The spreader slide was initially pushed backward slightly to touch the sample and then spread it evenly at the edge of the spreader and across the slide. The slide was allowed to air dry for up to 5 minutes before placing it into the staining rack and stained with Leishman stain for 2 minutes. The stained smear was then flooded with neutral distilled water for 8 minutes. The slide was then rinsed with distilled water and allowed to

air dry and viewed under the microscope using oil immersion objective. Initially, observation start from the tip end followed by application of systemic meander method to carefully count the different leukocyte and obtained correct result.

Statistical Analysis
Mean values and standard errors were calculated and the results were treated statistically using t-test assessing the mutual statistical differences between male and female animals Snedecor and Cochram (1982).

RESULT
The result of erythrocyte values which includes packed cell volume (PCV), total red cell (RBC) count, haemoglobin (Hgb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) for the sheep was presented as mean and standard error of the mean (SEM). The overall result obtained of both ewe and ram shows that, the mean for PCV recorded in this study was 32.46 ±1.08. PCV was observed to be high in ewe with mean of 33.08±1.3 compare to the ram which was found to be 31.1±1.7. The mean for total RBC count recorded in this study for both sexes was 12.07±1.03. Total RBC count was also observed to slightly high in female (ewe) with a mean value of 13.7±1.85 than male (ram) with a mean value of 11.1±1.51. There is significant different at p>0.05 between the values of the male compared to that of the female. Total haemoglobin recorded in this study for both sexes was 13.7±1.85. There is variation in haemoglobin values with ewe having high value 14.01±1.11 when compared with that of the ram 12.5±0.2. There is significant different at p>0.05 between the values of the male compared to that of the female. Mean corpuscular volume was also measured, the obtained values for both sexes was 26.89±4.07. There is significant difference between MCV values between the sexes with ram having high value of 28.02±3.37 when compared with that of ewe, which happen to be 23.61±10.04. Mean corpuscular haemoglobin was also calculated and the obtained result from both sexes was shown to be 11.43±2.30. Variation in MCH value was also observed with ram having high values of 11.26±5.31 when compared with the females which has values of 10.15±3.93. There is significant different at p>0.05 between the values of the male compared to that of the female. Mean corpuscular haemoglobin concentration was also measured, the values obtained from both sexes was42.55±0.5. The values differ between the sexes with ram having the values of 40.19±0.06 compared to that of ewe with values of 42.35±5.95. There is significant different at p>0.05 between the values of the male compared to that of the female (Tab: 1).

TABLE 1: Comparison of erythrocyte values (Mean ± S.D) between male and female sheep

PARAMETERS		MEAN± SEM
Packed Cell Volume%	All	32.46±1.08
	Male	31.1±1.7
	Female	33.08±1.3
Red Blood Cell Count ($\times 10^3/mm^3$)	All	12.07±1.03
	Male	11.1±1.51
Hgb (g/dl)	Female	13.7±1.85
	All	13.8±1.01
	Male	12.5±0.2
	Female	14.01±1.11
MCV= PCV×10/RBCg/dl	All	26.89±4.07
	Male	28.02±3.37
	Female	23.61±10.04
MCH= Hb×10/RBCg/dl	All	11.43±2.30
	Male	11.26±5.31
	Female	10.15±3.93
MCHC=Hb×10/PCVg/dl	All	42.55±0.5

Male	40.19±0.06
Female	42.35±5.95

RESULT

The result of total leukocyte count was also presented as mean and standard error of the mean (SEM). The overall result for both male and female shows the mean for leukocyte count was 6.78±0.32. This result shows that total WBC count is slightly higher in female (ewe) with recorded value of 7.17±0.51 than male (ram) with recorded value of 6.4±0.3. There is significant different at p>0.05 between the values of the male compared to that of the female.

Differential leukocyte count was also presented as mean and standard error of the mean (SEM). Relative values in percentage was used in this study since the sample collected was from the apparently health animal without sign or symptoms of diseases and was intended to use as reference values. The relative value for neutrophil in this study was 44.02±1.58 in both sexes. This result shows that relative value of neutrophil is slightly high in male (ram) with obtained value of 45±2.17 than the female (ewe) with recorded value of 43.04±2.32. The relative mean value of lymphocyte in both sexes was 50.4±2.08. This result also shows that relative value of lymphocyte count is slightly high in female (ewe) with obtained value of 51.42±3.0 compare to the male (ram) with obtained value of 49.5±2.9. The mean value for monocyte in both sexes was 0.27±0.86. Monocyte count was also observed to be slightly high in female (ewe) with a value of 0.42±0.15 then the male (ram) with a value of 0.42±0.15. The mean value for eosinophil in both sexes was 0.2±0.11. Eosinophil count was observed to be high in male (ram) with a value of 0.36±0.21then the female (ewe) with a value of 0.04±0.04. There is significant different at p>0.05 between the values of the male compared to that of the female. There was no single basophil and band neutrophil seen in the sample during the differential cells count in this study (Tab: 2).

