Thesis Ref. No _____

EFFECTS OF LACTATION AND PREGNANCY ON HEMATOLOGICAL AND SERUM BIOCHEMICAL PROFILES OF CROSS BREED HOLSTEIN FRIESIAN COWS IN BISHOFTU, ETHIOPIA

MSc THESIS

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ADDIS ABABA UNIVERSITY

COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

DEPARTMENT OF BIOMEDICAL SCIENCES (BMS)

JUNE, 2018

BISHOFTU, ETHIOPIA

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By

Temesgen Kifle

A Thesis submitted to the College of Veterinary Medicine and Agriculture, Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Science in Animal Physiology

June,2018

Bishoftu, Ethiopia

Addis Ababa University

College of Veterinary Medicine and Agriculture

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As members of the Examining Board of the final MSc open defense, we certify that we have read and evaluated the Thesis prepared by Temesgen Kifle entitled "Effects of lactation and pregnancy on hematological and serum biochemical profiles of Cross breed Holstein Friesian cows in Bishoftu, Ethiopia" and recommend that it be accepted as fulfilling the thesis requirement for the degree of Masters of Science in Animal physiology.

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STATEMENT OF AUTHOR

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ACKNOWLEDGMENTS

First and foremost, I would like to provide thanks to the Almighty God for the blessing and supplying strength, believes, hope, patience and protection to me and my families throughout my life. Had not been the willing of him, nothing would have been possible to me.

Next I am very much indebted to my advisor Dr. Fikru Regassa for his unreserved advice, encouragement, useful comments, suggestions and constructive criticisms.

I would like to thank the Angacha Woreda Administration and Livestock and Fishery office on behalf of Bureau of Livestock and Fishery of Southern Nations and Nationalities Peoples Region (SNNPR) for granting the study leave and securing my salary throughout the study period. I am also indebted to Dr. Hamid Jemal ,Head Bureau of Livestock and Fishery of SNNPR for facilitating suitable ground for continuing our study without any disturbance on reason of study leave and securing salary for all those who are in MSc program.

I would like to acknowledge the Addis Ababa Public Health Institute (Pasteur)National Referral Laboratory for Clinical Chemistry their cooperation. My special thanks also goes to Mis.Genet Asheber and Meron Silash ,Coordinator of NRLCC and Chief laboratory technician respectively for their cooperation and assisting laboratory work. I would like to express my deepest and sincere gratitude to Managers and Coordinators of the farms in which I have collected blood samples for this study.Those are Mrs. Hika Waktole,Mr Behailu, Mr Tadessa,Mr.Sisay, W/ro Worke and Dr Samson Terafe for their cooperation and all farm attendants creating suitable Environment for collecting blood samples based on my research deseign. My special thanks goes to Addis Ababa University, College of Veterinary Medicine and Agriculture (AAU, CVMA), Bishoftu, Ethiopia, academic and support staff members of the college, for their positive cooperation during my research work. Again, I would like to express my deepest gratitude and appreciatation to Mr Mokkonen Sorsa(PhD candidate) and Dr Mahari Teklu who supported me by materials and Moral encouragement. My final gratitude is reserved to my wife Meseret Binkir, and the rest of all my beloved families for their invaluable material and moral support in all facets of life and their constant encouragement to prepare this paper.

LIST OF TABLE OF CONTENTS

LIST OF ABBREVIATIONS

ALT	Alanine aminotransferase
ALP	Alkaline Phosphatase
AST	Aspartate aminotransferase
Ca	Calcium
CLSI	Clinical and Laboratory Standards Institute
dl	deciliter
ESR	Erythrocyte Sedimentation Rate
FAO	Food and Agricultural Organization
fl	femtoliter
GGT	Gamma Glutamyl Transferase
gm	gram
gm/dl	gram per deciliter
Hb	Hemoglobin
IFCC	International Federation of Clinical Chemistry
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
mg/dl	mill gram per deciliter
mm/24hrs	millimeter per24hrs
mmol/l	millmol perliter
Mg	Magnesium
Na	Sodium
NRCCL	National Referral Clinical Chemistry Laboratory

P	phosphorus
PCV	Packed Cell Volume
Pg	picogram
%	percent
RAR	Research Animal Resource
RBC	Red Blood Cells
SD	Standard Deviation
TP	Total protein
U/L	Unit per liter
WBC	White Blood Cells

LIST OF APPENDIXES page number

LIST OF TABLES

ABSTRACT

The study showed that all hematological indices associated with RBC differed significantly ($p<0.05$) at different physiological status. In line with this, mean RBC values during different physiological stages differs significantly. The value recorded during early and late stage of lactation differed significantly ($p<0.05$) from mid stage of lactation. The mean value of PCV of non-pregnant dry cows (27.11±6.07) was significantly lower than the mean value of PCV of the early lactating group (33.18±4.81) and the mean value of the PCV of the late lactating group (32.44± 3.4) respectively. The mean value of the WBC decreases when pregnancy advanced and the mean value of WBC increases when the lactation approach to dry period (from early lactation to late lactation). The mean value of AST in early and late pregnancy were significantly ($p<0.05$) higher than that of mid pregnancy(20.5±11.7)The mean values of AST in lactation stages that are early, mid and late were 78.3±22.7, 63.12± 19.87 and 62.2±14.4 respectively. The mean values of glucose in pregnant groups increase as the pregnancy advanced and the mean value of glucose in late pregnant group 38.13±5.31 was significantly differs from the mean values of both mid and early pregnancy 24.92±4.12 and 32.36± 4.87 respectively. The mean value of urea in early lactation 32.05±5.73 was significantly higher than both in mid lactation 23.65±7.89 and late lactation 15.8±6.58. The mean value of creatinine in late lactation 1.34±0.44 was significantly higher than the mean value of early lactating groups 0.87±0.16 and the mean value of mid lactating group 1.21±0.42. The mean value of total billrubin mid pregnancy groups 0.24±0.098 was significantly differs from the mean values of both early pregnant group 0.072± 0.06 and late pregnant groups 0.11±0.08. The mean values of total protein in early lactation groups 8.3±1.46 was significantly ($p<0.05$) differs from the mean values of both mid lactation 7.0±1.9 and late lactation 5.47±1.0. This study showed a significant differences in the mean serum electrolyte values of pregnant and lactating cows in between all groups of study were due to irregular and i mbalanced supplement of minerals in feed. Results revealed that most of the measured blood constituents were differed significantly during the period of lactation and pregnancy in Holistian fresain cow. In conclusion, the established reference values will be a useful guide for interpreting serum biochemical and hematologic data in pregnant and lactating cow.

Key words: Hematological, Lactation, Parameters,Pregnancy,Reference value, biochemical

1.INTRODUCTION

Physiological equilibrium is maintained mainly by the blood in the body (Geneser,1986), but many physiological conditions may alter this equilibrium. Hematology and serum chemistry are becoming increasingly important diagnostic tools in veterinary medicine. Blood parameters in animals are used as an aid for clinical diagnosis of infectious and several parasitic diseases and to assess the metabolic correlation of animals. The level of biochemical parameters of the blood is also important in determining the health and illness status of animals and in making clinical evaluation. The determinations of the main blood serum biochemical parameters are very helpful for the veterinarian so as to confirm the clinical diagnosis, to estimate the severity of the case, to apply the appropriate treatment and to evaluate the outcome (Roubiens *et al.*, 2006). Blood biochemical parameters vary during different physiological stages of animals (Ahmad *et al*., 2003). Pregnancy and lactation are two most important stages in the life of dairy animals, which affect metabolism resulting in the alteration of the haemato-biochemical profile (Krajnicakova *et al*.,2003;Iriadam, 2007). There are numerous reports on the effects of different phases of the repr oductive cycle and pregnancy on haemato-biochemical indices in domestic animal species including buffalo (Jain *et al*., 2009).

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 (Pyne and Maria ,1981; Dutta *et al.,*1988). So it should be understood how normal values turn into abnormal values due to many factors, such as management, feeding and illness (Utlu *et al.*, 2004)

All animals require minerals such as calcium (Ca), magnesium (Mg),and phosphorus (P) for growth, reproduction and lactation, which often affect specific requirements, and serve as catalytic components of enzymes or regulate several mechanism involved just in pregnancy and lactation (Tanritanir *et al*.,2009

Metabolic disturbances, caused by inappropriate feeding without manifestation of clinical symptoms are significant in animal husbandry and may cause insufficiently developed breeding cattle (Radostits *et al*, 2003). Therefore the determination of normal values of hematological and blood biochemical value are important for the clinical interpretation of laboratory data. These indices may vary depending on factors such as sex, age, weather, stress, season and physical exercise (Sherman & Mary, 1994; Kaneko *et al.*,1999; Nazifi *et al*., 2003; Yokus *et al*., 2006).

Considering the high economic values of Holistiean fresiean cross breed cattle among livestock farming systems, it is important to perform clinical and paraclinical exams in order to guarantee sanitary strategies control, prevention or treatment of diseases and to assure good management practices. It is well recognized that hematological parameters in healthy cattle show several variations in relation to breed (Zumbo *et al*., 2007), age (Piccione *et al*., 2010,), reproductive status, housing, starvation, environmental factors, stress and transportation. These differences have underscored the need to establish appropriate physiological baseline values for livestock which could be used in the realistic evaluation of the management practice, nutrition and diagnosis of health condition as well as in determining the physiological status of animals (Šimpraga *et al*., 2013).

Further, with the ever-increasing interest in Holistian fresian cross breeds cattle as a possible solution to increase efficiency of production in our environmental conditions, there is an urgent need of basic physiological database on such breeds so as to plan and implement health and disease monitoring program to ensure sustainable development of dairy husbandry and economic viability of commercial dairy farming in Ethiopia (Azage,1989; Azage and Alemu,1999).

However, Holstein Friesian cross bred, which are considered to be world's highest milk producers amongst cross-bred cows, remains unexploited for heamato-biochemical profile. Further, no attempt has been made till date to delineate the underlying cause of any problem of the economically important cross-bred in the light of blood biochemistry in Ethiopia. So that reproductive disorders can be handled efficiently before the manifestation of clinical symptoms and providing new and useful information about the guidelines for the management strategies

during different physiological phases aimed to prevent economic losses prior to reaching highest peak of devastating level.

