AKENTEN APPIAH-MENKA
UNIVERSITY OF SKILLS TRAINING AND ENTREPRENEURIAL
DEVELOPMENT

EFFECT OF PHYSIOLOGICAL STATE ON HAEMATO–BIOCHEMICAL PROFILE OF DJALLONKE SHEEP IN ASANTE MAMPONG

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A THESIS IN THE DEPARTMENT OF ANIMAL SCIENCE EDUCATION, FACULTY OF AGRICULTURE EDUCATION, SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF EDUCATION AGRICULTURE (ANIMAL SCIENCE) IN THE UNIVERSITY OF EDUCATION, WINNEBA

OCTOBER, 2022

DECLARATION

STUDENT'S DECLARATION

I, do hereby declare that with the exception of references to other people's work which have been cited and duly acknowledged, this thesis is the result of our own work and it has neither in whole nor partially been presented in this University or elsewhere.

Student's Name: KELVIN BOAKYE

Signature ………………………..

Date ……………………………

SUPERVISOR'S DECLARATION

I, hereby, declare that the preparation and presentation of the thesis was supervised following the guidelines for supervision of project work laid down by the Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development

Supervisor's Name: DR. ISMAIL COFFIE

Signature………………………..

Date …………………………..

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1

DEDICATION

I dedicate this dissertation to my beloved parents.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ABBREVIATIONS AND ACRONYMS

2,3 - DPG, 2,3	Diphosphoglycerate
BCS	Body Condition Score
CBC	complete blood count
EDTA	Ethylenediaminetetraacetic Acid,
EPO-	Erythropoietin
Hgb-	Hemoglobinb
HgbA	Hemoglobin A,
HgbC	Hemoglobin C
M : E ratio	Myeloid : Erythroid Ratio
PCV	Packed Cell Volume
RBC	Red Blood Cell
SEG	Segmented
WBC	White Blood Cell.

ABSTRACT

A three-month study was conducted to determine the effect of physiological state on the haemato-biochemical profiles of Djallonke sheep in Mampong Ashanti. A cross-sectional study was employed. A total of 24 Djallonke ewes of different physiological states categorized into age, parity, body condition score (BCS), and pregnancy stages (first trimester, second trimester, and third trimester) were used for haemato-biochemical for the study. Haematological indices were determined by five (5) parts (differentials) fully automated haematological system whereas serum biochemical profiles were analyzed using Mindray BS 130 fully automated blood chemistry analyzer. The haematology assessment indicated that erythrocyte indices were influenced ($P<0.05$) by age, BCS, parity and pregnancy stages. The differences did not show sequential variations with age, BCS, parity and stages of pregnancy. Leucocyte indices were affected by age, BCS, parity and pregnancy stages. Sheep at 4 years had the highest white blood cell followed by 6 months, 6 years, 2 years and 3 years in descending order. Six years ewes had the highest ($P<0.05$) heterophils followed by 6 months, 4 years 3 years and 2 years in descending order. Sheep of BCS 3.2 had the highest haematocrit followed by 2.8, 3.0, 3.5, 2.0 and 4.0 in descending order. The WBC and heterophils were higher in the first trimester of gestation compared to the second and third trimesters. Serum biochemistry was affected ($P<0.05$) by the fixed factors such that sheep of 4 years had the highest total protein concentration followed by 6 months old, 2, 3 and 6 years in descending order. Sheep of BCS 4.0 had the highest ($P<0.05$) glucose concentration followed by BCS 3.0, 2.5, 3.5, and 2.0 in descending order. The differences in serum biochemical indices did not follow a sequential pattern with age, BCS, parity and stages of pregnancy. Body condition score had a slight ($P>0.05$) influence on total cholesterol and triglycerol concentrations. It is recommended that Physiological states (age, BCS, parity. And pregnancy stages) of Djallonke sheep have an immense effect on the haemato-biochemical indices of Djallonke sheep and hence, these factors should be considered when assessing the performance of the animals.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the study

Blood indices are important in veterinary medicine as indicators of the physiological, metabolic, and clinical status of farm animals (Mirzadeh *et al*., 2010). Physiological equilibrium is maintained mainly by the blood in the body but many physiological conditions may alter this equilibrium (Mirzadeh *et al*., 2010) Hence, the haematological values during different physiological situations should be known for diagnosis of various pathological and metabolic disorders which can adversely affect the productive and reproductive performance of female animals leading to heavy economic losses (Mirzadeh *et al*., 2010).

The results of complete blood count (CBC) indices are often helpful in the diagnosis, monitoring, and prognosis of the disease (Roland *et al.,* 2014). Haematological indices reflect the responsiveness of an animal to its internal and external environment (Yaqub *et al*., 2013).

The selection of animals that are genetically resistant to specific illnesses and environmental circumstances may benefit the understanding of the link between blood properties and the environment provided by haematological investigations, which are of physiological importance. Animal performance results from the interplay of the environment and genetics.

Parity is particularly important for understanding physiological changes in healthy cows and biochemical variance in cows of different parities (Cozzi *et al*., 2011). Parity, age, and pregnancy status, according to several studies account for the causes of variations in the haematological and biochemical traits of dairy cows (Cozzi *et al*., 2011; Constable *et al*. 2017). According to Cozzi *et al*. (2011), older cows typically have a low percentage and high concentration of total protein.

1.2 Problem Statement

Haematological measurements have been used to identify cattle productivity limitations (Duskaev *et al.,* 2018), however, such indices are underutilized for breed and parity of cows in Ashanti Region. Although blood profiles are used to determine nutritional status, physiological vitals, disease diagnosis, prognosis, and as indicators of an undesirable reaction (Hallek *et al.,* 2018), data on these haemato-biochemical indices in cattle kept in the hot-humid zone are rare.

Several studies have been conducted to assess the effect of parity and age on variations in the haematological and biochemical traits of dairy cows (Cozzi *et al*., 2011) however, very little is known about these indices in sheep at different parity in Ashanti Region.

As blood profiles reflect the responsiveness of an animal to its internal and external environments (Yaqub *et al*., 2013), and hence, enhance the assessment of enzymes, electrolytes and other physiological/health indicators (Szablewski, 2011**).** The assessment of haemato-biochemical indices for physiological and prognostic decision-making in different breeds and parities of sheep cannot be underestimated in the study area. Therefore, a baseline assessment of erythrocytes and leucocytes, serum biochemistry, involving Liver Function Test (LFT), Kidney Function Test (KFT) and electrolytes with respect to breed and different physiological states (including parity) would be vital for the subsequent development of reference ranges in sheep flocks in the hot-humid environment of Ghana.

1.3 Objective of the Study

1.3.1 Main Objective

The main objective of the study was to find out the effect of physiological states on the haemato-biochemical profiles of Djallonke sheep in Mampong Ashanti

1.3.2 Specific objectives

The specific objectives were to determine:

- The effects of age and body condition score (BCS) on haemato-biochemical indices of Djallonke sheep in Mampong Ashanti.
- The effect of parity and pregnancy stages on haemato-biochemical profiles of Djallonke sheep in the study area.
- The effect of pregnancy stages (first, second and third trimesters) on haemato-biochemical indices of Djallonke sheep in Mampong Ashanti.

1.4 Significance of the study

This study would make available information on haemato-biochemical profiles of cattle of different breeds and physiological states. This information on the effect of physiological status on haemato-biochemical indices, and metabolic profile of Djallonke sheep could readily serve as reference data and enhance further research. Information from this research would play a crucial role in the growth, development and improvement of the health status of cattle in Ghana.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Djallonke Sheep Production in Ghana

Animal production is an integral part of Ghana's agricultural economy and a major source of livelihood for many rural households in the Northern, Upper East and Upper West Regions of Ghana. In Ghana, animals are raised under the extensive, semi-intensive and/or intensive system. However, the extensive system is the commonest method and it is practised most especially in rural communities. Animal production holdings are commercial, family or individually owned (Birteeb *et al*., 2013). The Djallonke sheep breed is the oldest in Africa and is more resistant to several livestock diseases than the Sahelian breed native to the same area, including haemonchosis and trypanosomosis (Yaro, 2017). Djallonké sheep are primarily distinguished by an early reproductive development together with a high rate of prolificacy and a high level of resistance to animal trypanosomiasis, a condition that is frequently prominent in tropical regions (Traoré *et al*., 2008; Birteeb *et al*., 2013). Despite this potential, the Sahelian sheep breed, which is larger and can coexist in the same zones, the Djallonké sheep are hardier and are more appealing to sheep meat dealers and producers (Birteeb *et al*., 2013).

