

STUDY ON HEMATOLOGICAL PARAMETERS IN RAMSWondimagegn Tuffa¹ and Yibrah Tekle^{2*}¹College of Veterinary Medicine and Agricultural, Addis Ababa University, Bishoftu.²Animal Health Researcher, Southern Agricultural Research Institute, Hawassa.***Correspondence for Author: Yibrah Tekle**

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ABSTRACT

The normal hematological profiles of rams were studied at Department of Veterinary Surgery and Obstetrics, Faculty of Veterinary Science, Mekelle University, Mekelle. The rams were apparently healthy and maintained under uniform management and feeding conditions. A quantity of 5ml of blood was collected from the jugular vein in ethylene diamine tetra acetate (EDTA) vacutainer tube from each ram with weekly interval for 8 weeks. The mean values of red blood cell count (RBC), hemoglobin (Hb) concentration, Erythrocyte sedimentation rate (ESR) and packed cell volume (PCV) were $10.61 \pm 0.57 \times 10^6/\text{mm}^3$, $10.79 \pm 0.19 \text{g/dl}$, $19.66 \pm 1.07 \text{mm}^2/\text{hr}$ and $31.23 \pm 0.46\%$, respectively. The mean total leucocyte (WBC) count was $11.30 \pm 0.82 \times 10^3/\text{mm}^3$ of blood. The differential leucocyte count (DLC) like lymphocyte, monocyte, neutrophils, eosinophil and basophil were $44.68 \pm 1.93\%$, $5.47 \pm 0.63\%$, $45.75 \pm 1.83\%$, $3.31 \pm 0.40\%$ and $0.72 \pm 0.19\%$, respectively. A positive correlation was observed between PCV and Hb ($r=0.35$) and PCV and RBC ($r=0.37$) in the present study.

KEYWORDS: Differential leukocyte count, Hematological parameters, Hemoglobin concentration, Rams.**INTRODUCTION**

Small ruminants represent an important component of the Ethiopian livestock production system, providing 12% of the value of livestock products consumed at the farm level and 48% of the cash income generated (Demelash et al., 2006). Ethiopia is the second in Africa and sixth in the world in terms of sheep population (Demelash et al., 2006). In view of this, in Ethiopia, the population of sheep and goat is 28 (FARM-Africa, 1996) and 19.2 million (CSA 1995 as cited in FARM-Africa, 1996), respectively. In Ethiopia; according to the 1998 livestock census, goat and sheep population in Tigray region is about 1.47 and 0.94 million, respectively (BoANRD, 1999 as cited in Gebremedhin *et al.*, 2002).

In Tigray, sheep are raised for several purposes. The first things that come to mind are for meat and wool. Sheep skins with their hair kept intact serve as sitting rugs in many rural households of Tigray. The ability of the small ruminants to survive and produce on low cost feed, their adaptability to diverse and difficult environmental conditions makes the small ruminant production typically suitable to small-scale farmers (Njwe, 1992). For the small subsistence farmer or pastoralist who considers his livestock as a kind of investment or "money in the bank", sheep represent a current account or working capital in relation to large livestock which can be considered as equity investment (McDowell and Hildebrand, 1980). In addition, sheep provide security of continued food supply in times of crop failure; their meat

is directly consumed or the animal is sold for cash to purchase grain or is exchanged for grain products. Sheep milk is especially suitable for yoghurt and cheese production because of its high protein and solid content (Haenlein, 1998). The region has, however, benefited little from this enormous resource owing to a multitude of problems, disease being the most important.

Hematological evaluation is useful for the assessment of many disease conditions. Primary alterations in hematological parameters result from disorders within the hemic system itself. Perhaps more importantly, secondary hematological alterations commonly occurs as the result of abnormalities in other body systems, so hematological evaluation can provide important diagnostic clues to many diverse conditions. Because similar hematological abnormalities may occur in response to widely different processes, determination of hematological parameters alonerarely provides a definitive diagnosis. However, when hematological alterations are interpreted in conjunction with other patient information, such as the clinical history, physical examination findings, and other relevant laboratory data, appropriate diagnostic decisions can often be made. On perusal of literature, the data on the hematological parameters of sheep was meager. Hence, this study was aimed to record the normal physiological blood parameters in rams of local breed.