Therefore present study was undertaken to explore the effects of lactation and pregnancy on major hematological and biochemical parameters of Holstein Friesian cross bred cows maintaine d in various farms in Bishoftu to establish reference values.

The specific objectives of this study were to:

- Assess major hematological and biochemical parameters in cross bred Holstein Friesian during in different stages of lactation.
- Assess major hematological and biochemical parameters in cross bred Holstein Friesian during different stages of pregnancy.
- Establish reference values of hematological and serum biochemical parameters of cross breed Holstein friesian cows and heifers

2. REVIEW OF LITERATURE

2.1 Alteration of major hematological and biochemical profiles during lactation

It is well established that milk and milk components are directly and indirectly synthesized from blood. The rate at which blood flows to the mammary gland is one of the key-factors in determining milk synthesis. Approximately, 400 to 500 liters of blood circulate through mammary gland to produce one liter of milk (Fernandez and Hoeffler, 1998). There is a 2 to 6 folds increase in blood flow in the mammary gland starting 2 to 3 days prepartum. During lactation, the mammary gland secretory cells utilize 80% of the blood metabolites for milk synthesis depending on the infiltration of precursors of milk components like amino acids, glucose and fatty acids (Piccione *et al*., 2009). Hence, blood biochemical parameters including total protein, triglycerides, free fatty acids and urea are important indicators of the metabolic activity in lactating animals (Karapehlivan *et al*.,2007). Since the milk yield and composition varies across the length of lactation stage, it is, therefore, imperative, to study haematolo-biochemical constituents during different stages i.e. early, mid and late stage of lactation.

Immediately after the calving, high rates of body condition score losses are associated with a severe negative energy balance status, indicated by alterations in blood metabolite and hormone profiles (Wathes *et al*., 2009). Specifically, high levels of non-esterified fatty acids (NEFA) and β-hydroxybutyrate concentrations are indicative of lipid mobilization and fatty acid oxidation (Wathes *et al*., 2009; Sakha *et al*., 2006). It is well known that to meet the nutritional demands of milk synthesis, dairy cows need to mobilize body reserves, causing negative energy balance until nutrient intake covers the demands (Kessel *et al*.,2008). Even the cholesterol serum level seems to be influenced from calving and milk yield, in lactating dairy cows (Kweon *et al*., 1986).

2.2 Alteration of major hematological and biochemical profiles during pregnancy

Pregnancy is physiological status considered to modify metabolism in animals and induce stress (Iriadam, 2007;Tanritanir *et al*., 2009). The periparturient period is important in terms of its influence on the health and the subsequent performance of dairy cows, since cows develop serious metabolic and physiological changes during these periods (Tanaka *et al.*, 2011). In fact, it is well known that during the pregnancy all the metabolic pathways are involved in sustaining the fetus growth (Bell *et al*., 2000). The period of transition between late pregnancy and early lactation presents a huge metabolic challenge to the high-yielding dairy cow and the hemato-chemical profiles are important in evaluating the health status of animals during this transition (H agawane *et al*., 2009; Bell *et al.*, 2000).

Pregnancy has a great impact on the intensity of metabolism and on metabolic parameters in the blood. The activity of blood amino transferases is very important because they act as a catalyst in connection to the metabolism of amino acids and carbohydrates. Changes in their activity in the blood can be a consequence of their increased activity in cells, or damages in cell structure (Milinković-Tur *et al*., 2005).

Pregnancy is one of the physiological conditions leading to remarkable and dramatic changes in hematological and biochemical variables in all animal species. Pregnant dairy cows are at a light risk of metabolic and reproductive disorders and oxidative stress is considered to be involved in these events (Turk *et al*.,2005). Pregnancy and lactation are physiological statuses considered to modify metabolism in animals (Krajnicakova *et al.,* 2003; Iriadam, 2007). The last 2-3 months of gravidity are extremely critical in cattle. The following detrimental factors may negatively influence pregnant breeding cows and therefore the fetus , qualitatively not fully valued feed ration ,shortened dry period and a number of health disturbances (diarrhea, anemia, hepato dystrophy, ketosis, mastitis, acid-base balance disturbances, intrauterine infections

(Slanina,1977). Furthermore stages of lactation, milk yield levels, as well as the animal's body co nditions may affect blood values (Castello *et al.*,2005).

2.3 Blood analysis for the assessment of animal welfare and health.

Monitoring the health of dairy herds is central to the assessment of animal health and welfare. In dairy cattle farming it is critical to identify and monitor health status and disease incidence. In recent years there has been a growing concern for animal welfare, with consumers paying increas ing attention to farm management conditions and procedures, especially those that may inflict pain and suffering on animals (Godyń *et al*.,2013). The health status of animals maintained under specific conditions is one of the criteria for welfare assessment. It is important to monitor housin g and feeding conditions, which translates directly into milk yield, reproductive parameters, abse nce of disease and mortality, mental comfort, absence of stress, good appetite, and determines the body's homeostasis or the maintenance of proper animal welfare levels. One of the most important challenges in modern herds is to maintain proper house climate, and the interrelations between air temperature and humidity are of prime importance for animal welfare and productio n profitability (Herbut and Angrecka 2012). Changes taking place in the animal's body in respon se to external factors such as nutrition, housing system and microclimate can also affect the level of different blood parameters (Nina *et al*.,2009).

Testing of physiological parameters is essential to monitoring the health status of dairy herds. The most popular blood diagnostic test is the blood count determination, which includes erythroc yte, leukocyte and thrombocyte counts, as well as hemoglobin content, hematocrit value and red blood cell parameters. The content of blood components varies depending on factors that inhibit or stimulate the circulatory system. The main function of blood is to maintain the body's physiological balance, while the hematological blood indicators are the main determinant of the animals' environmental adaptation and thus their welfare (Anderson *et al*.,1999,Sattar and Mirza,2009). In the assessment of animal welfare the use of physiological indicators is common. Within these, blood reference values have been an important tool for practitioners in order to monitor health and welfare at individual or group level, or the immediate response of an animal towards a determined stressor (Singh *et al*.,1991 , Geffré *et al* 2009, Ramin *et al.*,2007). For

example, changes in plasma concentration of glucose, urea and proteins have been associated to significant metabolic cost by the animal, while immune suppression can indicate a potential health risk, or a reduction in growth rate (Barnett and Hemsworth, 1990).

To identify those individuals that have health alterations, values from the blood analysis are usually compared with the population means or ranges of standard values (Herdt, 2000). The use of inappropriate reference values increases the risk of erroneous conclusions by the clinician or the researcher, and may lead to further unnecessary or inappropriate analysis (Turk *et al.*, 2005). Hemato-biochemical values obtained abroad may not be fully applicable under local conditions due to genetic factors, differences in environmental and husbandry practices and animal's function (Yokus *et al.*, 2006, Kessel *et al.*, 2008). During the dairy cow production cycle, the transition period is critical due to the tumultuous endocrine and metabolic changes that accompany parturition and the initiation of lactation. Biochemical tests evaluate the body's internal condition, the function of different organs (including kidneys and the liver) and the course of metabolic changes in the body (Kaneko *et al.*,2008).

2.4 Reference values for hematological and serum biochemical profile of holstein friesian cows

Establishing reference values for assessing blood biochemical profile of animals is imperative to confirm clinical diagnosis and estimate the severity of diseases (Piccione *et al*., 2010). Further, biochemical parameters are commonly employed as useful indicators of health as well as nutritional status of many species, and thus help in diagnosis of metabolic diseases and management of infertility as well as low productivity in farm animals (Pugh and Baird,2012). It is unequivocal that several factors viz. age, sex, pregnancy and lactation affect the metabolism and thereby the blood biochemical profile of animals (Jain *et al*., 2009).

The metabolic profile is based on a range of laboratory blood tests and represents the diagnostic starting point for the determination of the nutritional and health status of the animal. The changes in biochemical and hematological constituents are important indicators of the physiological or pathological state of the animal (Ahmad *et al.*, 2003). The reference value s for the purpose of early detection of nutrient deficiency or metabolic disease can contribute to successful diagnosis and faster recovery of animal (Radostits *et al.*,2000).

Metabolic diseases occur sporadically. In cows with high milk production, metabolic diseases are much more common than in primitive breeds of the same species.Laboratory medicine is an important tool that helps practitioners monitor transition cow health at the individual and herd levels (Holman, 1946; Kramer, 2000). Through laboratory profiling, not only can sick animals be detected, but those herds with higher risks for developing metabolic, reproductive, or infectious diseases may be predicted (Holman, 1946; Kramer, 2000; Kaneko *et al.*,2008;Pugh and Baird, 2012). Any measurement that may be used to make a decision about an intervention must be compared with an appropriate reference value, or more precisely, an interval of reference value (Alonso *et al*.,1997).

Reference limits should be determined by each laboratory under their specific conditions of equipment, reagents, target population, and staff training. Alternatives to these reference limits are those provided by the reagent manufacturers, reference laboratories working in similar conditions, or textbooks (Roubies *et al*.,2006). However, these alternatives should be used with considerable caution. Several methodologies have been described to establish reference limits (CLSI, 2008; Geffré *et al*.,2009). However, the data that are the basis of the reference limits, including the sample size and the variable distribution, should be carefully considered (Vojta *et al*.,2011; Šimpraga *et al*., 2013).

The smaller the sample, the wider the confidence interval, and consequently, more caution must be applied when using them to interpret results. A sample size of 60 is proposed to be sufficient to establish reference limits for domestic animals (Geffré *et al*.,2009). If the variable does not fit the normal distribution, adjustments such as logarithmic or squared transformations are possible tools to normalize the data to calculate valid descriptive statistics (Vojta *et al*.,2011).

In veterinary medicine, a pragmatic approach must be applied due to the diversity of species and complexity in how they can be sub grouped. However, it is necessary to understand the characteristics of the reference population that is to be sampled in order to most accurately interpret the results. Due to the changes that take place during the transition period, it is necessary to determine different reference limits for both the precalving and postcalving periods.

The reference intervals data for blood variables in many domestic animals have been known for many years (Holman, 1946; Kramer, 2000; Kaneko *et al.*, 2008; Pugh and Baird, 2012).However, it should be noted that these reference intervals have been developed for universal use for sheep as a species, were collected over a long and unknown interval of time, and have not previously been verified for local use. When validating the reference interval, two main factors must be taken into account: 1) the proper selection of the method for determining the reference interval, and 2) the appropriate selection of the reference animal sample. Techniques for method assessment are relatively simple, if used in the same way as in the original study, but in veterinar y medicine this is almost never the case. It is also important to use the same analytical systems to ensure quality and accuracy, which is also seldom possible primarily because of the speed with which the technologies and methods have developed in the past 50 years (CLSI, 2008).