2.2 Haemato-biochemical Profile of Sheep

2.2.1 Haematology

Blood indices are important in veterinary medicine as indicators of the physiological, metabolic, and clinical status of farm animals (Mirzadeh *et al*., 2010). Physiological equilibrium is maintained mainly by the blood in the body but many physiological conditions may alter this equilibrium (Mirzadeh *et al*., 2010). Hence, the haematological values during different physiological situations should be known for the diagnosis of various pathological and metabolic disorders which can adversely affect the productive and reproductive performance of cows, leading to heavy economic losses (Mirzadeh *et al*., 2010).

The results of complete blood count (CBC) indices are often helpful in the diagnosis, monitoring, and prognosis of disease (Roland *et al*., 2014).
Haematological indices reflect the responsiveness of an animal to its internal and external environment (Yaqub *et al*., 2013).

2.2.1.1 Red blood cells (Erythrocytes) as a haematological index

Erythrocytes are generally considered to be discocytes, with some degree of concavity. In adult ruminants (cattle, sheep and goats erythrocyte lifespan varies from 125 to 160 days (Reece *et al*., 2015). Ovine red blood cells (RBCs) are some of the smallest mammalian RBCs and do not aggregate or deform as readily as RBCs of other species. Except for the Angora breed, the RBCs of most sheep breeds are discoid in shape. Angora goats frequently have fusiform-shaped RBCs mixed in with discoid RBCs (Jain, 1986). As in other species, accelerated erythropoiesis in sheep and goats is accompanied by reticulocytosis, macrocytosis, polychromasia, and basophilic stippling of reticulocytes (Jain & Kono, 1977). Stippling is best demonstrated with freshly prepared, EDTA - preserved, rapidly air-dried, Wright's or Giemsa-stained RBCs. The erythrocyte indices, including mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) (Christensen *et al*., 2008) are calculated from determinations of erythrocytes (RBCs), haematocrit (HCT) and haemoglobin (HGB) concentration. These three indices are important and each relates to a value for a single RBC (Reece *et al*., 2015).

An increase in the RBC count is known as polycythaemia. It occurs in both physiological and pathological conditions. When it occurs in physiological conditions it is called physiological polycythaemia and it is influenced by age, sex, altitude, muscular exercise, emotional condition, increased environmental temperature and feeding (Reece, 2015). A reduced red blood cell count implies a reduction in the level of oxygen that would be carried to the tissues as well as the level of carbon dioxide returned to the lungs (Ugwuene., 2011; Soetan, Akinrinde, and Ajibade, 2013.; Isaac *et al*., 2013). The degree of regeneration of hyperchromasia of RBC is also important (Merck Manual., 2012).

Merck Manual (2012) reported the range of normal number (count) of RBC value for cows as 5.0 - 10.0 X 106/mm3. Higher than normal numbers of RBC can be due to congenital heart disease, dehydration resulting from severe diarrhoea, low blood oxygen levels (hypoxia), and polycythemia vera among other. When an animal moves to a higher altitude, the RBC count increases for several weeks (Gernsten, 2009; Bunn, 2011).

Lower RBCs may be attributed to anaemia, bone marrow failure resulting from radiation, toxins or tumour, erythropoietin deficiency (secondary to kidney disease), haemolysis (RBC destruction) due to blood vessel injury or other causes, haemorrhage (bleeding), malnutrition, nutritional deficiencies or iron, copper, folate, vitamin B_{12}, vitamin B_6, over dehydration, pregnancy among others (Awuchi *et al*., 2020).

Drugs also decrease the RBC count (Gernsten, 2009; Bunn, 2011). Bloody diarrhoea, bleeding, and blood-sucking parasites are specific causes of chronic blood loss and abnormalities (Johnson and Morris, 1996; Chineke *et al*., 2006).

2.2.1.2 Haematocrit/Packed cell volume

The cellular components of blood (erythrocytes, leukocytes, and platelets, also known as thrombocytes) occupy the lower portion (a capillary column) and, taken together, are known as the haematocrit (HTC). When a column of blood is centrifuged, the components are separated according to their relative specific gravities Phillips, (Reece *et al*., 2015). Packed cell volume (PCV) which is also known as HTC or erythrocyte volume fraction (EVF) (Etim *et al*., 2016) represents the percentage (%) of red blood cells in the blood (Purves, Sadava, Orians and Heller, 2003).

A PCV/HCT value of < 24 %o is considered anaemic whereas ≥ 24 % is considered normal as described by Kessell (2015). A PCV/HTC range of 28.4 ± 0.61 to 31.4±0.50 % has been reported in cows in literature (Sattar and Mirza, 2009). PCV has been determined using micro-haematocrit centrifugation technique as described by Brar *et al*. (2011). Haematocrit value is a reliable and significant measurement parameter for the health status of the animal.

High PCV reading may indicate an increase in the number of circulating RBCs (Patel *et al*., 2016) stated the RBCs of PCV are involved in the transport of oxygen. Increased HTC shows better transportation and thus results in increased primary and secondary polycythemia. A low haematocrit with a low MCV and with a high RDW suggests chronic-iron-deficient anaemia resulting in abnormal haemoglobin synthesis during erythropoiesis (Coffie *et al*., 2020).

Elevated haematocrit is most often associated with dehydration, which is a decrease in the amount of water in the tissues. These conditions reduce the volume of plasma causing a relative increase in RBC concentration, usually termed haemo-concentration (Suski *et al*., 2006). Low haematocrit requires increased production of red blood cells, so dietary modifications may include increased protein and iron. Elevated glucose levels cause RBCs to swell and may cause a falsely elevated haematocrit (Lockwood, 2015).

2.2.1.3 Haemoglobin

Haemoglobin (HGB) is the iron-containing oxygen-transport metalloprotein in the red blood cells of all vertebrates (Jagadeesan *et al*., 2014). Haemoglobin carries oxygen from the respiratory organs (lungs) to the rest of the body tissues where it releases the oxygen to burn nutrients to provide energy for the correct functions of the organism and takes away carbon dioxide to bring it back to the respiratory organ to be dispensed from the organisms. Bovine haemoglobin has also been used extensively as an oxygen-carrying substitute in humans and small animals (Wood and Quiroz-Rocha, 2010). Cattle HGB ranges that have been reported include 8.0 - 15.0 g/dlL (Day, 2003), 7.0 - 13.9 g/dL (Kalaitzakis *et al*., 2011), 8.4 - 12.0, 9.0 - 14.0 g/dL (Roland *et al*., 2014).

Increased haemoglobin occurs when there are burns, heart failure, dehydration, erythrocytosis, haemoconcentration, and "high altitudes polycythemia vera (Lockwood, 2015).

A decrease in circulating RBCs affects the haemoglobin level. Conditions with abnormal types of haemoglobin often result in lower total haemoglobin because the red blood cells with abnormal haemoglobin are readily damaged. According to Lockwood (2015), specific disorders that result in decreased haemoglobin include thalassaemia sickle cell disease-an abnormally-shaped haemoglobin known as sickle haemoglobin (hgbS), iron deficiency anaemia, physiological anaemia of pregnancy, haemolytic disorders, malignancies (such as leukaemia, carcinoma, lymphomas), Hodgkin's disease, pregnancy, nutritional deficit, fluid retention and intra venous overload. In chronic haemorrhage, RBC count, HCT, and HGB are decreased while reticulocytes as well as MCV are increased. In ruminants, only a moderate rise in reticulocytes is observed in animals responding to anaemia (Roland *et al*., 2014). Merck Manual (2012) reported the normal range of cattle MCV value as 39 - 55(fl). Etim *et al*. (2014) also documented the normal range of MCV (fl) values in sheep as 23 - 48 and cows as 40 - 60.