MATERIALS AND METHOD

Study Area

The study was conducted during the period from December, 2007 to February 2008 at Department of Veterinary Surgery and Obstetrics, Faculty of Veterinary science, Mekelle University, Mekelle.

Animals and Experimental Design

The rams used in the present study were procured from Atsebi region. They were dewormed soon after purchased and were allowed to stabilize for one week. The rams were maintained under uniform managerial and feeding conditions.

Blood Sample Collection and Laboratory Analysis

An amount of 5ml of blood was collected from each ram from the external jugular vein following proper restraint and with minimal excitement. The blood was collected in ethylene diamine tetra acetate (EDTA) vacutainer tube and transported to the laboratory for investigation. The sample was processed within 15 minutes of collection of blood. The packed cell volume (PCV) of each sample was determined by the Microhematocrit method. The hemoglobin (Hb) concentration was determined by using Sahli's method. The erythrocyte (RBC) and leucocyte (WBC) counts were estimated by Neubauer haemocytometer. Differential leucocyte counts (DLC) were determined by the thin slide method. The erythrocyte sedimentation rate (ESR) was recorded by using Wintrobe tube method.

Study Animals

The study was conducted on four rams belonging to Atsebi area. The rams were managed under intensive managerial system throughout the study.

Study Protocol

Estimation of haemoglobin (Hb) concentration by method

The graduated tube of hemoglobinometer was filled with decinormal (N/10) solution of hydrochloric acid up to mark 10. By using a clean and dry Hb pipette, the blood was sucked from the vial containing anticoagulant up to mark 20. The blood was expelled directly into the graduated tube containing the hydrochloric acid solution. The last trace blood was removed by drawing the solution up in the pipette and was expelled several times. The contents were mixed thoroughly with the stirrer after every addition and the color was matched with the standard. Adding was continued until the color in the tube corresponds exactly to the color of the standard. The corresponding reading was taken to which the solution has risen in sahli's tube and it was recorded the concentration of Hb as g/dl of blood.

Determination of packed cell volume (PCV) (Hematocrit method)

A capillary hematocrit tube approximately 7cm in length and having a bore of about 1mm was used. The tubes were filled by capillary action. For it, the tube was hold

near a horizontal plane to facilitate filling. The blood was wiped from outside the tube with a piece of gauze while it was wet. The opposite (vacant) end of the tube was sealed with special clay. The cover plate of centrifuge was removed and the sealed capillary tubes was placed on the head in the slots with open ends towards the hub and sealed ends as close as possible to the rim of the head to prevent breaking during centrifugation. Since it was difficult to make the capillary tubes, these were identified by noting the number of slots for these tubes. The cover was replaced securely and it was centrifuged for 5 minutes at 10,000 to 13,000 rpm. The tubes were removed and it was read as percent by using a hematocrit reader.

Erythrocyte sedimentation rate (ESR) by Wintrobe tube method

The Wintrobe hematocrit tube was filled to the 0 mark on the left side scale. The tube was set in a vertical position in an appropriate rack. The upper level of sedimenting erythrocyte was read in mm on the left scale at the time interval (24hr). The results were expressed as fall of RBCs in mm/24hr.

Total erythrocyte count (TEC) by Hemocytometer method

Blood with an anticoagulant was drawn exactly to the 0.5 mark using gentle suction on the mouth piece. If the blood was drawn slightly past the line, it was permissible to expel excess blood by stroking the tip of the pipette with the finger. The tip of the pipette was cleaned to remove extra blood before it was inserted into erythrocyte diluting fluid. The diluting fluid (normal saline) was drawn into the pipette with steady suction up to 101 marks above the bulb. The pipette was brought to a horizontal position and a finger was placed over the tip before the rubber tubing was removed.