Recently, many authors (Alonso *et al*.,1997;Roubies *et al.*,2006) have indicated that haematologi cal and biochemical blood values do not exhibit merely a small degree of breed specificity. They are also statistically significant dependent on living conditions, region, husbandry and rearing loc ation, and especially diet (Vojta *et al*.,2011; Šimpraga *et al*.,2013). This fact is particularly impor tant in organic farming (Vojta *et al*.,2011). Significant differences were also identified concernin g sex, reproductive status (Roubies *et al*.,2006;Bonev *et al*.,2012) and the age of the animals (Ro ubies *et al*.,2006; Piccione *et al*.,2009).

In 2008, a document was published, guiding scientists and laboratories in determining reference intervals (CLSI, 2008). Geffré *et al*.,(2009) published a review of this document and concluded that this guideline is applicable to veterinary medicine. Both documents recommend testing all reference intervals, and their validation in each laboratory while using their own, conventional techniques for the variables and in a population as similar as possible to the one for which routin e blood analysis is being done. They also give rigorous rules for the preanalytical, analytical and statistical methods for determining reference intervals. Friedrichs *et al.*,(2012) concluded that ad

option of these guidelines by the entire veterinary community would provide easier communicati on and information dissemination.

It is particularly important for herd health monitoring, as rearing and feeding conditions often ca use variations in the concentrations of many serum/plasma metabolites, and their "exit" from the reference range in the published literature (Braun *et al*., 2010)

To interpret biochemical data correctly, the results obtained in the laboratory must be compared with values corresponding to reference values (Yokus *et al.*,2006). The blood biochemicals profiles are considered important in evaluating the health status of animals, the estimation of biochemical constituents are the prerequisites to diagnose several pathophysiological and metabolic disorders in cattle (McDowell, 1992).

Animal health can be defined as the absence of disease determined by clinical examinations combined with various diagnostic tests. Laboratory medicine is an important tool that helps practitioners monitor the animal's health at individual and herd levels (LeBlanc *et al.*, 2006). Through laboratory profiling, not only sick animals can be detected, but those herds with higher risks for developing metabolic, reproductive or infectious diseases can also be predicted (Barnouin *et al*.,1997; LeBlanc *et al*., 2005). Serum biochemical and haematological reference values are used to prove normality and to diagnose disease and physiological alterations. Published data propose erratic reference values that are often obtained from a relatively small number of animals. Textbook reference intervals produced by European or US Veterinary Laboratories were established for animals other than buffaloes and are often based on animals living under good husbandry conditions in temperate climates. However, those sample reference groups may differ from those of the developing countries. Reasons for potential differences could include genetic factors, nutritional quality and quantity, water availability, sweat electrolyte losses, parasitism and hot climate(Kaneko *et al*., 1997).

Blood indices are getting increasingly importance in veterinary medicine as indicators of the physiological, nutritional, metabolic, and clinical status of farm animals (Braun *et al*.,2010).
Hence, reference values for such indices become imperative for any disease diagnosis,

prevention and controlling program as it forms the very basis for clinical interpretation of laboratory data (Friedrichs *et al.*,2012). It is well known that various factors *viz.* age, sex, breed and physiological stages affect the cellular hemodynamic (Alonso *et al*.,1997) .

However, pregnancy and lactation are the two most important stages in the life of dairy animals that affect metabolism resulting in alteration of the hematological parameters (Vojta *et al*., 2011).International Federation of Clinical Chemistry (IFCC 1979) specified some factors that must be established when using reference values, as the characterization of the reference population with respect to age and sex. The criteria used for including or excluding individuals from the reference sample group were the physiological and environmental conditions under which the reference population was studied and the specimens collected from the reference sample group.

2.5 The importance of hematological examination

2.5.1 Red blood cell (RBC) count

The process of blood cells formation is called haemopoiesis. Within the embryo, blood cells are produced from the various sites of the mesenchyma of the yolk sac. Foetus active takes place in the liver, spleen, lymph nodes and thymus gland. In the adult, bone marrow is the only organ of red cell formation. That is stimulated by the erythropoietin hormone which the main site of its production from the kidney and some amount is also formed in the liver (Bhattacharyya, 2000).Red cells, the most common type of blood cell, are responsible for transport oxygen throughout the body. A red blood cell count (RBC) is ordered to check whether the number of red cells in the blood is abnormally high or abnormally low (Rose and Hodgson,1994).

2.5.2 Hemoglobin concentration

The Hb values recorded during early and late stages of pregnancy were significantly low ($p<0.05$) in comparison to mid pregnant stage in crossbred cows (Mir *et al*., 2008). The decrease in the content of Hb during pregnancy could be due to the dilution of blood which occurs as a consequence of increase of plasma volume (Singh *et al*.,1991). Sattar & Mirza (2009) showed that the Hb concentration was significantly higher ($p\leq0.05$)in non pregnant heifers compared

with pregnant lactating cows , non pregnant dry cows and non pregnant lactating cows , while the lowest values were observed in non pregnant lactating cows. Similarly Neelu *et al*.,(1996) reported significantly higher values for Hb in heifers than other groups of Sahiwal cows .

Esievo & Moore (1979) reported a decrease in Hb concentration in non-pregnant lactating Holstein fresian cows during early lactation. While Ahmad (1995) reported the highest Hb concentration in Sahiwal cows during last trimester of pregnancy (pregnant dry cows). Unlike these results, Geoge *et al*.,(1999) reported a decrease in Hb with advancing lactation and pregnancy with high increase at parturient stage in dairy cattle. The highest mean Hb concentrations were found in the samples taken in the day of parturition, they then gradually decreased and 4 days after parturition reached the lowest mean Hb concentration in dairy cows (Piccione *et al.,*2010).

2.5.3 Hematocrit (Packed cell volume)

The benefit of these indices is that you can quickly narrow down the potential causes of anemia; the disadvantage is that these numbers assume a similar population of cells. For example, if the MCV is 90, does that mean all you cells are 90 of all the factors disease conditions are rampant with a significant impact on the performance of animals (Ademosum,1994).

For the intervention of such disease problem either by treatment, control or prevention, appropriate diagnostic procedures are essential. So that physiological parameters, hematological and serum biochemical tests have to be implemented. The hematological and serum biochemical profits would substantiates the physical clinical examination together with anamnesis to provide excellent bases for correctly diagnosing the diseases, the extent of organ damage and the response of the defense mechanism. These days, many countries have established the normal reference values of clinical, hematological and serum biochemical parameters for their local animal species (Ademosum, 1994).

The diagnosis of diseases is mainly dependent upon deviations from the normal range of physiological values. However, in the absence of established reference values for any blood parameters, one can be exposed to misinterpretations in diagnosing and treating different disease

conditions. This again entails standardizing blood serum biochemistry profiles to once environmental conditions for use and application of research and educational practices (Falconer and chapman,1977).

A part from disease conditions, factors like climate, exercise, altitude, pregnancy, age and sex are capable of inducing changes on the normal physiological parameters of all domestic animals including equines (Dacie,1991 ; Jain,1993; Sherman and Mary,1994; George *et al*.,1999). Nutrition or feed is another important factor that affected the blood constituents of animals living in different geographical zones (Ahmd *et al*.,1995).

2.5.4 Red cell indices

The most important red cell indices are Mean corpuscular volume (MCV), mean corpuscular he moglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC)**.**The mean value essentially evaluates variation in RBC size, iron deficiency results in a decrease MCV (microcytosis) because inadequate hemoglobin concentration. The MCH is an estimation of the amount of hemoglobin in the blood per erythrocyte. Iron deficiency results in a decreased MCH. The MCHC is the most accurate of erythrocyte indices. Reticulocytosis (erythroid regeneration or iron deficiency) results in a decreased MCHC while hemolysis causes an increased MCHC (Gavan *et al.*,2010).

The MCV values reported in mid pregnancy were significantly ($p<0.05$) lower when compared with the values reported in the early and late stages of pregnancy. The MCH values did not differ significantly among different stage of pregnancy in contrast with MCH, the mean values of MCHC indicated that the value in the mid stage of pregnancy differed significantly ($p\leq0.05$) from the values observed in the early and late stage of pregnancy in crossbred cows (Mir *et al*., 2008; Opara *et al.*,2006).Sattar & Meriza (2009) noted the highest MCV, MCH,and MCH C values in parturient cows and the lowest value were observed in pregnant lactating cows.
Kupezynski & Chudoba-Drozwska, (2002) noted that the MCV , MCH increase in delivery while the MCHC decrease in the same period and Nina (2009) showed a slightly higher MCV and MCH in pregnant than non-pregnant in red deer. Kumar & Pachauri, (2000) reported the highest MCV and MCH and the lowest MCHC in non-pregnant dairy cows compared with other groups.

2.5.5 Erythrocyte sedimentation rate (ESR).

Erythrocyte sedimentation rate (ESR) is a test on blood collected with anticoagulant to determine the health status of animals. The highest ESR was observed in pregnant dry cows and the lowest values were observed in parturient cows. However, the mean ESR values observed very high when compared with the non-pregnant cows (Pampori, 2003). Mir *et al*.,(2008) noted a decrease in ESR value in the late stage of pregnant cows compared with the mid stage of pregnancy in cows. The highest ESR was reported in pregnant dry cows and the lowest values were observed in parturient cows, the difference was statistically significant (Sattar & Mirza, 2009). Ahmad, (1995) reported ESR values (5.9±0.40) to (17.1±0.89)mm/24 hr in Sahiwal cows during the last trimester of pregnancy (pregnant dry cows).

2.5.6 White blood cells count (Total and differential leucocytic count).

White blood cells are cells of the immune system, responsible for protecting the body from infection and malignancy. A white blood cell count (WBC) is ordered to check whether the number of white cell in the blood is abnormally high or abnormally low. The test is performed to find out many white blood cells do the animals have.