2.2.1.4 Platelets as a haematological index

Platelets are anuclear cytoplasmic fragments of megakaryocytes and play an essential role in haemostasis (Cimmino & Golino, 2013). Bovine platelets are small compared to those of other species and have an average range of mean platelet volume (MPV) of 4.0-4.8

femtolitres (Roland *et al*., 2014). The normal range of platelet count in cows is 300-800 (Merck Manual, 2012).

Platelet parameters that can be estimated include the total number of platelets, MPV, thrombocrit or plateletcrit (PCV), and platelet distribution width (PDW) (Habibu *et al*., 2018). In sepsis however the platelet count increases(Guclu *et al*., 2013). The PDW and MPV increase in sepsis with the appearance of large and heavy platelets in circulation, PDW increased with sepsis (Golwala *et al*., 2016).

2.2.2 Leucocyte indices

White blood cells, leukocytes, are classified into two primary groups:

(l) **Granulocytes** (granules in leukocyte cell cytoplasm which include neutrophils, eosinophils and basophils (Lockwood, 2015) The three types of granulocytes are named according to which component of the haematoxylin-eosin (H&E) stain (haematoxylin, basic and coloured blue; eosin, acidic and coloured red) is taken up by the granules (Reece, 2015). Since these cells have a multilobed nucleus, they are also called polymorphonuclear leukocytes or "polys'" Neutrophils have segmented nuclei, and are sometimes referred to as segmented neutrophils or "segs" (Lockwood, 2015). Neutrophils are neither markedly acidophilic nor basophilic and incorporate both basic and acidic components into their granules. Basophils only accept the basic (haematoxylin) component, and eosinophils only accept the acidic (eosin) component (Reece, 2015).

(2) **Agranulocytes** (no granules and non-lobular nuclei) are of two types, including lymphocytes and monocytes which are sometimes referred to as mononuclear leukocytes (Lockwood, 2015)."The determination of the percentage distribution of WBCs is known as d differential white blood cell count (Reece, 20l5).

The different types of leukocytes in' humans have many similarities with those in animals (Reece, 2015). In some respects, ruminant WBC responses are similar to those in other species, but they also have some distinct features (Tornquist and Rigas, 2010). Sheep have a similar N:L to cattle (Tornquist and Rigas, 2010).

An increase in leukocyte number is called leucocytosis, which usually occurs in bacterial infections. A decrease in numbers is called leukopenia which is usually associated with the early stages of viral infections. Leukaemia is a cancer of WBCs and is characterized by leukocytosis (Reece, 2015).

Cattle, sheep and goats typically have a lower peak level of leukocytosis in the face of acute inflammation than do other domestic animals (Valli, 2007). For cattle, and goats WBC count of 20,000 - 30,000/μL and 22,000 - 27,000/μL, respectively, are considered extreme leukocytosis (Jain, 1993).

Physiologic leukocytosis is a transient increase in neutrophils and lymphocytes caused by epinephrine released in the face of excitement or fear. The neutrophils are mature and the changes are present for a short period on the order of 30 minutes following the stimulus (Tornquist and Rigas, 2010).

A stress leukogram is frequently present in ruminants that have been exposed to endogenous or exogenous glucocorticoids. Leukogram changes include mild mature neutrophilia, lymphopaenia, eosinopaenia, and variable monocytosis (Raskin, Latimer, & Tvedten, 2004). In cattle, the N: L ratio is frequent. Reserved to greater than 1.0 (Tornquist and Rigas, 2010).

Blood parasites are known to cause leukocytosis. Among them is Anoplasma phagocytophilum which is an obligate intracellular bacterium formerly known as Ehrlichia phagocytophila (Whist *et al*., 2003; Woldehiwet, 2006).

2.2.2.1 Monocyte

Monocytes are the largest white blood cells which become macrophages when activated and engulf pathogens and debris through phagocytosis. Monocytes are also involved in presenting antigens to B and lymphocytes. Monocytes range from 1 to 6% of the leucocytes (Etim *et al*., 2014) or may range from 0 to 800 cells/µ or 0 to 0.8 X 10^9/L (Radostits *et al*., 1994). Monocytosis is sometimes seen as part of a stress response in ruminants, but not as frequently as in other species. It may also be noted in several inflammatory conditions (Weiss and Perman, 1992). Monocytopaenia may be associated with endotoxaemia and other peracute and acute inflammation (Jain, 1993).

2.2.2.2 Lymphocytes

Lymphocytes originate from a lymphoid stem cell, known as a lymphoblast, in lymph tissue, such as lymph nodes, spleen, tonsils, and various lymphoid clusters in the intestine and elsewhere (Reece, 2015). A range of 19.0 to 92.0 %; 40.0 to 70.0%, and 60 to 65% of leucocytes have been reported by Thanakun *et al*. (2017), Etim *et al*. (2014) and Reece (2015), respectively. Radostitis *et al*. (1994) reported a lymphocyte number range of 2.7 to 7.5 X 10^9/L. A gradual decrease in lymphocyte numbers is then seen as cattle age (Mohri *et al*., 2007; Tornquist and Rigas, 2010).

2.2.2.3 Basophil

This white blood cell enters damaged tissues and releases histamine and other chemicals that promote inflammation in the body to fight pathogens. Basophils constitute < 1% of WBC (Reece, 2015). The mast cells and basophils play an exceedingly important role in some types of allergic reactions because the type of antibody that causes allergic reactions, the immunoglobulin E (IgE) type, has a special propensity to become attached to mast cells and basophils (Guyton and Hall 2006; Tornquist and Rigas, 2010). Basophilia has been reported in cattle with tick infestations (Tornquist and Rigas, 2010) and in goats experimentally infected with nematodes (Richard and cabaret, 1993).

2.2.2.4 Eosinophil

The nuclei of most ruminant eosinophils are a band or bilobed and surrounded by numerous, intensely red-stained, small, round, refractile, cytoplasmic granules against the background of a sparse basophilic cytoplasm. Within the ovine eosinophilic granules, there are unique dense crystalline structures. The ovine and caprine immunological response to seasonal parasitism is accompanied by eosinophilia (McBean *et al*., 2016). This seasonal eosinophilia of parasitaemia should not be assumed to be normal resulting in inclusion in reference intervals. Investigators concerned with seasonal parasitism eosinophilia have observed it to be earlier and greater in some sheep in a flock. Eosinophils have been reported in a variety of endoparasite infections in cattle (Conboy and Stromberg, 1991) and small ruminants (Tornquist and Rigas, 2010).

2.2.3 Serum biochemical parameters of sheep

Pregnancy and lactation are very critical physiological states in small ruminants during which nutritional requirements are increased manifold. A substantial cost to the animal is imposed by pregnancy as nutrients are required to the extent of 75% towards the end of pregnancy compared to non-pregnant animals and for a successful outcome of pregnancy major changes in the dam's physiology and metabolism are needed. Consequently, significant changes may occur in the ewes in late gestation and early lactation periods, leading to metabolic disorders. Moreover, substantial losses of body minerals occur during pregnancy and lactation. Therefore, estimating the concentration of macro and micro minerals in the serum during different physiological states becomes imperative (Elnageeb and Adelatif, 2010). The blood metabolic profile provides an important diagnostic tool to assess the nutritional status and general health of an animal and gives us an idea about the overall well-being of an animal much earlier than apparent changes become visible in the animal (Zivkovic and German, 2009).

Parity has been seen to affect the pattern of metabolic changes in cows (Meikle *et al*., 2004). However, knowledge about such report is limited in ewes.

2.2.3.1 Glucose

Prepartum and postpartum differences in serum glucose concentrations have been reported (Pal and Bhatta, 2013). Blood glucose concentration ranges from 1.9 to 3.9 mmol/L by multiplying by a factor of 0.0555 (Szablewski, 2011). It has been reported that there is a peak in the concentration during parturition followed by a decrease after parturition (Studer *et al*., 1993). Increase in the blood glucose concentration is due to the gluconeogenesis and glycogenolysis (Szablewski, 2011) stimulated by glucocorticoids and catecholamines during parturition (Fonseca *et al*., 2004; Studer *et al*., 1993).