The pipette was shaken for at least 2-3 minutes by holding it horizontally between the thumb and middle finger. The ruled area of hemocytometer and the special cover glass were cleaned. The special cover glass was placed with the longer edges parallel and on the supporting ribs of the counting chamber. At least one third of the contents of the pipette were discarded and the tip was wiped off so that no fluid was adhered. With a fore finger over the upper end of the pipette, the tip was touched to the space between counting chamber and the cover glass. The space was filled by the fluid by capillary action. The pipette was removed after charging hemocytometer. It was allowed about 3 minute for the cells to settle but it was avoided evaporation. Under low power magnification of light microscope, the hemocytometer was fixed to locate the central square of the 9 large squares and it was observed for even distribution of cells. All the erythrocyte in 5 of the 25 small squares in the central area was counted under high power. The cells were counted beginning at the left top row of 4 small squares, then from right to left for the

next row and so on. Duplication was avoided in counting cells that touch the line.

Calculation

$$\begin{aligned} \text{Erythrocyte/mm}^3 &= \text{cells counted} \times 10(0.1\text{mm depth}) \\ &\times 5(1/5 \text{ of sq. mm}) \times 200(1:200 \text{ dilution}) \\ &= \text{sum of cells in 5 small square} \times 10,000. \end{aligned}$$

Total leucocyte count (TLC)

The blood was mixed with an anticoagulant carefully by swirling movement before the pipette was filled. After attaching rubber tubing to a leucocyte diluting pipette, the blood was drawn exactly to the 0.5 mark and the blood was wiped from the outside. The blood was diluted with WBC diluting fluid (N/10HCl) by drawing it steadily to the 11 mark above the bulb. It was made a 1:20 dilution. Before filling the counting chamber first 2-3 drops was discarded from the pipette. It was allowed at least 2 minutes for lysis of erythrocytes and for leucocyte to settle. The number of cells in each of the four large corner squares was counted under low power magnification (x10). The rule for including or excluding cells touching the line was the same as for erythrocyte count.

$$\begin{aligned} \text{Calculation TLC/mm}^3 &= \frac{\text{cells counted} \times 20(1:20 \text{ dilution})}{\text{4(no. of sq.mm counted)}} \\ &= \text{sum of the cells in 4 corner squares} \times 50. \end{aligned}$$

Differential leucocyte count (DLC)

The procedure for DLC involves the following steps

- A. Preparing of blood smear.
- B. Fixation of blood smears.
- C. Staining of blood smear.
- D. Counting of leucocyte.
- A. Preparing of blood smear.

A clean grease-free slide with smooth edges was used. The blood sample was mixed properly by gentle agitation and then with the help of applicator stick, small drops were placed near one end of the slide. The end of a second slide (Spreader slide) was placed on the surface of the first slide which was held at an angle of about 30 degree. The spreader slide was drawn gently into the drop of blood, when the blood has spread along 2/3 or more width by a capillary action. The spreader slide was pushed forward with a steady and even motion. The blood smear was dried rapidly by waving in air.

B. Fixation

The dried blood smear was immersed in methanol for 5-15 minutes and it was allowed to air dry.

C. Staining

The fixed blood smear was placed in a couplin jar containing fresh stain which was prepared by diluting 1 volume of commercial stock solution of Giemsa with 9 volume of distilled water. The smear was allowed to

stain for 30 minutes. It was washed in tap water, dried and examined.

D. Counting leucocyte

The blood smear was inspected under low power to note distribution of cells. A portion of the smear near the thin end where the erythrocytes were not overlap was selected. The oil immersion objective was switched for making the rest of examination. The DLC was done by counting and classifying at least 100 leucocytes. The examination was started along the outer margin of the smear for about 3 fields, a short distance was moved inward (3 field), parallel the margin for 3 fields and then it was moved back to the edge of the blood smear.

Data Analysis

The data was analyzed as per the prescribed of statistical methods. All the data that were collected were entered to MS excel and analyzed by using that the prevalence of PPR in goats by SPSS version 11.

RESULTS

The hematological parameters (Mean \pm SE) of the rams are presented in (Table 1 and 2.).

RBC count

The overall mean of RBC count of rams were 10.61 \pm 0.57 \times 10⁶/mm³. There was no significant difference (p>0.05) in counts of RBC among the rams (Table 1).