Body produces more white blood cells when there is an infection or allergic reaction, even when animals are under general stress and during vaccination (Friedrick *et al*.,2005). A low number of WBC is called leucopenia. It may be due to bone marrow failure (for example, due to infection, tumor, or abnormal scarring), collagen-vascular diseases, and disease of the liver or spleen and radiation exposure of the animals. A high number of WBCs is called leukocytosis (Fry, 2010; Malikides *et al*., 2001). It may be due to infectious diseases, inflammatory diseases (such as allergy), leukemia, severe emotional or physical stress and tissue damage (for example, burns). These lists are not all inclusive (Baily,1989).There are drugs that may increase WBC counts including aspirin , corticosteroids, epinephrine and others. Drugs that may lower WBC count

include; antibiotics, antihistamines, antithyroid drugs, barbiturates, diuretics and sulfonamides (Boudreaux and Ebbe, 1998).

2.6 The effect of pregnancy and lactation on some serum biochemical parameters.

Dairy cattle, like most lactating mammals, are usually in negative energy balance within the first few weeks of lactation (Mir *et al*.,2008). After the delivery, the cow requires high amount of energy from body reserves, but actually it is not able to cover the required nutrients consumed, what leads to the loss of body weight. The continuous increase in milk production has created new challenges for high-producing dairy cows, especially during the transition period. At the beginning of lactation, dairy cows have to cope with the high energy and protein demands for milk synthesis at the time when nutrient intake is low. Mobilizing energy and protein from body tissue stores and repartition of nutrients away from extramammary tissues are the primary alternatives to supply sufficient nutrients for milk production during the first weeks of lactation. Excessive body reserves, especially fat, can cause a series of metabolic disorders and consequent production losses (Friedrick *et al*.,2005). Furthermore, physiological and pathological changes associated with negative energy balance are important factors related to development of ketosis, displaced abomasum, and retained placenta (Opera *et al.*,2006).

2.6.1 Serum total protein (TP) concentration.

Blood serum total protein (TP) content is one of the indices of nitrogen metabolism (Kuleta *et al*., 1990).The reduction of protein level occurs in late pregnancy and the first weeks of lactation, the greatest decrease in the TP content occurs in parturition period in cows, Onita, *et al.*,(2009) reported that the Plasma TP represent one of the nitrogen metabolism indicators and depend on protein content of the fodder, lactation phase, cows age and season. The decrease of TP after parturition can be a consequence of displacement blood gammaglobulins in mammary gland. The TP level is an indirect expontent of feeding (Ghergariu & Baba, 1990). A significant ($p \leq 0.05$) increase in plasma TP content was observed during the mid and late pregnancy when compared with the values observed in early pregnancy. Piccione, *et al.*,(2009) showed that

Serum TP significantly ($p \leq 0.05$) increased during all the (early, mid and end) lactation and dry period compared to dioestrus, pregnancy and post partum periods.

Kaneko & Cornelius, (1980) had reported that the TP progressively decreases throughout gestation; their data suggest that the immunolglobins rapidly leave the plasma during the last month of gestation when colostrums are being formed in the mammary gland. The serum TP constitute a portion of the amino acid pool of the body and such as are believed to be indicative of the nutritional status of the animal except for the values of birth, TP levels were significantly lower ($p \leq 0.05$) in young animals and higher in mature animals. The TP concentration in cattle and sheep did not change with reproductive status (Yokus & Cakmr, 2006).

2.6.2 The serum urea concentration

The urea concentration in blood and milk is often used as criteria to evaluate protein /energy ration balance of cows (Moore & Varga, 1996). Doornenbal *et al*.,(1988) concluded that the urea serum concentration increased significantly ($p < 0.05$)with the age in beef cattle .While Bedo *et al*., (1997) reported that the blood urea was the highest in the early lactation , deceased in the mid and then increased again in the late milking period. Plasma urea concentration was determined as a significant indicator of dietary protein supply in both sheep and goats (Nazifi *et al.*,2003). The mean level of the blood urea nitrogen in prepartum period was greater than postpartum period. Urea production rises to 67% during pregnancy and fall to 36% following parturition and lactation (Ramin *et al*.,2007). Also Brozostowski *et al*.,(1996) observed an increase in urea level during early pregnancy where there was a decrease below the reference level in late pregnancy.

The marked elevation in urea level during the mid gestation is apparently associated with an increase in the formation of embryo tissues, also it is possible that protein catabolism and high demand for energy by the pregnant ewes during the mid gestation led to an increase in the urea level to an extent above the ability of the kidneys to eliminate excess amounts of plasma urea (Sakha *et al.*,2006). Urea and creatinine are waste products that the kidneys normally filter from the blood and these are interrelated, if the kidneys are not working properly (Digraskar *et*

al.,1991).The increased total blood volume especially in the late pregnancy induces an increase glomerular filtrations, which is also responsible on the increased volume of albumin and urea during the late gestation(Yokus *et al.*,2006).

Yokus *et al.*,(2007) show that the serum urea concentration significantly lowered during postpart um compared with prepartum in normal parturition dairy cows. Urea was significantly lower in the first lactation compared with the second lactation and compared with third lactation and in both pre calving and post calving groups as well (Gerardo *et al.*,2009).

2.6.3 The serum creatinine concentration.

Creatinine compound is the excretion form creatine compounds .Creatine is phosphorlyated in the muscle forming a reservoir of readily available high energy. Because creatine is contained almost entirely in striated muscle, the amount of creatinine released is related to muscle mass ((Doornenbal *et al.*,1988). The same authors reported that creatinine concentration decreased during lactation and increased during post weaning in beef cattle, while Peterson & Waldern, (1981) found no differences in creatinine levels among the stages of lactation but did reported that creatinine levels rose in dry cows with increasing days of pregnancy. In ewes, creatinine content of serum was statistically significant higher in dry period than in late gestation. The quantity of creatinine formed each day depends on the total body content of creatine which, in turn, depends on the dietary intake, rate of synthesis of creatine , and muscle mass (Piccione *et al*., 2009). The same authors supposed that protein mobilization start during gestation reaching its peak during late gestation, during which was observed the lower creatinine values. After calving protein mobilization probably is reduced and craetinine reached its peak during dry period.

2.6.4 The serum glucose concentration.

The blood glucose level is regarded as one of the indicators of energy status in ruminants. An exponent decrease in blood glucose concentration as the pregnancy approached in dairy cattle and the significant decrease in blood glucose level during the late pregnancy signifies the rapid utilization of glucose toward the fag of the pregnancy(Bulent *et al*., 2006). Herdt, (2000) claims that a change in glucose concentration is associated with possibly reflecting hormonal changes at

calving that promote gluconeogenesis and glucogenolysis. Abnormal functioning of hormone pro ducing organs may influence glucose levels (Coles,1986).

A relative increase in plasma glucose level during the advanced stages of pregnancy which indicates that the size of the fetus induces a change in maternal carbohydrate metabolism .The increase in glucose level could be associated with a decline in maternal insulin concentration with the advanced pregnancy (Blom *et al.*,1976); (Abdellatif *et al*.,2009).The increase in glucose level during the advanced stage of pregnancy may be attributed to the reduction in insulin secretion which minimizes peripheral glucose utilization and maximizes glucose extraction of the uterus (Connolly *et al*.,2004).It could be associated with an increase in the rate of secretion of glucocorticoid and adrenaline and stimulation of glucogenolysis in the liver (Swenson, 1993).. The hypoglycemia after parturition was attributed to the heavy drain of glucose for lactose synthesis (Nale, 2003).Basoglu *et al.*,(1996) show that there was a significant higher levels of glucose in periparturient cows than in early and late lactation.

The difference in glucose concentration between prepartum and postpartum periods reveals the c onsumption of glucose by fetus and milk yield, so glucose administration before and after parturition results reduction in hypoglycemia and pregnancy toxemia (Scott *et al.*,1995 ; Ramin *et al.*, 2007). Onita & Colibar, (2009) show that hypoglycemia occurs in the first 21 days postpartum with a return to normal at 41 days postpartum in dairy cows. It is known that cows with milk production have an energy and protein –deficit especially in the first day of lactation .In this condition, metabolism is based on mobilization and the usage of the lipid and usage of glucose in other organ than mammary gland is low.

2.6.5 The serum Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) concent rations.

The intensity of metabolic changes, mainly of proteins, is also reflected in the concentration of other biochemical indicators of the blood, such as total protein, urea or aspartate aminotransferas e (Piccione *et al*., 2009).Measurements of blood metabolites require more frequent sampling to capture the dynamic changes in the peri-parturient period.The concentration of AST in dairy cows at the beginning of lactation was significantly higher in comparison with the dry period.

AST is a widely distributed enzyme, which is found in many tissues and organs, with high activity in the liver (Pathan *et al*.,2011). Increased AST activity in the serum is a sensitive marke r of liver damage (Anderson, 1999). The activities of aminotranferase (AST and ALT) in blood associated with implantation, embryo survival, growth, uterine carbohydrate metabolism amino acid metabolism and glycogen deposition (Milinkovic-Tur *et al.*,2005). Ling *et al*.,(2003) determined that (AST) was low in dry period; however, it increased between 117 and 151 days of lactation in Holstein cows. On the other hand ElSherif & Assad (2001) found that (AST and ALT) levels were higher in lactating ewes than in dry ewes. While Jacob *et al.*,(2001) observed that (AST and ALT) values increased at postpartum, Also Sevinc *et al*.,(1999) determined a decrease in (AST and ALT) values at postpartum. Jovanovic *et al*.,(1997) observed an increase in (AST and ALT) levels during pregnancy.However, Khan *et al.*, (2002); Yokus *et al*., (2006) reported that serum activity levels do not change during pregnancy and the postpartum period.

2.6.6 Serum Alkaline Phosphatase concentration (ALP).

Alkaline phosphatase is one of a group of enzymes which catalyzes the liberation of phosphorus from phosphate esters. It is present in almost all tissues of the body, in mature animals, serum (ALP) originates mainly from the liver, however, in the growing animals most of the (ALP) originates from bone tissues therefore, the higher serum levels in young animals are indicative of rapid skeletal growth (Doornenbal *et al*.,1988). Roussel, (1982) also found a negative relationshi p between ALP activity and age .After parturition (ALP) levels dropped during lactation and increased again toward weaning (Doornenbal *et al*.,1988) .