2.2.3.2 Protein profile (Total protein, Albumin and Globulin)

Albumin can reflect hepatic insufficiency by decreasing its concentration (Whitaker, 2000). Hypoalbumin is more common in chronic liver disorders such as cirrhosis and this usually reflects severe liver damage and decreased albumin synthesis (Fauci *et al*., 2008). In hepatitis, albumin < 3g/dL should raise the possibility of chronic liver disease.

Serum globulins are a group of proteins made up of globulins (immunoglobulins) produced by B lymphocytes and globulins are produced primarily in hepatocytes. Globulins are increased in chronic liver diseases, such as chronic hepatitis and cirrhosis. In cirrhosis, the increased serum gamma globulin concentration is due to the increased synthesis of antibodies, some of which are directed against intestinal bacteria (Fauci *et al*., 2008).

2.2.3.3. Triglyceride

A triglyceride is a form of fat. Fat is stored as triglyceride and from the deposits, it is transported as free fatty acids bound to albumin. Animals with high triglyceride often have a high total cholesterol level, including high low-density lipoprotein (HDL) (good) cholesterol levels (Petkova, Kitanov and Girginov, 2008). High serum free fatty acid concentrations and low serum triglyceride and cholesterol concentrations have been observed in cattle with fatty liver (Petkova *et al*., 2008)

2.2.3.4 Total cholesterol

The serum concentration of cholesterol ranges from 1.0 to 4.6 mmol/L (Radostits *et al*., 1994). A range of 2.1 – 4.7 mmol/L (81.1 – 181.5mg/dL) has been reported by Meyer *et al*. (1992). Increased serum cholesterol concentration reflects the increased concentration of the cholesterol-rich lipoprotein such as LDL and HDL. These lipoprotein increases commonly occur secondary to endocrine, hepatic, or renal diseases. Inherited or primary disorders of cholesterol metabolism are rare in domestic animals (Evans, 2011).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Period and Location

The study was conducted in the sheep and goat section of the Department of Animal Science Education farm of Akenten Appiah Menkah University of Skills Training and Entrepreneurial Development (AAMUSTED), Asante Mampong. The data collection took 3 months. Asante Mampong municipal is located in the Northern part of Ashanti Region and Asante Mampong is its capital town. Mampong municipal shares a boundary with Sekyere south district in the south, Sekyere central district in the east and Adwira Sekyere Dumasi in the north. The population of the municipality according to the 2010 population housing census stands at 88051 with 45398 females and 42653 males. The Mampong municipal is located within longitude 00 05W and 10 30w and latitude 60 55N and 70 30N.

3.2. Study Design

A cross-sectional study (Lynn, 2009) was used for the study. This involved taking of blood samples from sheep of different physiological states on one visit for determination and assessment of the haemato-biochemical and progesterone profile of ewes in the study area.

3.4 Sample Population

The study population was Djallonke ewes/sheep in the Sheep and Goat Unit of the Department of Animal Science AAMUSTED. A total of 24 ewes were used for the study.

3.4. Sample Technique

Purposive sampling was used to select ewes based on different parities and physiological states. Simple random sampling was used to identify the animal group for blood sampling.

3.5 Management of the Experimental Animals

Animals were managed in the ruminant section of the Department of Animal Science Education. CAGRIC AAMUSTED Asante Mampong. The animals were grazed from 8:30 am to 4:30 pm. Clean water was provided for the sheep *ad-libitum*. Routine prophylactic treatment was adopted using the university veterinary technicians.

3.6 Method of Data Collection

A total of 24 sheep of the same breed and different physiological states categorized into age, parity, body condition score (BCS), and pregnancy stages (first trimester, second trimester, and third trimester) were used for haemato-biochemical studies. Data collected on each sheep included the effect of age, BCS, parity and different stages of gestation.

3.6.1 Measurement of haematological Parameters

Blood samples were collected from the jugular veins of sheep into haematological tubes containing EDTA and serum biochemistry tubes for the haemato-biochemical study. Full blood count (FBC) was determined by five (5) parts (differentials) fully automated haematological system (Mindray, Germany) per the manufacturer's instructions. Principles employed included the impedance method for RBC and platelets counting, cyanide-free reagent for haemoglobin test and flow cytometry + laser light scatter + chemical dye method for WBC differential analysis and WBC counting.

The haematological indices assessed were: Red blood cells count, Haemoglobin concentration, Packed cell volume, and Mean platelets volume. Leucocyte indices considered were white blood cell percentage, heterophils, lymphocyte, monocyte, eosinophils and basophils.

3.6.2 Measurement of serum biochemical parameters

3.6.2.1 Analysis of serum biochemical

The amount of enzymes, proteins, sugar, electrolytes, minerals and hormones present in the liquid component of the blood is measured by serum biochemistry. Utilizing the measurement of blood biochemical markers, it is also possible to assess the physiological and nutritional status of cows.

The Mindray BS 130 fully automated blood chemistry analyzer was used to perform liver function test (LFT), lipid profile, kidney function test (KFT) and electrolytes in order to measure the level of enzymes, metabolic processes and blood electrolytes. The details of the indices evaluated included lipids including total cholesterol (TCHOL) and triglycerides (TG); total protein (TPROT), globulin, and albumen. OneTouch Select

Simple portable blood glucose monitoring equipment (LifeScan Inc., China) was used to measure the blood glucose level in line with the manufacturer's instructions.

3.7 Statistical Analysis

The data on haematological and biochemical profiles were then subjected to Least squares (LS) analysis using Generalized Linear Model (GLM) Type III Procedure of IBM Statistical Package for Social Sciences (SPSS, 2021), version 28.0.1 using the model:

$Y_{ij} = \mu + A_i + B_j + G_k + P_l + e_{ijklm}$

Where: Y_{ij} = Dependent variables representing haemato-biological indices (RBC, WBC, HGB, PLT, LYM, HCT, PLT, GLU, TG. TPROT); μ = overall mean of traits; A_i = effect of i^{th} Age, i = 1, 2, 3 4 and 5; B_j = effect of j^{th} BCS, j = 1, 2, 3, 4, 5 and 6; G_k = effect of k^{th} Gestational or Pregnancy stages, k=1, 2, and 3; P_l = effect of l^{th} Parity, l = 1, 2 3 and 4; e_{ijklm} = error term.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Haematological Profile in Djallonke Sheep

The results on erythrocytes and leucocytes of Djallonke sheep in Asante Mampong are presented in Tables 1 and 2 respectively.

4.1.1 Effect of age on erythrocyte indices of Djallonke sheep in Asante Mampong

Age-influenced ($P<0.05$) red blood cell count in that sheep of 6 months had the highest red blood cell followed by 4 years, 6 years, 2 years and 3 years in descending order (Table 4.1). Age had a significant ($P<0.05$) effect on haemoglobin. Sheep under 6 years old had the highest haemoglobin followed by 6 months, then 4 years and then 2 years and 3 years in descending order. Haematocrit levels in Djalllonke sheep were significantly ($p<0.05$) influenced by age such that sheep of 6 months old had the highest followed by 4 years, then 3 years and 6 years in the descending order. Age-influenced ($P<0.05$) platelet such that a sheep of 6 years had the highest platelet level followed by 6 months, 4 years 3 years and 2 years in descending order. Nevertheless, sheep of 2 years and 3 years had similar ($P>0.05$) platelet counts.