Hemoglobin (Hb) concentration

The hemoglobin concentration of the rams was 10.79 \pm 0.09g/dl. The hemoglobin concentration was found to be positively correlated (r=0.35) with packed cell volume (PCV). There was no significant difference among the rams in Hb concentration.

Packed Cell Volume (Hematocrit)

The mean value of packed cell volume (PCV) of the rams was 31.22 \pm 0.46% (Table 1). The PCV also showed no significant difference among the rams. But packed cell volume was correlated positively with hemoglobin (Hb) (r=0.35) and RBC (r=0.37).

Erythrocyte sedimentation rate

The erythrocyte sedimentation rate (ESR) was 19.66 \pm 1.07mm/24hr (Table 1). There was no significant difference observed among the rams.

Total Leucocyte count (WBC)

The absolute leucocyte count was found as 11.30 \pm 0.82 \times 10³/mm³ of blood (Table 2). There was significant difference (P<0.05) among leucocyte count of the rams.

Differential leucocyte count (DLC)

The relative differential leucocyte count recorded as 44.69 \pm 1.96% lymphocyte, 45.75 \pm 1.83% neutrophil, 3.31 \pm 0.40 eosinophil, 0.72 \pm 0.19% basophil and

5.47±0.63% monocyte in the rams (Table 2). The overall mean of differential leucocyte count (DLC) showed no

significant difference among the rams.

Table 1. Erythrocyte parameters (Mean±SE) of adult rams.

S.No	Ram No	RBC[x10 ³ /mm ³]	Hb[g/dl]	PCV[%]	ESR[mm/24hr]
1	A n=8	12.19±2.17	11.19±0.16	32.81±0.74	15.88±0.92
2	B n=8	10.54±0.64	10.63±0.26	31.56±0.94	22.5±1.98
3	C n=8	9.10±0.42	10.75±0.13	30.06±0.77	19.25±2.09
4	D n=8	10.26±1.2	10.56±0.15	30.44±0.1	21.00±2.76
	Overall mean	10.60±0.57	10.79±0.09	31.22±0.46	19.66±1.07

Table 2. Total WBC and Differential leucocyte count (Mean±SE) of adult rams.

No	Ram No	WBC(x10 ³ /mm ³)	Differential leucocyte count (DLC) in percent				
			Lymphocyte	Neutrophil	Eosinophil	Monocyte	Basophil
1	A n=8	13.5±1.16	38.38±3.12	53.13±4.24	1.5±0.57	6±1.39	1±0.46
2	B n=8	8.46±1.02	47.75±2.62	41.5±2.16	3.88±0.95	5.88±0.85	1±0.46
3	C n=8	14.38±2.13	43.25±5.57	46.38±4.36	3.5±0.63	6.13±1.5	0.5±0.19
4	D n=8	8.87±0.93	49.38±2.89	42±2.35	4.38±0.75	3.88±1.25	0.38±0.38
	Overall mean	11.3±0.82	44.69±1.94	45.7±1.83	3.31±0.4	5.47±0.63	0.72±0.19

DISCUSSION

The result of the present study indicated that the RBC were within the normal range as recorded by Kelly (1973), Duncan (1986), Clarence *et al.*, (1991), Borjesson *et al.*, (2000) and Gupta *et al.*, (2003). The RBC value of the study was higher than the value recorded by Bone (1988) and Olayemi *et al.*, (2000). The variation in the RBC counts of rams might be attributed to the excitement or strenuous exercise during handling Coles (1980) and Gartener *et al.*, (1969) which lead to the release of adrenalin consequently the spleen contracts and ultimately release of RBC in to the circulation. It is only psychological tranquillization that can reduce the splenic influence Schalm *et al.*, (1975). The difference in RBC counts recorded by different authors could be due to breed difference, management and environmental factors (Egbe-Nwiyi *et al.*, 2000). RBC has a biconcave shape, the biconcavity increases the surface area, thus facilitating the exchange of oxygen and carbon dioxide carried by, and the red blood cells contain the enzyme carbonic anhydrase, which catalyzes the reversible reaction of carbon dioxide form bicarbonate ions, thereby readily eliminating carbon dioxide (Swenson, 1993).