The (ALP) activity values decreased successively during the milking period (Masek *et al*.,2007). A similar but non significant, decrease in the (ALP) activity was reported by (Sato *et al*.,2005) in cows.These authors observed higher levels of serum (ALP) in lactation periods than in dry periods and it was explained by increased bone specific (ALP) and liver (ALP) activities in lactation period and by the suggestion that ALP originating from mammary gland could influence serum (ALP) values (Sato *et al*.,2005); (Khan *et al.*,2002).

(Yokus & Cakmr,2006) reported that levels of (ALP) in mid –pregnancy and late pregnancy were higher than lactation period. Gurgoze *et al.*,(2009) show that (ALP) activities were higher at 14 days postpartum in Awassi ewes .This increase during lactation is due to an increase in the production of the bone isoenzyme and the activities of (ALP) were within the reference range during gestation and lactation dependent changes in Awassi ewes.

2.6.7 The effect of the pregnancy and lactation on some serum electrolyte concentrations.

Several minerals are required for life to exist. In animals, elements (Ca, P, Mg, Na, K, and Cl) are required to be present in the diet in fairly large amounts (grams to tens of grams each day for the dairy cow) and are termed macrominerals. Several other elements are termed microminerals or trace minerals because they are required in much smaller amounts (milligrams to micrograms each day). In most cases the mineral in the diet must be absorbed across the gastrointestinal mucosa and enter the blood if it is to be of value to the animal. The body needs the paracellular and transcellular mechanisms used by the gastrointestinal tract to absorb each of the various minerals needed. Unfortunately, particularly in ruminants, interactions between minerals and other substances within the diet can occur within the digestive tract that impair mineral absorption. The attributes of organic or chelated minerals that might permit diet minerals to circumvent factors that inhibit absorption of more traditional inorganic forms of these minerals. Once absorbed, minerals are used in many ways. One the effect macrominerals have on the acid–base status of the animal. Manipulation of dietary cation and anion content is commonly used as a tool in the dry period and during lactation to improve performance (Underwood & Suttle,1977)

Many macrominerals play a role in the body as cofactors of enzymes involved in controlling free radicals within the body and are vital to antioxidant capabilities. Those same minerals, when consumed in excess, can become pro-oxidants in the body, generating destructive free radicals. Complex interactions between minerals can compromise the effectiveness of a diet in promoting health and productivity of the cow (Rook & Thomas, 1983).

The physiological concentration of trace elements must always be maintained for proper maintenance of the cellular functions of the animal .The normal concentration of these elements in different tissues mainly depends on the dietary concentration, absorption and also on concentration at other tissue elements, homeostatic control mechanism of the body and the species of the animal involved (Underwood & Suttle, 1977).The requirement for maintaining homeostasis of calcium (Ca) and phosphorus (P) increase with the augmented milk production and with the advancement of pregnancy (Doubek *et al*.,1985).The highest Ca concentration was observed in dairy cows in the 4th -5th months of pregnancy and the lowest in dry cows, while the highest and lowest plasma concentrations of inorganic P were found in 4th- 5th months of pregnant cows and 2-6 week postpartum group respectively (Doubek *et al.*,1985).

The depressed trend in calcium level during the stage of lactation could be a result of the impaired absorption of food metabolites from the gastrointestinal precursor , excessive losses through urine , colostrums as it was much more drained in the colostrums during excessive milking and due to insufficient mobilization from the skeleton , while the serum P concentration in early stage of lactation was significantly ($p<0.05$) lowered than the normal healthy control and other groups(mid lactation , late lactation and dry pregnant) animals (Hagawane *et al*., 2009) The moderate depression in the levels of P might be due to the necessity of it for the colostrums synthesis (Rook & Thomas, 1983). Meglia *et al*., (2001) reported that blood levels of Ca & P are expected to decrease at calving due to the large demand of colostrums and milk production.

3. MATERIALS AND METHODS

3.1. Study area

The study was conducted in Bishofftu/Debre Zeit from October 2017 to April 2018 in five purposively selected Dairy Farms. For its suitable location, large population, satisfactory record keeping system, proper feeding, management and also for their kind cooperation. Cows were of different ages and production status. Those were the Farm of College of Veterinary Medicine, Ethiopian meat and milk industry development institute, Debre Zeit Ethiopian Agricultural research center, Alemayehu Atomsa small scale dairy enterprises farms and Genesis farms included in the study. Effects of lactation stages and pregnancy on some hematological and serum biochemical profiles of cross Holistien fresien cows used in current experimental study were according to the physiological stages and inclusion criteria of the farm. Effects of lactation stages and pregnancy on some Hematological and serum biochemical profiles of cross holistien fresion cows in different physiological states were included in this study.

The town is located 45kms south east of the capital of Ethiopia, Addis Ababa. It is situated at latitude of about 9° north and longitude of 4° east. The city lies at an altitude of 1850 meters above sea level in central high land of Ethiopia. The area has three distinct seasons, namely main rain, short rain and dry seasons. Based on weather data, the mean annual rainfall of the area is 866 ml with mean minimum and maximum temperatures of 14^{o}C and 26 ^{0}C, respectively, with mean relatively humidity of 61.3%. Farmers in the vicinity of Bishoftu town use a mixed crop and livestock farming system. Moreover, Bishoftu and its surrounding have variable and yet representative agro-ecologies of the country (NMSA, 2008).

3.2. Study animals

The cows were in various stages of lactation andpregnancy based on the length of their lactation, the animals were identified as in early (3-6w eeks), mid (15-20 weeks) and late (22-38 weeks) la ctational stage for this study purpose. Accordingly, they were categorized into three different groups of fifteen animals each viz. group I (early lactation), group II (mid lactation) and group III (late lactation).The same to lactation animals study groups categorized in to eary,mid and late pregnant animals. Animals were routinely vaccinated and treated with anthelmintic twice a year. All animals were clinically healthy, and handled carefully to reduce any possible effects of stress on the parameters analyzed.

Table 1.The number of study animals different age category and study groups with their physiological status and ranges.

Sr.No	Number of animals in group	Age of the animals in Grou ps(year)	Animals to be categorized gro up	Current physiolo gical status of ani mals	Range of current physiological status of animals
1	15	1.3-1.7	Control	Heifer(non pregnant)	-
2	15	3.6-6.8	Control	Dry cows(non pregnant)	-
3	15	2.3-8.5	Experimental	Early lactating	3-6 weeks
4	15	2.7-6.4	Experimental	Mid lactating	15-20 weeks
5	15	1.9-6.0	Experimental	Late lactating	22-38 weeks
6	15	1.8-5.8	Experimental	Early pregnant	Up to 3 months of pregnancy
7	15	2.4-9.0	Experimental	Mid pregnant	4-6 months of pregnancy
8	15	3.2-8.6	Experimental	Late pregnant	Up to 9 months of pregnancy

All these physiological stages were confirmed from record book of each animal in the farm and the status of pregnancy was assessed by rectal palpation and record book for about the date of AI service and any history of conception failure was identified strictly. The health status of the animals was assessed by clinical examination where animals which are alert, and aware of its surrounding, standing on all of its feet, no clinical signs of any disease are considered as apparently health. On the other hand separation of an animal from its group, reduction in feed intake, watering and poor body condition were considered as sign of disease and are excluded

All these physiological stages were confirmed by rectal palpation and record book of each animal in the farm. The criteria considered being animals apparently healthy, the following conditions are included. Healthy animal is alert and aware of its surrounding. It should stand on all of its feet. Separation of an animal from its group is often a sign of health problem.

Animals show some signs like reduction in feed intake, watering and poor body condition are excluded from study. All experimental animals are dewormed and deticked from both internal and external parasites with (triclabendazole and ivermectin) and clinical parameters and blood samples are taken after two weeks of deworming to avoid the confounding effects of parasitism on the normal value. All animals are vaccinated to prevent common viral and bacterial diseases by local vaccines which are produced by NVI. The animals are acclimatized for some days before the beginning of the experiment and handled based on guidelines of animal welfare in order to avoid stress. All animals are housed in proof compartments, provided with adequate feed and clean water adlibitum and supplied with concentrated feeds and mineral leaks. Animals showing exceptionally high or low clinical parameters reading are excluded. All these criteria are taken in to consideration while sampling.

3.3 Inclusion and Exclusion criteria for the investigated animals.

The most important inclusion criteria for this study purpose were free from disease of mastitis and past history of mastitis, free from TB (TB screening was performed),no history of coughing, good body condition score ,general attitude: alert, no loss of skin elasticity, normal mucous membrane: pink, no diarrhea in previous 7 days, no urogenital abnormalities in previous 7 days ,no muscular abnormalities in previous 7 days, no medication in previous 7 days, absence of skin lesions or alopecia, absence of intestinal and blood parasites, presence or absence of record book in the farm the most criteria of inclusion criteria of investigated animals. In addition to these no history of both vaginal and uterine prolapsed ,no history of metritis and endometritis (Discharge of pus from vulva) and no history of abortion were the most important criteria for inclusion of investigated animals for this research to prevent any confounding and interfering effects on expected results of this study.

3.4. Sample Collection and Processing

3.4.1 Clinical and physiological Examination

A complete physical examination is performed on all animals to detect any abnormal signs. Blood samples are only taken from apparently healthy animals. During the experimental period (from October 2017 to April 2018) animals are physiologically and clinically examined two times per week for the first two weeks before collecting the sample to prevent any disease interference that alters the targeted hematological and serum biochemistry test results. Clinically important physiological parameters recording include; rectal temperature, respiratory rate, heart rates, pulse rates, capillary refill time and gut sound taking from all cows in the farms. Data check list was prepared on which measured values were recorded. All clinically important physiological parameters were taken by one individual to avoid personal bias and approximately the same time during the day at 8:00 11:30 two weeks each before sample collection.Temperatur e was taken by digital thermometer. Respiratory rate is taken from the trachea by using stethoscope. Heart rate was also taken by stethoscope. Pulse rate is taken by coccygeal artey. Colour of mucus membrane was determined through the examination of conjunctiva and vaginal mucus membrane. All hematological and serum biochemical tests were also done using different techniques on blood samples collected in capillary tubes.