Table 4.1: Erythrocyte Indices in Djallonke Sheep of Ashanti Region

Fixed Factors	RBC*(N=24)*	HB (g/dL)	HCT (%)	PLT (x10^3/µL)
AGE *(N=24)*	*0.000**	*0.000**	*0.000**	*0.000**
0.6	5.87±0.04[a]	11.43±0.13[b]	23.46±0.15[a]	792.33±25.07[b]
2.0	3.50±0.03[d]	8.08±0.11[d]	13.06±0.13[d]	162.83±21.58[d]
3.0	3.10±0.04[e]	5.78±0.14[e]	11.56±0.15[e]	189.83±26.13[d]
4.0	5.71±0.04[b]	10.50±0.12[c]	22.06±0.14[b]	399.08±23.39[c]
6.0	5.60±0.04[c]	12.43±0.13[a]	21.96±0.15[c]	854.33±25.07[a]
BCS *(N=24)*	*0.000**	*0.000**	*0.000**	*0.000**
2.0	2.80±0.04[e]	6.90±0.13[e]	10.30±0.15[e]	246.60±25.31[d]
2.8	5.82±0.05[b]	13.15±0.16[a]	22.80±0.19[b]	754.10±31.60[a]
3.0	5.74±0.03[c]	11.55±0.10[b]	22.50±0.12[c]	573.10±19.78[b]
3.2	6.41±0.05[a]	11.00±0.16[c]	25.20±0.18[a]	604.60±30.20[b]
3.5	4.93±0.04[d]	8.35±0.13[d]	19.40±0.15[d]	453.10±24.66[c]
4.0	2.80±0.04[e]	6.90±0.13[e]	10.30±0.15[e]	246.60±25.31[d]
PARITY*(N=24)*	*0.000**	*0.000**	*0.000**	*<0.001**
1	5.50±0.03[a]	11.27±0.10[a]	21.60±0.11[a]	596.81±18.63[a]
2	2.94±0.08[d]	4.69±0.27[c]	11.20±0.31[d]	295.06±53.01[c]
3	4.70±0.02[c]	10.47±0.09[b]	18.30±0.09[c]	513.81±14.73[b]
4	5.11±0.03[b]	10.47±0.09[b]	19.80±0.10[b]	490.81±17.43[b]
PREGNANCY STAGE *(N=24)*	*<0.001**	*<0.001**	*<0.001**	*<0.001**
1	4.83±0.02[a]	9.38±0.07[b]	18.79±0.09[a]	420.81±13.81[b]
2	4.72±0.02[b]	9.73±0.05[a]	18.29±0.06[b]	499.31±9.70[a]
3	4.72±0.02[b]	9.73±0.05[a]	18.29±0.06[b]	499.31±9.70[a]

**=P-values; RBC= Red blood Cells;HB= Haemaglobin; HCT=Haematocript; PLT=Platelets; Values in the parenthesis=Number of observation: [abcd]=Different Superscripts along the same column were statistically different ($p < 0.01$).*

The observed RBC records with respect to the fixed factors considered were lower than the reference ranges reported by Radostits *et al*. (2007) and Constable *et al.* (2017). This might be attributed to insufficient feed required by the Djallonke ewes.

The significant differences in erythrocyte indices are comparable to the finding of Etim *et al*. (2013) who observed great variation in the haematological profiles in goats for age and other physiological stages. Age is one of the contributing factors to changes in the physiological status of animals (Etim *et al*., 2014). The low haematocrit with a low MCV and a high RDW suggests chronic-iron-deficient anaemia resulting in abnormal haemoglobin synthesis during erythropoiesis (Etim *et al*., 2013).

4.1.2 Effect of body condition score (BCS) on erythrocyte indices in Djallonke sheep

The current findings show that body condition score (BCS) had a significant influence ($P<0.05$) on the red blood cell of Djallonke sheep (Table 4.1). Sheep of BCS 3.2 recorded the highest red blood cell followed by 2.8, 3.0, 3.5, 2.0 with BCS of 4.0 recording the least. Body condition score had a significant influence ($P< 0.05$) on haemoglobin such that sheep with BCS 2.8 had the highest haemoglobin followed by 3.0, 3.2, 3.5, 2.0 and 4.0 in the descending order. Haematocrit levels in Djallonke sheep were influenced ($P<0.05$) by body condition score such that sheep of body condition score 3.2 had the highest haematocrit followed by 2.8, 3.0, 3.5, 2.0 and 4.0 in the descending order. Sheep of BCS 2.0 and 4.0 had similar ($P> 0.05$) red blood cells, haemoglobin and haematocrit. Platelet was influenced ($P< 0.05$) by BCS in that BSC 2.8 had the highest platelet followed by 3.2, 3.5 2.0 and 4.0 in the descending order. Sheep of 3.2 and 3.0 had similar ($P>0.05$) platelet and BCS 2.0 and 4.0 also had similar platelet. The significantly higher erythrocyte values recorded for sheep of BCS 2.8, 3.0, 3.2, and 3.5 compared to 2.0 and 4.0 is indicative of better performance in good-conditioned sheep as opposed to poor or obese conditioned ones. It has been reported that increased BCS is genetically and favourably linked with good health and performance traits for the assessment of animals' outputs (Berry *et al*., 2003; Bastin *et al*., 2010). Although BCS is a subjective measure of available tissue reserves, it can be used to indicate the energy balance of the dairy cow (Loker *et al*., 2010). The current findings are consistent with the assertion that animals having BCS around 3.00

and 3.75 points have better performance (Gumen *et al*., 2003; Lopez *et al*., 2005; Watters *et al.*, 2010; Gergovska *et al*., 2011).

4.1.3 Effect of parity on erythrocyte indices in Djallonke sheep

Current findings show that parity had a significant influence ($P<0.05$) on red blood cells. Sheep of parity 1 had the highest red blood cell with parities 4, 3 and 2 following in descending order (Table 4.1). Parity influenced ($P<0.05$) haemoglobin such that sheep of parity 1 had the highest haemoglobin followed by parity 3 and parity 4. Sheep at parities 3 and 4 had similar haemoglobin. Haematocrit levels were highest ($P<0.05$) in sheep of parity 1 followed by parity 4, parity 3 and parity 2 in descending order. Parity influenced ($P<0.05$) platelet count such that sheep of parity 1 had the highest platelet followed by parities 3, 4 and 2 in the descending order. Sheep of parities 3 and 4 had similar platelet.

Parity indicates the number of births an animal has experienced and it is correlated with age since animals must attain a certain age before reproduction. Differences in erythrocyte indices were consistent with the findings of Vallejo-Timaran (2020). The RBC, haemoglobin hematocrit and platelet component favoured sheep of parity 1 and these values fell within the physiological reference ranges (Etim *et al*., 2014). Health concerns have been reported to increase with the parity of animals. According to Bradley and Green (2005) increase in infections of multiparous female animals may be often, but not exclusively, due to an increased likelihood of infections with age. Indeed, it is not surprising to have good blood indices in parity 1 compared to others observed in the current findings.

4.1.4 Effect of pregnancy stage on erythrocyte indices in Djallonke sheep

The pregnant stage had a significant influence (P<0.05) on sheep red blood cells such that the first trimester had the highest red blood cell followed by the second and third trimesters in descending order (Table 4.1). Sheep of the second and third trimesters had similar red blood cells. the second recorded the highest haemoglobin levels followed by the third trimester and first trimester in descending order. Ewes in the second and third trimesters had similar haemoglobin counts. Stages of pregnancy influenced (P<0.05) haematocrit count in that sheep in the first trimester had the highest haematocrit count followed by second and third trimesters in that order. Ewes in the second and third trimesters also had similar (P>0.05) haematocrit values. Second-trimester sheep had significantly (P<0.05) the highest platelets count. Third and first trimesters ewes followed by the first trimester in descending order. Sheep in the second and third trimesters had similar platelet.

4.2 Leucocyte Indices in Djallonke Sheep

4.2.1 Effect of age on leucocyte indices in Djallonke sheep

Age influenced (P<0.05) white blood cells in that sheep at 4 years had the highest white blood cell followed by 6 months, 6 years, 2 years and 3 years in descending order. Sheep of 4 months being the highest white blood cell was similar to those obtained at ages 6 months, 6 years and 2 years (Table 4.2). Lymphocyte levels were significantly influenced by age such that sheep of 2 years had the highest lymphocyte followed by 3 years, 4 years, 6 months and 6 years in descending order.

Age had a significant influence on heterophils such that sheep of 6 years had the highest heterophils followed by 6 months, 4 years 3 years and 2 years in descending order. Sheep of 4 years, 3 years and 2 years also had similar ($P>0.05$) heterophils. Age imposed a significant influence ($P<0.05$) on monocytes. Sheep at 4 years had the highest monocyte followed by 3 years, 2 years, then 6 months and 6 years in the descending order. Monocyte levels observed in sheep at 4 were similar ($P>0.05$) to those obtained at ages 3, 2, 6 years and 6 months. Sheep of 2 and 3 years also had similar monocytes whereas sheep of 6 months and 6 years also had similar monocytes. Age influenced ($P <0.05$) basophil such that sheep at 3 years had the highest basophil followed by 2 years, 4 years, 6 months and 6 years in descending order. Monocyte level in ewes of 3 years was similar to those obtained in 2 years, 4 years, 6 months and 6 years (Table 4.2).