Anemia is a condition which can occur due to an abnormally low number of circulating RBC, abnormally low hemoglobin or both (Porth, 1990). Polycythemia is an increased erythrocyte mass per volume of blood. There are two kinds of polycythemia; relative and absolute. In relative polycythemia, the hematocrit is increased because the aqueous portion of the blood is decreased. This situation is the result of dehydration and is by far the most common type of polycythemia seen. Rehydration resolves the problem. Absolute polycythemia is due to an actual increase in the total erythrocyte mass in the body. Example of this type of polycythemia include that associated with tissue hypoxia

secondary to high altitude and that associated with pulmonary disease that interferes with oxygenation of hemoglobin (Oyewale, 1991).

Packed cell volume (hematocrit) in the present study (Table 1), is similar to observations made by Kelly (1973), Duncan (1986), Bone (1988), Clarence *et al.*, (1991) and Gupta *et al.*, (2003) where as higher and lower than the present value was reported by Olayemi *et al.*, (2000) [23-26.85] and Borjesson *et al.*, (2000) [33.2-56.3] respectively.

The hemoglobin levels were in agreement with those reported by (Kelly 1973), (Duncan 1986), (Clarence *et al.*, 1991), (Borjesson *et al.*, 2000) and (Gupta *et al.*, 2000). The higher and lower values than the present study was noticed by (Olayemi *et al.*, 2000) and (Bone 1988) respectively. The hemoglobin is composed of a pigment and a simple protein. The protein is globin and a histone. The red color of hemoglobin is due to heme, a metallic compound containing an iron atom. Hemoglobin has four polypeptide chains alpha, beta, gamma and delta. Each of the four chains unites with a heme group, resulting in the hemoglobin molecule (Swenson, 1993). The variation in the hemoglobin count could be attributed by excitement (Swenson, 1993).

The mean value of 19.66±1.07mm/24hr was obtained for erythrocyte sedimentation rate (ESR) which was higher than the value reported by Gupta *et al.*, (2003) as 3.00-08.50mm/24hrs. Collection of blood after intake of meal can result in elevation of ESR. The temperature of the environment, diameter and length of the pipette are factors that influence the rate of ESR (Sengupta, 2002).

The mean value for leucocyte count (WBC) was within the values reported by (Holman, 1944), (Kelly, 1973), (Duncan, 1986), (Schalm *et al.*, 1986) and (Borjesson *et*

al., 2000). The leucocyte count in the present study (Table 1) was lower than the value as reported by (Oduye, 1976) [$15.25 \times 10^3/\text{mm}^3$] and (Olayemi *et al.*, 2000) [$11.78-18.9 \times 10^3/\text{mm}^3$]. The white blood cells are much less numerous than erythrocytes in the circulating blood. Erythrocytes function in the blood stream whereas leucocytes carry on their functions predominantly in the tissues. There are approximately 1200 erythrocytes to every leucocyte in the blood stream of sheep Swenson (1993). Since the animals are apparently healthy, any value beyond the upper limit may be regarded as leukocytosis and any value below the lower limit may be termed leucopenia.

The lymphocytes and neutrophils constitute the majority of WBC. The lymphocytes in the study were similar to the result reported by (Bone 1988) and were higher to observations of (Duncan 1986) and (Olayemi *et al.*, 2000). (Kelly 1973), (Swenson 1993) and (Borjesson *et al.*, 2000) recorded higher values than the present study.

Lymphocytosis is of rare occurrence than neutrophilia (Kelly, 1973). The commonest form of lymphocyte is of small size (less than $10\mu\text{m}$ in diameter) with a large, almost circular or slightly indented nucleus and a narrow peripheral zone of blue stained cytoplasm. The cytoplasm may contain a group of comparatively large, dark blue or red (azurophilic) granules. The large lymphocytes have proportionately more cytoplasm, which stains pale blue and may contain small vacuoles, thus causing confusion with monocytes (Kelly, 1973).