3.4.2 Blood collection for Hematological analysis

Blood samples are drawn from animals at rest, undisturbed or under least excitement. 15 to 20 milliliters of whole blood is collected aseptically from the jugular vein using disposable needles and EDTA 10% for hematology and another sample was placed in plain tubes without anti-coagulant. Immediately after blood collection, the capped tubes containing anti coagulant and the blood samples are inverted gently ten times to mix. In the laboratory hematological analysis is performed after mixing the blood using an automated KJMR-IV blood mixer. Hematological analysis including total white blood cell count (WBC) including eosinophils, basophils, neutrophils,lymphocytes and monocytes. Red blood cell count (RBC), hemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglob in (MCH) and mean corpuscular hemoglobin concentration (MCHC) is performed within few hours after collection using an automated analyser (POCH 100 IV Diff, sysmex Corporation,Ko be Japan,2005)in laboratory of National Clinical Chemistry of Referral Laboratory in Pasteur Institute (Public Health Institute) in Addis Ababa. The results obtained were compared with the values of control groups and effects of lactation stages and pregnancy on hematological and serum biochemical profiles of cross Holistien Fresien cows were obtained and some reference values were established to interpret laboratory data in specific to Bishoftu area.

3.4.3 Blood collection for serum biochemistry analysis

Biochemical tests were performed on serum samples prepared from blood obtained in plain vacutainer tubes, centrifuged at 3200rpm for 10-15 minutes and stored at -20 degree celicius until the performance of the test. The concentration of total protein, total bilirubin, gucose, urea, creatinine and the activities of Alanine aminotrasferase(ALT/GPT),Aspartate aminotransferase(AST/GOT),Alanine phosphatase(ALP),Gamma glutamyl transeferase were also deterimined using automated system photometer 5010(Robert Riele GmbH and cKG,Berlin Germany,2002.S erum electrolytes ;sodium, potassium, chloride, phosphorus, magnesium and calcium were analysed using Roche AVL 9180 snap pack Electrolyte Analyser (Roche Diagnostic Corporation9115 Hague road,USA,2002). The results are automatically analysed and printed out with the sample identification number.

All animal experiments were conducted under ethics approval College of Veterinary Medicine and AAU Ethical clearance council.

3.5 Quality Control

The accuracy and reliability of the procedures, instrument and the reagents are ensured by quality controls. The quality controls have been performed before analyzing the samples, after replacement of reagents, at maitanance, if there is any doubt about the accuracy of the analyses of values and as required by regulations. To ensure the accuracy of the test results, biochemical analyzers and reagents are checked daily with quality control kits of known values for the various constituents.

3.6 Statistical analysis

Obtained laboratory data was stored in Microsoft Excell-2007 and imported to the software STAT/IC 12.0 for analysis. Descriptive statistical analysis was done to measure the mean, SEM, 95% confidence interval (CI) and p value of different parameters. Data were expressed as mean ± SD, the data were analysed by (ANOVA). Significant differences were assessed using independent sample t-test. Differences in the means between the two groups were examined with t test. The arithmetic means (±SD) serum biochemical parameters in different groups were calculated. T-TEST was done to assess the biochemical parameters in between groups of equal variances to check the significance differences between groups. In all cases the level of significance was determined at $p<0.05$.

4. RESULTS

The assessment of the correct health status of an animal is often difficult without recurs to an examination of its blood. Thus, hematological and serum biochemical analysis were important and or great adjunct in the diagnosis, prognosis and treatment of various animal diseases.

The study showed that all hematological indices associated with RBC differed significantly ($p<0.05$) at different physiological status. In line with this, mean RBC values during different physiological stages differs significantly. During lactation stage the mean RBC values were 5.66 ±0.61 ,4.75±0.98 and 5.57± 0.86, respectively in early, mid and late stage of lactation. The value recorded during early and late stage of lactation differed significantly ($p< 0.05$) from mid stage of lactation (table 1) During pregnancy stage the mean RBC values were 4.66± 0.82, 6.31± 0.33 and 4. 91±0.69, respectively in early mid and late stage of pregnancy. The values recorded during mid stage of pregnancy differed significantly ($p< 0.05$) from both early and late stage of pregnancy. To confirm the effect of lactation stage and pregnancy on mean values of RBC the mean values of the two control group (both heifers and Non pregnant dry cow mean values of RBC) were recorded. The means values of RBC of heifers (6.49±0.76) was significantly ($p<0.05$) higher than the mean values of RBC of non-pregnant dry cows (control). The comparison between experimental and control group by using t-test was significant ($p<0.05$) indicating the physiological stages (pregnancy and lactation) affects the parameters table 2.

Table 2.The effects of lactation stages and pregnancy on hematological parameters of cross Holistien Fresien cows and heifers in Bishoftu (Mean±SD). n=number of animals

Parameters	Control(n=30)		Experimental groups(n=90)			
	Heifers(n=15)	Non-pregnant dry cows(n=15)	Early(n=15) pregnancy(up to three months)	Mid (n=15) Pregnancy(up to six months)	Late (n=15) Pregnancy(up to nine months)	Early (n=15) Lactation (3-6 week)
RBC(×106/µl)	6.49±0.76	5.32±0.65	4.66±0.82	6.31±0.33	4.91±0.69	5.66±0.61
PCV(%)	30.32±2.53	27.11±6.07	32.07±7.6	29.34±4.54	26.91±4.33	33.18±4.81b
MCH(pg)	29.44±3.53	25.32±4.28	30.31±3.91	31.93±4.08	26.01±4.26	13.21±2.06
WBC(×103/µl)	6.41±0.74	6.39±0.29	6.27±1.35	5.78±1.09	5.34±0.68	7.66±1.08
Hb(gm/dl)	10.78±1.88	8.74±1.67	9.22±1.18	9.46±1.39	8.70±1.44	10.68±2.06
MCV(fl)	57.42±5.05	55.38±3.75	53.65±6.68	48.37±11.29	27.39±3.24	45.18±6.75
MCHC(%)	31.36±4.61	30.35±4.08	20.00±1.88	15.83±2.57	18.24±2.06	27.33±3.1
ESR(mm/24 hrs)	6.11±1.39	7.19±0.52	10.86±2.72	10.66±2.52	11.45±1.79	6.70±0.52
Basophils(%)	1.00±0.00	1.00±0.0	1.00±0.00	1.00±0.0	1.00±0.00	1.00±0.0
Neutrophils(%)	37.17±5.67	36.17±3.07	36.17±3.08	37.33±4.71	40.50±2.66	36.20±4.45
Monocytes(%)	5.17±1.48	5.67±1.62	6.00±1.67	6.00±1.09	6.17±1.72	5.77±1.72
Lymphocytes(%)	55.33±5.42	55.67±3.92	55.37±2.56	55.67±2.06	55.33±1.36	58.33±4.32
Eosinophils(%)	1.83±0.69	1.66±0.47	1.83±0.75	1.86±0.51	2.16±0.75	1.83±0.75

The mean values of RBC of heifers (6.49±0.76) was significantly ($p<0.05$) higher than the mean values of RBC of early pregnant group (4.66±0.82) the mean value of RBC of late pregnant group (4.91± 0.69) the mean value of RBC of early lactating group (5.66±0.61),the mean value of mid lactate group (4.75±0.98) and the mean value of late lactating group (5.57±0.86), respectively.

The PCV recorded in the present study were not significantly different between the different physiological stages and in between control groups. The mean value of PCV of non -pregnant dry cows (27.11±6.07) was significantly lower than the mean value of PCV of the early lactating group (33.18±4.81) and the mean value of the PCV of the late lactating group (32.44± 3.4) respectively The mean value of PCV of early pregnant group (32.07±7.6) was significantly higher than the mean value of PCV of the late pregnant group (26.91±4.33).

The MCH recorded in present study were that of the mean value of MCH of the three stages of lactation Early (13.21 ±2.06), mid (12.95 ±1.65),late (13.37±1.53) were significantly lower than that of the mean value of control group and the mean value of three stages of pregnant group like early (30.31±3.91), mid (31.93±4.08) , late (26.01±4.26).

The effects of pregnancy stages and lactation on the parameter of WBC on cross holistien fresien cows in Bishoftu were indicated in the table 1. During normal physiological state, the mean value of WBC of late lactating group (10.14±7.16) was significantly higher than the mean value of the rest of control groups and experimental groups. The mean value of the WBC decreases when pregnancy advanced and the mean value of WBC increases when the lactation approach to dry period (from early lactation to late lactation).

The mean value of total WBC of the heifers (control) (6.41±0.74) is higher than the mean value of non pregnant dry cows (6.39±0.29). The mean values of WBC of both control groups (heifers and non pregnancy dry cows) were higher than all pregnant groups and lower than mean value of some lactating experimental groups(Early lactation and mid lactation groups).

The mean value of Hb in early lactating groups 10.68+2.06 was significantly ($p<0.05$) differs from the mean values of Hb of both mid lactation groups (8.16±1.06) and late lactating groups (7.78±1.08).The mean values of the Hb of the heifer (control) group(10.78±1.88) was significantly higher than the mean value of Hb of the three pregnant groups 9.22±1.18, 9.46± 1.39 and 8.70±1.44 for early, mid, and late pregnant group ,respectively (Table 2).

The mean value of MCV decreased significantly when pregnancy advanced and the mean value of MCV of early pregnant cows (53.65±6.68) was significantly higher than the mean value of late pregnant cows, (27.39± 3.24). The mean value of MCV of heifers (control) group (57.42± 5.05) was higher than the mean values of all experimental groups and in particularly the mean value of MCV of non pregnant dry cows (Table 2).

The mean value MCHC of late pregnancy (18.24± 2.06) was significantly higher than the mean value of early pregnancy (20.00±1.88) and the mean value of mid pregnancy (15.83± 2.52). The mean values of MCHC of the three lactation stages namely early lactation (27.33±3.1), mid lactation (27.81±1.99) and late lactation (28.19±2.85) were significantly ($p<0.05$) higher than that of the mean value three stages of pregnancy early pregnancy (20.00±1.88) mid pregnancy (15.83±2.57) and late pregnancy (18.24±2.06) and non significantly lower than that of control groups heifers (31.36±4.61) and dry cows (30.35±4.08) (Table 2).

The mean values of ESR of the three stages of pregnancy early, mid and late pregnancy 10.86± 2.72,10.66±2.52 and 11.45±1.79 respectively recorded. These mean values of ESR of experiment al groups were significantly higher than the mean values control groups of heifers and dry cows 6.11±1.39 and 7.19±0.52 respectively. The mean values of ESR of three stages of pregnancy early, mid and late stages 10.68±2.72, 10.66±2.52 and 11.45±1.79 values were significantly higher than the mean values of the three stages of lactation early, mid and late stages 6.70±0.52, 6.49±0.46 and 6.97±1.78 respectively.