The differences in leucocyte numbers with increasing age are consistent with the findings of Roland *et al.* (2014). The Djallonke sheep are hardier and resistant to some endemic diseases (Annan-Prah, 2011) and this might have accounted for their high leucocyte indices with age as reported by Kalaitzakis *et al*. (2011) and Roland *et al*. (2014) as an indication of physiological adaptation and not, as a result, severe infection (Tornquist and Rigas, 2010; Reece *et al*., 2015).

Table 4.2: Leucocyte Indices in Djallonke sheep Asante Mampong

FIXED FACTORS	WBC $(x10^9/L)$	LYMPH %	HETERO %	MONO %	BASO %	EOSIN0 %
AGE *(N=24)*	*0.001**	*0.000**	*<0.001**	*0.000**	*0.079*	*0.031**
0.6	23.95±2.38[a]	62.83±0.19[d]	27.67±2.04[a]	0.17±0.37[ac]	-0.75±0.00	10.08±1.49[a]
2.0	18.60±2.05[ab]	78.33±0.16[a]	15.17±1.76[c]	1.17±0.32[ab]	2.25±0.00	3.08±1.28[c]
3.0	10.90±2.45[c]	73.33±0.19[b]	16.17±2.13[c]	1.17±0.39[ab]	3.25±0.00	6.08±1.55[ab]
4.0	24.90±2.22[a]	64.08±0.17[c]	21.92±1.91[bc]	6.67±0.35[a]	1.25±0.00	6.08±1.39[ab]
6.0	19.60±2.38[a]	60.83±0.19[e]	28.67±2.04[a]	0.17±0.37[ac]	0.25±0.00	10.08±1.4[a]
BCS *(N=24)*	*> 0.001**	*0.000**	*0.124**	*0.000**	*0.081*	*0.019**
2.0	12.70±2.40[c]	74.63±0.19[a]	18.50±2.06	0.03±0.38[c]	1.92±0.00	4.92±1.50[bd]
2.8	22.40±3.00[b]	62.13±0.23[d]	27.00±2.58	5.03±0.47[a]	0.08±0.00	5.92±1.85[dc]
3.0	21.70±1.88[b]	67.13±0.15[c]	22.00±1.61	3.03±0.29[b]	0.08±0.00	7.92±1.17[bd]
3.2	35.10±2.90[a]	58.63±0.22[e]	24.50±2.46	4.03±0.45[a]	1.92±0.00	10.92±1.79[a]
3.5	12.70±2.34[c]	70.13±0.18[b]	21.00±2.01	0.97±0.37[d]	1.92±0.00	7.92±1.46[bc]
4.0	12.70±2.40[c]	74.63±0.19[a]	18.50±2.06	1.00±0.37[ce]	1.92±0.00	4.92±1.50[d]
PARITY *(N=24)*	*0.010**	*0.000**	*0.077**	*0.000**	*0.071*	*0.031**
1	4.80±5.03[b]	73.17±0.39[a]	20.04±4.32	3.22±0.79[d]	3.08±0.00	6.92±3.14[a]
2	20.90±1.77[a]	64.42±0.14[d]	25.29±1.52	1.28±0.28[b]	0.08±0.00	8.92±1.10[a]
3	20.50±1.40[a]	70.42±0.11[b]	22.30±1.20	0.28±0.22[c]	1.08±0.00	5.92±0.87[b]
4	23.70±1.65[a]	66.42±0.13[c]	21.30±1.42	4.28±0.26[a]	1.08±0.00	6.92±1.03[a]
PREGNANCY STG *(N=24)*	*<0.001**	*0.000**	*0.308**	*<0.001**	*0.141*	*0.350**
1	23.90±1.31[a]	71.26±0.10[a]	20.80±1.13	1.12±0.21[b]	0.50±0.00	6.33±0.82
2	18.10±0.92[b]	66.76±0.07[b]	22.30±0.79	2.12±0.14[a]	1.50±0.00	7.33±0.57
3	18.10±0.92[b]	66.76±0.07[b]	22.30±0.79	2.12±0.14[a]	1.50±0.00	7.33±0.57

**= P-value; WBC=White blood cell; LYMPH=Lymphocyte;HETERO=Heterophils; MONO= Monocyte;BASO= Basophil;EOSINO=Eosinophil*; *Values in the parenthesis=Number of observation;* [abcd]*=Different Superscripts along the same column were statistically different ($p < 0.05$).*

Age had a significant influence (P<0.05) on eosinophils such that sheep of 6 months and 6 years had the highest followed by 3 years, 4 years and 2 years in descending order. Sheep of 6 months and 6 years had the highest eosinophil and this value was similar to those obtained at ages 3 years, 4 years and 2 years. Ectoparasites have also been reported to elicit eosinophilia (Jacquiet *et al*., 2005) and exposure to parasitic infestations increased with increasing age, hence, the observed trend in the current findings. However, one study showed that cattle experimentally infested with ticks did not have increased peripheral eosinophil numbers when compared to controls (Tornquist and Rigas, 2010). Eosinopaenia may be a component of a stress response in ruminants.

4.2.2 Effect of body condition score (BCS) on leucocyte in Djallonke sheep

Body condition score (BCS) indicates the level of body reserves an animal possesses (Constable *et al*., 2017). And can be reliably used for the assessment of animals' outputs and physiological conditions (Berry *et al*., 2003; Bastin *et al*., 2010).

Current findings show that body condition score (BCS) influenced (P<0.05) white blood cells. Sheep with BCS 3.2 had the highest WBC followed by 2.8, 3.0, 2.0, 4.0, and 3.5 in descending order (Table 4.2). Sheep of BCS 2.8 and 3.0 had similar (P>0.05) white blood cells. Sheep with body condition score of 2.0, 3.5 and 4.0 also had similar (P>0.05) white blood cells. Lymphocyte levels were significantly (P<0.05) influenced by body condition score such that sheep with body conditions 2.0 and 4.0 had the highest followed by 3.5, 3.0, 2.8 and 3.2 in the descending order (Table 4.3).

Body condition score had a significant influence (P<0.05) on monocyte such that sheep with body condition score of 2.0 had the highest monocyte followed by 3.2, 2.8, 3.5, and 4.0. Sheep with body condition scores of 2.0 and 3.2 had similar (P>0.05) monocytes.

Body condition score did not significantly (P<0.05) influence heterophils. Body condition score imposed a significant influence on (P<0.05) on eosinophil such that sheep of body condition score 3.2 had the highest eosinophil followed by 3.2, 3.0, 2.0, 2.8 and 4.0 in the descending order. Sheep with body condition scores of 2.8 and 4.0 also recorded similar (P>0.05) monocyte. The sheep of body condition score of 4.0 was similar to those obtained in the body conditions 2.0 and 2.8. Sheep of body condition score of 3.0 was also similar (P>0.05) to those obtained in the body condition scores 2.0 and 2.8. The current findings reveal that BCS around 3.0 to 3.5 have better leucocyte indices and vice versa. This confirms the previous finding that BCS around 3.00 and 3.75 points have better performance (Watters *et al.*, 2010; Gergovska *et al*., 2011).