TABLE 3: Comparison of leukocyte values (Mean±S.D) between male and female sheep

PARAMETERS		MEAN± SEM
White Blood Cell Count ($\times10^3/mm^3$)	All	6.78±0.32
	Male	6.4±0.3
	Female	7.17±0.51
Neutrophils%	All	44.02±1.58
	Male	45±2.17
	Female	43.04±2.32
Lymphocytes %	All	50.4±2.08
	Male	49.5±2.9
	Female	51.42±3.0
Basophils	All	0
	Male	0
	Female	0
Monocytes %	All	0.27±0.86
	Male	0.12±0.06
	Female	0.42±0.15
Eosinophils%	All	0.2±0.11
	Male	0.36±0.21
	Female	0.04±0.04

DISCUSSION

Blood is a fluid substance that primarily serves to carry oxygen and nutrients to tissues while shuttling away metabolic waste such as carbon dioxide. Blood is composed of cells, water, proteins, hormones, glucose, fats, amino acids, vitamins, and minerals (Knudsen Sand et al., 2015). Blood testing is commonly used to help diagnose disease or

pinpoint abnormalities such as dehydrated, anemic (having inadequate numbers of red blood cells), or dealing with an infection in animals. It can also help determine the state of the animal health during regular physical or clinical examination (Gardhouse et al., 2015). Hence, Complete blood count (CBC) is necessary before instituting any treatment that involve administration of chemotherapeutic agent (topically or systematically) or carrying out surgical operation in animals (Thompson et al., 2016). There is significance difference (P> 0.05) packed cell volume (PCV), red blood cell (RBC), haemoglobin (Hgb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), complete and differential white blood cell (WBC) counts. In this finding, the result of packed cell volume (PCV) and the total RBC count is significantly high in ewe with a statistic value of 33.08±1.3 PCV, 13.7±1.85 RBC count then ram with a statistical value of 31.1±1.7 PCV, 11.1±1.51 RBC count. This is in line with finding reported by Njidda et al., (2014) which shows variations in packed cell volume and total RBC count with high recorded values in ewe than ram. There is no variation in normal value of both PCV and RBC count with the reference value (RBC 9-15 and PCV 27-45%) Merck manual (Thoen, 2017). There is also variation in haemoglobin (Hgb) with high 11.26±5.31 in male and 10.15±3.93 female, mean corpuscular volume (MCV) 28.02±3.37 in ram and in ewe, which happen to be 23.61±10.04, mean corpuscular haemoglobin (MCH) 11.26±5.31 in ram and 10.15±3.93 in ewe, mean corpuscular haemoglobin concentration (MCHC) 40.19±0.06 in ram and 42.35±5.95 in ewe. There is no variation between the obtained and reference values of Hgb 9-15, MCV 28-40 and MCH 8-12. There is slight decrease in MCHC 40.19±0.06 when compared with the references values 31-34 Merck manual (Thoen, 2017). This may be due to error in sample processing or over hydration concurrent with withdrawal from feed (Ganguly et al., 2017). Diseases such as iron and mineral deficiencies or parasitic infestation may also cause decrease in MCHC values (Roland et al., 2014). Absent of clinical signs and symptoms of diseases as well as normal erythrocyte indices especially PCV and RBC count explained the true health status of the animals with no alteration in the abilities of RBC and hemoglobin to carries oxygen to different body's tissues for normal functions (Beeson et al., 2016). White blood cells (also called leukocytes or leucocytes and abbreviated as WBCs) are the cells of the immune system that are involved in protecting the body against both infectious disease and foreign invaders (Sajjad et al., 2017). All white blood cells are produced and derived from multipotent cells in the bone marrow known as hematopoietic stem cells (Birbrair and Frenette, 2016). Leukocytes are found throughout the body, including the blood and lymphatic system (Wong et al., 2016). The major role of the white blood cells is to defend the body against invading organisms such as bacteria, viruses, and fungi (Rajanbabu et al., 2015). There are different types of leukocytes and each performed specific function depending on the nature and severity of the diseases, types of nutrient and physiological status of the animals (Shrivatav et al., 2012). Variation from normal values of total WBC count is due to problem associated with either individual or all white blood cells (Cwynar, Kolacz, & Czerski, 2014). The result of complete leukocytes count in this study shows total count in for both male and female was 6.78±0.32. The obtained value was slightly high in female (ewe) with 7.17±0.51 than male (ram) with 6.4±0.3 but the values from both sexes were with the normal range 4-12%. There is significant different at p>0.05 between the values of the male compared to that of the female. For differential leukocyte count, the result shows high neutrophil count in male (ram) 45±2.17 compared with the female (ewe) 43.04±2.32, the values obtained from both sexes were within the normal range 10-50%. Lymphocyte count also is slightly high in female (ewe) 51.42±3.0 compare to male (ram) 49.5±2.9, values from both sexes were within the normal range 40-75%. The mean value for monocyte was also observed to be high in female (ewe) 0.42±0.15 compare to male (ram) 0.42±0.15, the values from both sexes were still within the normal range 0-6%. Eosinophil count was observed to be high in male (ram) with a value of 0.36±0.21then the female (ewe) with a value of 0.04±0.04, but the values obtained from both sexes were within the normal range 0.05-0.65%. There is significant different at p>0.05 between the values of the male compared to that of the female. There was no single basophil and band neutrophil seen in the sample during the differential cells count in this study. The number of leukocytes in the blood is often an indicator of disease and thus the white blood cell count is an important subset of the complete blood count (Watz et al., 2016). White blood cells make up approximately 1% of the total blood volume in a healthy adult animal, making them substantially less numerous than the red blood cell at 40% to 45% (Reiner et al., 2011). However, this 1% of the blood makes a large difference to health, because immunity depends on it.

CONCLUSIONAND RECOMMENDATIONS

From the present study, it can be concluded that the hematological parameters for sheep studied fall within normal range with slight variation. The observed differences may be due to nutritional, environmental or unknown effect. Study of haematological parameters across the age group, breed, season and region is recommended to reaffirm the normal reference hematological value.

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