The eosinophil, basophil and monocyte values obtained in this study were comparable to those previously reported (Kelly, 1973; Duncan, 1986; Bone, 1988; Swenson, 1993 and Borjesson *et al.*, 2000). The monocyte in the study was higher than the value reported by (Duncan, 1986), (Olayemi *et al.*, 2000) and (Borjesson *et al.*, 2000). The higher and lower eosinophil values than the present study was noticed by (Duncan, 1986) and (Olayemi *et al.*, 2000) respectively. The production and utilization kinetics of eosinophils are similar to those of neutrophils, but eosinophils respond to different stimuli. Their granulation is cell specific and to some extent, species specific. They are capable of killing bacteria but are less efficient than neutrophils. They have receptors for complement 3b, which is probably the means by which they bind to tissue parasites. Eosinophils are late arrivals in the immune response to parasites and their chemotaxis can be explained on the basis of specific lymphokines from prior sensitized T lymphocytes. The increase in mature eosinophils seen in mature sheep with hemochromatosis is probably part of the syndrome of iron deficiency and not directly related to parasitism. In diseased animals, eosinophils frequently have more marked toxic changes than neutrophils. These changes consist of reduced granulation, increased diffuse cytoplasmic basophilia and large vacuolation. The toxic changes are seen most often in diseases involving the gut, lung and skin and are not species specific.

Eosinophils are most prominent in these three tissues and this may provide an anatomic basis for the preference (Valli *et al.*, 1990) (Olayemi *et al.*, 2000) and (Borjesson *et al.*, 2000) reported lower value of basophils than the value in the study. Their maturation sequence and kinetics are similar to neutrophils with peak peripheral blood arrival of labelled cells seven days after injection of tritiated thymidine. The circulation time is unknown but is probably short. Newly released cells are specifically directed; they do not have a random and age-dependent removal as do neutrophils. Basophils do not segment as fully as neutrophils and in this respect resemble eosinophils. Basophils are about a third greater in diameter in blood films than are neutrophils and retain smooth nuclear boundaries unlike the irregular constrictions of mature neutrophils. These characteristics are of interest because basophils are easily degranulated in slide preparation and may be mistaken for neutrophils (Valli *et al.*, 1990). Basophils are relatively scarce in the blood of the large animal species, although these cells are more frequently encountered in large animals than in dog and cats (Jain, 1986). Changes in the number of basophils are difficult to interpret. Stress causes a reduction in the number of basophils. Basophilia may be seen with allergic dermatitis and delayed-hypersensitivity reactions in which production of IgE and lymphokines occurs (Morris *et al.*, 1986).

The monocyte in this study was higher than the value reported by (Duncan 1986), (Olayemi *et al.*, 2000) and (Borjesson *et al.*, 2000). Monocytes are produced in the bone marrow from the colony-forming unit-granulocyte: macrophage which differentiates into either myeloblasts or monoblasts (precursors of monocytes) (Jain, 1986). Monoblasts undergo mitosis to promonocytes and then divide one to two more times to produce monocytes. Once released into the blood, monocytes circulate for 1 to 3 days and then enter body cavities and tissues and transform into macrophage. Tissue macrophages survive in tissues for weeks to years. Once in tissue, these macrophages are described as "fixed" or "free". Free macrophages are found within the peritoneal and pleural cavities in joints, in alveolar spaces and at area of inflammation. Fixed macrophages include Kuffer's cells of the liver, osteoblasts, microglial cells and macrophages found in the spleen, bone marrow and lymph nodes (Morris *et al.*, 1991).

5. CONCLUSION AND RECOMMENDATION

The result of the present study showed that, the hematological parameters found during the study were comparable to values recorded by different authors. There was slight fluctuation in the hematological parameters among the rams during the time of the research which could be due to undetected minor infections and weather extremities. Breed difference, management and environmental factors could be the reasons for changes in hematological records among the rams during the time of the research. Therefore, based up

on this conclusion the following recommendations forwarded.

✓ The result of this study could be used as a reference point in diseases that alter the hematological parameters.

✓ Since there was no recorded data on hematological parameters, the study provide normal hematological value of rams.

✓ The hematological values of the study could be helpful to compare the hematological parameters of other breed of different region.

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