4.2.3 Effects of parity on leucocyte in Djallonke sheep of Asante Mampong

Parity had a significant influence (P<0.05) on white blood cells in that sheep of parity 4 had the highest blood cell followed by parities 1, 3 and 2 in descending order. A sheep of parity having the highest white blood cell was found to be similar to those obtained in parities 1 and 3 (Table 4.2). Parity had a significant (P<0.05) influence on lymphocytes such that sheep of parity 2 had the highest followed by parities 3, 4 and 1 in descending order. Parity did not significantly influence heterophils. Parity had a significant influence (P< 0.05) on monocyte such that a sheep at parity 4 had the highest monocyte count followed by parities

1, 3 and 2 in descending order. Parity had a significant influence (P< 0.05) on basophil such that sheep of parity 2 had the highest basophil followed by parities 3 and 4 in the descending order. Sheep of parity 2 had the highest basophil and this value was similar to those obtained in parities 1, 3 and 4. Parity had a significant influence (P <0.05) on eosinophil such that sheep of 1 had the highest eosinophil followed by parities 2, 4 and 3 in the descending order. Sheep of parity 1 had the highest eosinophil and this value was similar to those obtained in parities (2), third (3) and 4. The increase in WBC, lymphocytes and monocytes with increasing parities was consistent with the findings of (Etim *et al*., 2014) and these are due to increasing infection rates with age and parity (Etim et a., 2014).

4.2.4 Effects of pregnancy stage on leucocyte in Djallonke sheep

The pregnancy stage had a significant influence (P< 0.05) on white blood cells such that sheep in the first trimester had the highest white blood cell followed by second and third trimesters in descending order (Table 4.2). Sheep in the second and third trimesters were found to have similar (P>0.05) white blood cells. The pregnancy stage had a significant influence (P<0.05) on lymphocytes such that sheep in the first (1) trimester had the highest lymphocyte followed by second and third trimesters in descending order. Sheep in the second and third were found to have similar white blood cells. The pregnancy stage had a significant influence (P<0.05) on monocyte such that sheep in the second and third trimesters had the highest monocytes (2.12 ± 0.14 %) followed by the first trimester (1.12 ± 0.21 %) in descending order. The pregnancy stage had a significant influence (P<0.05) on basophils such that sheep in the second and third trimesters had the highest basophil followed by the first trimester in descending order. Sheep in the first, second and third trimesters were found to have similar basophils. Sheep in the second and third trimesters were found to have similar ($P>0.05$) monocyte. The pregnancy stage had little ($P>0.05$) influence on heterophils and eosinophils.

Higher than the normal range (1 – 6 %, Etim *et al*., 2014) of monocyte levels indicate stress among the ruminant in gestational stages, across the physiological states. Lymphocytes and eosinophil levels fell within the reference ranges of Roland *et al*. (2014) and, therefore, might not be attributed to a healing phase of infectious diseases, during chronic antigenic stimulation due to infectious agents, neoplasia, and hypoadrenocorticism as in lymphocytosis (Fowler, 1998; Keller *et al*., 2006).

The higher level of lymphocytes, heterophils, monocytes and eosinophils in increasing pregnancy or gestational stages might be attributed to stress and changes in hormonal levels and increased demand for the foetus as pregnancy advances. Similar observations have been made by reported by Radostits *et al*. (2007) and Constable *et al.* (2017). Nevertheless, basophil levels were not influenced by the fixed factors considered in this study.

4.3 Serum Biochemistry in Djallonke Sheep.

Results on Protein indices of sheep in Ashanti Region are presented in Table 4.3.

4.3.1 Effect of age on protein indices in Djallonke sheep of Asante Mampong

Age influence ($P< 0.05$) total protein concentration in that sheep of four (4) years had the highest followed by the six (6) months old, two (2) years, three (3) years and six (6) years in descending order (Table 4.3). Sheep of 6 months and 2 years had similar protein. Age also affected (P<0.05) albumen concentration such that sheep at 6 months old sheep had the highest albumen concentration followed by the 6 years, 3 years, and 4 years in descending order.

Table 4.3: Protein Indices in Djallonke sheep

FIXED FACTORS	TOTAL PROTEIN (g/L)	ALBUMIN (g/L)	GLOBULIN (g/L)
AGE (N=24)	*0.042**	*<0.000**	*0.082**
0.6	63.99±9.27[b]	28.28±1.31[a]	35.51±10.30
2.0	58.41±9.88[b]	21.28±1.40[b]	37.39±10.96
3.0	56.94±4.95[c]	23.43±0.70[b]	33.50±5.50
4.0	71.59±5.51[a]	21.88±0.78[b]	49.81±6.11
6.0	56.59±9.27[c]	25.18±1.31[b]	31.21±10.30
BCS ***(N=24)***	*0.022**	*0.035**	*0.128**
2.0	50.60±5.90[b]	23.02±0.83[b]	27.41±6.50
2.8	63.42±6.00[ab]	23.72±0.85 [ab]	39.86±6.66
3.0	64.50±4.09[a]	24.70±0.58[a]	39.81±4.54
3.2	67.42±7.00[a]	24.10±1.00 [ab]	43.36±4.54
3.5	59.70±11.00[ab]	24.82±1.60 [a]	34.60±12.21
4.0	63.42±6.00 [ab]	23.72±0.85 [ab]	39.86±6.66
PARITY ***(N=24)***	*0.768**	*0.321**	*0.740**
1	54.06±11.30	24.89±1.60	28.90±12.52
2	62.53±2.18	24.04±0.31	38.50±2.41
3	64.83±4.84	22.99±0.70	42.00±5.40
4	62.53±2.18	24.04±0.31	38.50±2.41
PREGNANCY STG	*0.228**	*0.612**	*0.307**
1[ST] TRIMESTER	57.72±3.51	23.80±0.50	33.91±3.90
2[ND] TRIMESTER	62.77±2.31	24.10±0.33	38.67±2.56
3[RD] TRIMESTER	62.77±2.31	24.10±0.33	38.67±2.56

=P-value; STG=Stage; BCS=Body Condition Score; Values in the parenthesis=Number of observation.* [abcd]*= Superscripts that are different along the same column were statistically different (p < 0.05).*

However, sheep of 2 to 6 years had similar albumen concentrations. Age had little or no effect on the globulin concentration of the sheep studied. Total protein gives information about kidney damage, liver damage, and nutritional health (Stojević *et al*., 2005). The current findings did not show a sequential pattern in protein indices with age. Nevertheless, total protein was highest at age 4 whereas albumin levels were lower at 2 and 4 years. Albumin can reflect hepatic insufficiency by decreasing its concentration (Whitaker, 2000). Hypoalbuminaemia is more common in chronic liver disorders such as cirrhosis and usually reflects severe liver damage and decreased albumin synthesis (Fauci *et al*., 2008).

4.3.2 Effect of body condition score (BCS) on protein indices in Djallonke sheep

Current findings show that body condition score had a significant influence ($P < 0.05$) on total protein concentration (Table 4.3). Sheep with body condition score of 3.2 had the highest protein concentration and this value was similar to those obtained in the body conditions 2.8, 3.0, 3.5 and 4.0. Body condition scores 2.0, 2.8, and 3.5 also had a similar protein concentrations. Body condition score had a significant influence ($P < 0.05$) albumen concentration such that sheep of body score 3.5 had the highest followed by 3.0, 3.2. 2.8, 4.0 and 2.0 in descending order. Sheep with body condition scores of 2.8 to 4.0 had similar albumen concentrations. Body condition score did not significantly influence globulin concentration.

Body reserves reflect the quality of nutrition the animal is exposed to and therefore, the observed differences in the protein indices might be attributed to nutritional factors and the animal's ability to utilize the feed (Stojević *et al*., 2005) which also influences BCS.

4.3.3 Effect of parity on protein indices in Djallonke sheep

The parity of dam had little ($P > 0.05$) influence on albumen and globulin concentration.

4.3.4 Effect of pregnancy stage on protein indices in Djallonke sheep

The pregnancy stages of sheep did not influence albumen and protein concentration (Table 4.3). Variation in protein indices might be accounted for by a different factor other than pregnancy stages.

4.4 Glucose and Lipid Profiles in Djallonke Sheep

The results on Glucose and Lipids concentration in Djallonke sheep are presented in Table 4.4

4.4.1 Effect of age on glucose and lipids profiles in Djallonke Sheep

Glucose levels were significantly ($P<0.05$) influenced by age such that sheep of 3 years old had the highest glucose concentration followed by 4 years, 2 years and 5 years in the descending order.