The present finding shows that the mean value of basophils both experimental and control groups show no significant ($p>0.05$) difference among all groups. The mean value of neutrophils of late pregnant cows (40.50±2.66) was significantly ($p<0.05$) higher than the mean values of neutrophils of late lactating groups (34.00±3.68) (Table 2).

There was not significant($p<0.05$) differences in mean monocyte count between the experimental groups and the control groups . The mean Lymphocytes count in both early lactation and mid lactation 58.33 ±4.32 and 58.50 ±4.50 respectively were significantly ($p<0.05$) higher than other groups. The mean Lymphocytes count in late lactation (48.83± 2.93) was significantly ($p<0.05$) lower than other groups. There was significant difference in mean Eosinophils count among all groups (table2).

Table 3.Reference values hematological parameters of cross Holstein Friesian non pregnant cows and heifers in Bishoftu (Reference Interval).

Parameters	Heifers(n=15)			Non-pregnant dry cows(n=15)		
	Mean	SD	95% CI	Mean	SD	95% CI
RBC(×106/µl)	6.49	0.76	6.07-6.91	5.32	0.65	4.96-5.68
PCV(%)	30.32	2.53	28.92-31.73	27.11	6.07	23.74-30.47
MCH(pg)	29.44	3.53	27.48-31.4	25.32	4.28	22.94-27.69
WBC(×103/µl)	6.41	0.74	5.99-6.82	6.39	0.29	6.23-6.56
Hb(gm/dl)	10.78	1.88	9.74-11.83	8.74	1.67	7.8-9.7
MCV(fl)	57.42	5.05	54.61-60.22	55.38	3.75	53.31-57.46
MCHC(%)	31.36	4.61	28.81-33.92	30.35	4.08	28.09-32.61
ESR(mm/24hrs)	6.11	1.39	5.34-6.89	7.19	0.52	6.9-7.48
Basophils(%)	1.00	0.00	0.0-1.63	1.00	0.0	0.0-2.0
Neutrophils(%)	37.17	5.67	32.6-43.7	36.17	3.07	32.76-43.93
Monocytes(%)	5.17	1.48	4.56-12.67	5.67	1.62	4.68-12.89
Lymphocytes(%)	55.33	5.42	53.67-72.78	55.67	3.92	53.78-72.98
Eosinophils(%)	1.83	0.69	1.64-12.45	1.66	0.47	1.53-12.75

Table 4. Reference values Hematological parameters of cross Holstein Friesian early and mid pregnant cows and heifers in Bishoftu (Reference Interval).

Parameters	Early(n=15) pregnancy(up to three months)			Mid (n=15) Pregnancy(up to six months)		
	Mean	SD	95%CI	Mean	SD	95%CI
RBC(×106/µl)	4.66	0.82	4.2-5.12	6.31	0.33	6.12-6.50
PCV(%)	32.07	7.6	27.85-36.28	29.34	4.54	27.82-31.85
MCH(pg)	30.31	3.91	28.14-32.48	31.93	4.08	29.67-34.19
WBC(×103/µl)	6.27	1.35	5.52-7.02	5.78	1.09	5.17-6.39
Hb(gm/dl)	9.22	1.18	8.56-9.87	9.46	1.39	8.69-10.24
MCV(fl)	53.65	6.68	49.94-57.35	48.37	11.29	42.12-54.63
MCHC(%)	20.00	1.88	18.95-21.04	15.83	2.57	14.4-17.26
ESR(mm/24hrs)	10.86	2.72	9.35-12.36	10.66	2.52	9.27-12.06
Basophils(%)	1.00	0.00	0.56-1.7	1.00	0.00	0.65-2.1
Neutrophils(%)	36.17	3.08	31.6-46.7	37.33	4.71	32.6-45.2
Monocytes(%)	6.00	1.67	4.67-13.3	6.00	1.09	4.84-12.9
Lymphocyte(%)	55.37	2.56	53.68-72.	55.67	2.06	51-68
Eosinophils(%)	1.83	0.75	2.0-14.4	1.86	0.51	1.86-13.6

Table 5. Reference values hematological parameters of cross Holstein Friesian Late pregnant and early lactating cows and heifers Bishoftu (Reference Interval).

Parameters	Late (n=15) Pregnancy(up to nine months)			Early (n=15) Lactation (3-6 week)		
	Mean	SD	95%CI	Mean	SD	95%CI
RBC(×106/µl)	4.91	0.69	4.52-5.29	5.66	0.61	5.32-6.0
PCV(%)	26.91	4.33	24.51-29.32	33.18	4.81	30.52-35.85
MCH(pg)	26.01	4.26	23.65-28.37	13.21	2.06	12.06-14.35
WBC(×103/µl)	5.34	0.68	4.96-5.71	7.66	1.08	7.06-8.26
Hb(gm/dl)	8.70	1.44	7.90-9.51	10.68	2.06	9.54-11.82
MCV(fl)	27.39	3.24	25.59-29.19	45.18	6.75	41.43-48.92
MCHC(%)	18.24	2.06	17.1-19.39	27.33	3.1	25.61-29.05
ESR(mm/24hrs)	11.45	1.79	10.45-12.44	6.70	0.52	6.41-6.99
Basophils(%)	1.00	0.00	0.65-2.1	1.00	0.00	0.56-1.7
Neutrophils(%)	40.50	2.66	32.6-45.2	36.20	4.45	31.6-46.7
Monocytes(%)	6.17	1.72	4.84-12.9	5.77	1.72	4.67-13.3
Lymphocyte(%)	55.33	1.36	51-68	58.33	4.32	53.68-72.
Eosinophils(%)	2.16	0.75	1.86-13.6	1.83	0.75	2.0-14.4

Table 6. Reference values Hematological parameters of cross Holstein Friesian mid lactating and late lactating cows and heifers in Bishoftu (Reference Interval).

Parameters	Mid(n=15) Lactation (15-20 week)			Late(n=15)Late Lactation (22-38 week)		
	Mean	SD	95%CI	Mean	SD	95%CI
RBC(×106/µl)	4.75	0.98	4.2-5.3	5.57	0.86	5.09-6.04
PCV(%)	31.67	3.33	29.82-33.52	32.44	3.4	30.55-34.33
MCH(pg)	12.95	1.65	12.03-13.86	13.37	1.53	12.52-14.22
WBC(×103/µl)	8.45	3.46	6.53-10.37	10.14	7.16	6.17-14.1
Hb(gm/dl)	8.16	1.06	7.57-8.75	7.78	1.08	7.18-8.38
MCV(fl)	45.23	5.43	42.2-48.24	44.48	6.09	41.11-47.86
MCHC(%)	27.81	1.99	26.71-28.92	28.19	2.85	26.6-29.77
ESR(mm/24hrs)	6.49	0.46	6.24-6.75	6.97	1.78	5.98-7.96
Basophils(%)	1.00	0.0	0.58-1.9	1.00	0.0	0.68-2.3
Neutrophils(%)	36.33	2.00	31.6-45.7	34.00	3.68	32.6-43.2
Monocytes(%)	5.33	0.81	4.67-12.3	5.17	1.16	4.44-12.9
Lymphocyte(%)	58.50	4.5	53.68-72.	49.83	2.93	51.2-63.6
Eosinophils(%)	2.00	0.63	2.0-12.4	1.83	0.40	1.86-13.6

Table 7.The effects of lactation stages and pregnancy on serum biochemical profiles of cross Holstein Friesian cows a
of pregnancy and lactation, dry and non-pregnancy in Bishoftu (Mean±SD). n=number of animals in th

Parameters	Control(n=30)		Experimental groups(n=90)			
	Heifers(n=15)	Non-pregnant dry cows(n=15)	Early(n=15) pregnancy(up to three months)	Mid (n=15) Pregnancy(up to six months)	Late(n=15) Pregnancy(up to nine months)	Early(n=15) lactation (3-6) week
AST(U/L)	51.64±9.2	57.91±17.3	51.02±8.1	20.5±11.7	38.19±10.12	78.3±22.7
ALT(U/L)	22.14±4.05	30.65±7.75	51.02±7.8	16.31±2.71	26.03±4.24	26.23±5.96
ALP(U/L)	36.6±5.66	28.93±8.51	47.24±19.1	33.4±6.55	31±8.97	49.73±18.56
GGT(U/L)	27.93±6.66	41.53±9.66	24.26±2.37	33±10.39	22.73±4.28	28.2±6.1
Urea(g/dl)	25.33±4.78	24.08±6.15	32.31±7.77	26.35±4.39	23.19±3.55	32.05±5.73
Creatinine(g/dl)	1.1±0.23	1.05±0.19	0.72±0.18	1.06±0.23	0.75±0.18	0.87±0.16
Glucose(mg/dl)	30.88±4.1	36.82±7.47	38.13±5.31	32.36±4.87	24.92±4.12	45.7±11.57
Total Bilirubin(mg/dl)	0.26±0.067	0.23±0.1	0.072±0.06	0.24±0.098	0.11±0.08	0.21±0.074
Total Protein(g/dl)	7.7±1.25	7.61±1.75	7.98±1.76	6.04±0.86	7.79±1.38	8.3±1.46
Calcium(mmol/l)	2.4±0.74	1.82±0.4	2.16±0.18	2.01±0.35	2.6±0.60	2.29±0.19
Magnesium(mmol/l)	0.94±0.27	0.62±0.24	1.02±0.3	0.93±0.13	0.85±0.12	1.19±0.39
Phosphorus(mmol/l)	1.76±0.54	1.41±0.35	2.58±0.68	1.97±0.52	2.31±0.32	2.32±0.58
Potassium(mmol/l)	3.54±0.43	3.23±0.81	3.36±0.74	3.88±0.62	2.82±0.52	4.45±0.63
Sodium(mmol/l)	128.33±12.99	128.26±13.06	129.06±10.43	115.8±9.39	123.73±10.24	136.06±11.97
Chlorine(mmol/l)	96.14±5.29	83.72±11.08	83.39±9.19	97.29±9.31	84.95±10.05	82.35±9.76

The present findings show that the mean value of AST in both experimental and control groups in between each group of the study. The effects of lactation stages and pregnancy on serum biochemical profile especially AST enzyme was clearly indicated in table 7.The mean values of AST on pregnancy that means early, mid and late were 51.02±8.1, 20.5±11.7 and 38.19±10.12 respectively. The mean value of AST in early and late pregnancy were significantly ($p<0.05$) higher than that of mid pregnancy (20.5±11.7)The mean values of AST in lactation stages that are early, mid and late were 78.3±22.7, 63.12± 19.87 and 62.2±14.4 respectively. The mean values of AST in lactation deceases towards the end of lactation. The mean value of early lactation (78.3±22.7) was significantly higher than the mean values of mid and late lactation 63.12±19.87 and 62.2±14.4 respectively. The mean values of non-pregnancy dry cows 57.91±17.3 was significantly ($p<0.05$) higher than the mean values of the three stages of pregnancy early, mid and late stages of pregnancy 51.02±8.1,20.5±11.7 and38.19±10.12 respectively.