The significant differences in glucose concentrations between ages 2 and 5 years fell within the physiological range of 1.7 to 3.6 mmol/l (Constable *et al*., 2017) but higher than the value (2.00 mmol/l) reported by Wada *et al*. (2018). The glucose levels in the Djallonke sheep of ages 3 and 4 years group were higher than the upper limits of glucose concentration reported by Radostits *et al*. (2007) and Constable *et al*. (2017). An increase in the blood glucose concentration is due to gluconeogenesis and glycogenolysis (Szablewski, 2011) stimulated by glucocorticoids and catecholamines during parturition (Fonseca *et al*., 2004).

Sheep of 4 years and 5 years had similar (P>0.05) glucose concentrations. Age had little or no (P>0.05) effect on total cholesterol and triglycerol concentrations in this study.

Table 4.4: Glucose and Lipid Profile in Djallonke Sheep of Asante Mampong

FIXED FACTORS	GLUCOSE (mmol/L)	TOTAL CHOLESTEROL (mmol/L)	TRIGLYCEROL (mg/L)
AGE (N=24)	*0.000**	*0.276**	*0.582**
2	2.78±0.07[c]	1.20 ± 0.08	0.18 ± 0.04
3	4.37±0.32[a]	0.93 ± 0.19	0.12 ± 0.09
4	3.97±0.18[b]	1.20 ± 0.08	0.18 ± 0.04
5	2.77±0.07[bd]	1.25 ± 0.08	0.18 ± 0.047
BCS ***(N=24)***	*0.000**	*0.089**	*0.408**
2.0	1.10±0.18[e]	1.36 ± 0.18	0.23 ± 0.09
2.5	3.39±0.15[c]	0.82 ± 0.17	0.11 ± 0.08
3.0	3.43±0.07[bc]	1.07 ± 0.09	0.10 ± 0.04
3.5	3.35±0.11[dc]	0.97 ± 0.12	0.12 ± 0.06
4.0	4.39±0.31[a]	1.45 ± 0.13	0.25 ± 0.06
PARITY (N=24)	*0.000**	*0.002**	*0.068**
1	2.43 ± 0.17[cd]	1.13 ± 0.10[a]	0.21 ± 0.05
2	3.53 ± 0.18[b]	0.85 ± 0.26[a]	0.08 ± 0.12
3	4.57 ± 0.34[a]	1.46 ± 0.13[a]	0.24 ± 0.06
4	3.07 ± 0.08[c]	1.33 ± 0.19[a]	0.08 ± 0.09
5.	2.60 ± 0.15[d]	0.89 ± 0.11[a]	0.20 ± 0.05
PREGNANCY STAGE (N=24)	*0.000**	*0.012**	*0.001**
1	3.56±0.10[a]	1.29 ± 0.07[a]	0.20 ± 0.03[a]
2	3.56 ± 0.10[a]	0.94 ± 0.12[b]	- 0.00 ± 0.06[b]
3	2.88 ± 0.08[b]	1.16 ± 0.21[a]	0.30 ± 0.06[a]

**=P-value; BCS=Body Condition Score; Values in the parenthesis=Number of observation; [abcd]=Different Superscripts along the same column were statistically different ($p < 0.05$).*

4.4.2 Effect of body condition score on glucose and lipid profile in Djallonke sheep

Current findings show that body condition score (BCS) had a significant ($P<0.05$) influence on glucose such that sheep with a body condition score of 4.0 had the highest followed by BCS 3.0, 2.5, 3.5, and 2.0 in the descending order. Sheep with a body condition score of 2.5 had similar ($P<0.05$) glucose levels to those obtained in the body condition scores of 3.0 and 3.5. Glucose concentration increased with increasing BCS 1 to 4. The glucose levels in sheep of BCS 2.5 to 3.5 fell within the normal physiological range (Constable *et al*., 2017), whereas BCS 2 had the lowest ($P<0.05$) concentration below the normal range (Etim *et al*., 2014; Constable *et al*., 2017). Sheep with BCS recorded higher than the upper limits of glucose concentration reported by Radostits *et al*. (2007) and Constable *et al*. (2017). Indeed, the normal physiological range of glucose concentration in sheep (Constable *et al*., 2017) was recorded in BCS 2.5 to 3.5 for the best performance.

Body condition score had a slight ($P>0.05$) influence on total cholesterol and triglycerol concentrations.

4.4.3 Effects of parity on glucose and lipid profile

Parity imposed a significant ($P< 0.05$) influence on glucose such that a sheep at parity 3 had the highest followed by parities 2, 3.5, then 5 and 1. Sheep of parity 1 was similar to sheep of parity 5. A sheep of parity 1 was similar to sheep of parity 5. It is important that although there are differences in glucose concentration with respect to parity all fell within the reference range reported by (Etim *et al*.. 2014; Constable *et al*., 2017). This is an indication that there might not be a health implication.

Parity had a significant influence (P< 0.05) on total cholesterol levels such that sheep of parity 3 had the highest total cholesterol level followed by parities 3.5, 5 and 2 in descending order. A sheep at parity 3 was found to be similar to those obtained at parities 1, 2, 3, and 5. Despite the variations in cholesterol concentrations at different parities, the values fell within the physiological range reported by (Radostits *et al*., 1994). Increased serum cholesterol concentrations reflect the increased concentration of cholesterol-rich lipoproteins such as LDL, and HDL. These lipoproteins increase commonly occur secondary to endocrine, hepatic, or renal diseases. Inherited or primary disorders of cholesterol metabolism are rare in domestic animals. However, parity had a slight (P>0.05) influence on triglycerol.

4.4.4 Effect of pregnancy stage on glucose and lipid profile

The pregnancy stage had a significant influence on glucose such that sheep in the first and third trimesters had the highest followed by the third trimester. Sheep in the first and second trimesters had similar glucose concentrations. The observed variations in the blood glucose levels were within normal ranges reported by Radostits *et al*. (1994) and Merck Manual (2012). Mean serum glucose concentrations within normal ranges have been reported (Radostits *et al*., 1994; Merck Manual, 2012).

Pregnancy stage influenced (P< 0.05) triglycerol such that sheep in the third trimester had the highest followed by first and second trimesters in the descending order. However, the concentrations fell within the reference range (Merck Manual, 2012: Etim *et al*. 2013) sheep in the third and first trimesters had similar triglycrol levels. A triglyceride is a form of fat. Fat is stored as triglycerides and from the deposit, it is transported as free fatty acids bound to albumin. Animals with high triglycerides often have a high total cholesterol level, including high low-density lipoprotein (LDL) (bad) cholesterol and low high-density lipoprotein (HDL) (good) cholesterol levels (Petkova, Kitanov and Girginov, 2008).

The pregnancy stage had little effect on serum total cholesterol and triglycerol concentration in Djallonke sheep.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Based on the results from the study, it was concluded that:

- Erythrocyte indices were influenced by age, body condition score (BCS), parity and pregnancy stages. The differences did not show sequential variations with age, BCS, parity and stages of pregnancy.

- Leucocyte indices were affected by age, BCS, parity and pregnancy stages. Sheep at 4 years had the highest white blood cell followed by 6 months, 6 years, 2 years and 3 years in descending order. Six years ewes had the highest heterophils followed by 6 months, 4 years 3 years and 2 years in descending order. Sheep of BCS 3.2 had the highest haematocrit followed by 2.8, 3.0, 3.5, 2.0 and 4.0 in descending order. The WBC and heterophils were higher in the first trimester of gestation compared to the second and third trimesters.

- Serum biochemistry was affected by the fixed factors such that sheep of 4 years had the highest total protein concentration followed by 6 months old, 2, 3 and 6 years in descending order. Sheep of BCS 4.0 had the highest glucose concentration followed by BCS 3.0, 2.5, 3.5, and 2.0 in descending order. The differences in serum biochemical indices did not follow a sequential pattern with age, BCS, parity and stages of pregnancy. Body condition score had little influence on total cholesterol and triglycerol concentrations.

5.2 Recommendation

It is recommended that:

- Physiological states (age, BCS, parity, and pregnancy stages) of Djallonke sheep have an immense effect on haemato-biochemical indices of Djallonke sheep and hence, these factors should be considered when assessing the performance of the animals.

- Further study should be conducted to determine the effect of feed supplementation on the haemato-biochemical indices in Djallonke sheep.

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