The mean value of ALT enzyme was affected by different physiological stage especially lactation and pregnancy were shown below in table 7. The mean values of ALT was different in different lactation stages and differs on different pregnancy stages and also if differs in control and experimental groups. The highest mean value was recorded during early pregnancy and significantly higher than all the values of the studied groups. The significantly ($p<0.05$) lowest mean value was recorded during the mid pregnancy (16.31±2.71) and significantly lower than other values of the studied groups. The mean value of mid lactating groups 35.61±5.9 was significantly higher than the mean values of both early lactating group 26.23±5.96 and late lactating groups 27.25±9.13.

The mean values of ALT in early, mid and late pregnant group of are 51.02±7.8, 16.31±2.71 and 26.03±4.24 respectively. The mean value of the ALT in early pregnancy was significantly ($P<0.05$) higher than the mean values of both mid and late pregnancy. In addition the mean value of early pregnancy (51.02±7.8) was significantly higher than the mean values of both control groups (heifers and dry cows) 22.14± 4.05 and 30.65±7.75 respectively. The mean value of ALT in mid lactating groups (35.61±5.9) was significantly higher than the mean value of both control groups (heifers and non pregnant dry cows 22.14±4.05 and 30.65±7.75) respectively (shown in table 7).

The mean values of ALP during different physiological stage was different from each other and from their control groups. The mean values of ALP during different stages of lactation decreases toward the end of lactation. The mean values of Alp during different stages pregnancy decreases whet the pregnancy advanced.The mean values of ALP in early lactating group (49.73±18.56) was significantly higher than the mean value of both mid and late lactating groups 36.4±9.35 and 34.06±18.9 respectively.The mean values of ALP in early pregnancy 47.24±19.1 was significantly ($P<0.05$) higher than both the mid and late pregnancy 33.4±6.55 and 31± 8.97 respectively. The mean value of ALP in early pregnancy 47.24±19.1 was significantly higher than the mean values of both control groups (heifers and dry cows 36.6±5.66 and 28.93±8.51 respectively.

The present findings of the mean values of GGT were shown in table2.The mean values of GGT in lactation stages were early, mid and late stages 28.2± 6.1, 38.4± 13.58 and 40.26± 7.03 respectively. The mean values of GGT enzymes in lactation stages increases towards the end of lactation. The mean value of late lactation stage 40.26±7.03 was significantly higher than the mean value of early lactation stage, 28.2±6.1and the mean value of lactation stages in mid lactations stages 38.4± 13.58 was significantly higher than the mean value of early lactation stage 28.2±6.1.The mean values of GGT enzyme during pregnancy were early, mid and late 24.26±2.37, 33±10.39 and 22.73±4.28 respectively. The mean values of GGT in mid pregnancy (33±10.391 was significantly higher than the mean values of both early and late pregnancy 24.26±2.37 and 22.73±4.28 respectively. The mean values of GGT in non pregnant dry cows 41.53±9.66 was significantly higher than mean values of pregnancy stages and mean values of heifers (control) 27.93±6.66.

The mean values of urea in different physiological states of experimental and control groups are shown table2. The mean value of the urea during lactation stages and pregnancy stages including the control groups are recorded in presently in the table 2. The mean values of urea in pregnancy stages early, mid and late were 32.31±7.77, 26.35±4.39 and 23.19±3.55 respectively. The mean values of urea in d/t pregnancy stages decreases when pregnancy advanced. The mean value of urea in lactation stages are early, mid and late were 32.05±5.73, 23.65±7.89 and 15.8±6.58 respectively.

The mean value of urea significantly decreases towards the end of lactation. The mean value of urea in early lactation 32.05±5.73 was significantly higher than both in mid lactation 23.65±7.89 and late lactation 15.8±6.58.The mean value of urea in early pregnancy stage 32.31±7.77 was significantly ($p<0.05$) higher than the mean values of mid pregnant groups, late pregnant group, heifers (control) and non pregnancy dry cows (control) 26.35±4.39, 23.19±3.55, 25.33±4.78 and 24.08±6.15 respectively.

The mean value of creatinine in our present findings was shown is table7. The mean value of creationine during d/t stages of lactation early, mid and late were 0.87± 0.16, 1.21± 0.42, and 1.34±0.44 respectively.The mean value of creatinine in late lactation 1.34± 0.44 was significantly higher than the mean value of early lactating groups 0.87±0.16 and the mean value of mid lactating group 1.21± 0.42 was significantly higher than the mean value of early lactating group 0.87±0.16.The mean value of creatinine in different stages of pregnancy groups early, mid and late were 0.72±0.18, 1.06±0.23 and 0.75±0.18 respectively. The mean value of creatinine in mid pregnancy 1.06±0.23 was significantly differs from the mean values of creatinine in early and late pregnancy. The mean values of control groups (heifers and dry cows) 1.1±0.23, 1.05± 0.19 both values differs significantly from most of the mean values of experimental groups. The mean values of creatinine in lactation stages increases towards the end of lactation.

The mean values of glucose in the present findings were analyzed in the table 7. The mean values of glucose in pregnant stages were early, mid and late pregnancy 24.92± 4.12, 32.36±4.87 and 38.13±5.31 respectively. The mean values of glucose in pregnant groups increase as the pregnancy advanced and the mean value of glucose in late pregnant group 38.13±5.31 was significantly differs from the mean values of both mid and early pregnancy 24.92±4.12 and 32.36± 4.87 respectively.The mean values of glucose in lactation stages were early, mid and late lactation 45.7±11.57, 34.95±7.06 and 28.5±7.05 respectively. The mean values of glucose in lactation stages decreases towards the end of lactation. The mean values of glucose in early lactation 45.7±11.57 was significantly differs from the mean values of glucose in both mid lactation 34.95± 7.06 and late lactation 28.5±7.05. The mean values of glucose in dry cow (control) 36.82±7.47 was significantly differs from mean values of early pregnant 24.92±4.12 and late lactation groups 28.5±7.05.

The mean values of total billrubin in different experiencetal and control groups were shows in table 7. The mean value of billrubin in stages of pregnancy were early, mid and late pregnancy 0.072±0.06, 0.24±0.098 and 0.11± 0.08 respectively. The mean value of billrubin mid pregnancy groups 0.24±0.098 was significantly differs from the mean values of both early pregnant group 0.072± 0.06 and late pregnant groups 0.11±0.08. The mean value of billrubin of early pregnant group 0.072± 0.06 was significantly lower than the mean value of billrubin of the both control groups heifer and dry cows 0.26±0.067 and 0.23±0.01 respectively.

The mean values of total protein in the stages of lactation early, mid and late as follow 8.3±1.46, 7.0±1.9, and 5.47±1.0 respectively. The mean values of total protein in the lactation stages decreases towards the end of lactation. The mean values of total protein in early lactation groups 8.3±1.46 was significantly ($P<0.05$) differs from the mean values of both mid lactation 7.0±1.9 and late lactation 5.47±1.0. The mean values of total protein in pregnancy groups were early, mid and late 7.98±1.76, 6.04±0.86 and 7.79±1.38 respectively and the mean values of total protein in mid pregnancy groups 6.04±0.86 was significantly differs from both early pregnancy and late pregnancy 7.98±1.75 and 7.79±1.39 respectively.

The present funding shows the mean values of calcium in different studied groups were shows in table 7. The mean values of calcium in lactating groups early, mid and late lactating were 2.29± 0.19, 1.76±0.5 and 1.37±0.24, respectively. The mean values of calcium in lactating groups decreases towards the end to lactation. The mean values of calcium in early lactating groups 2.29± 0.19 was significantly ($P<0.05$) differs from the mean value of mid and late lactation groups (1.76±0.56 and 1.37± 0.24 respectively. The mean values of calcium in late lactation groups 1.37± 0.24 was significantly differs from the mean value of calcium in mid lactating groups 1.76±0.56 .The mean value of calcium in pregnant groups were early ,mid and late were 2.16±0.18, 2.01± 0.35 and 2.6±0.60 respectively. The mean values of calcium in late pregnant 2.6±0.60 was significantly differs from the mean values of both mid pregnancy groups 2.01± 0.35 and early pregnancy groups 2.16±0.18. The mean values of calcium in both experimental groups significantly differ from both control groups in different physiological stages in all studied groups.

The mean values of magnesium in early lactation groups 1.19±0.39 was significantly differs from the mean value of mg in mid lactation groups 0.63±0.45. and early lactation 1.19±0.39 were significantly differs from the mean value of mg in non pregnant dry cows 0.62±0.24. The mean values of magnesium in early pregnancy 1.02±0.3was significantly differs from the the mean value of mg in late pregnant groups 0.85±0.12. The mean values of magnesium in early pregnancy 1.02±0.3and early lactation groups 1.19±0.39 was significantly differs from the mean values of magnesium in non pregnant dry cows 0.62± 0.24.

The mean values of phosphorus in three stages of lactation were early, mid and late lactation 2.32±0.58, 3.27±0.58, and 2.11±0.98 respectively. The mean values of phosphorus is early lactating group 2.32±0.58, was significantly differs from the mean value of phosphorus in mid lactating groups 3.27±0.58 in addition the mean value of phosphorus in late lactation 2.11±0.98 was significantly differs from the mean value of phosphorus in mid lactating 3.27±0.58. The mean values of phosphorus in the three stapes of lactation early, mid and late 2.32±0.58, 3.27± 0.58 and 2.11±0.98 were significantly ($p<0.05$) differs from the mean values of phosphorus in control groups both in heifers 1.76±0.54 and dry cows 1.41±0.35. The mean values of phosphorus in three stages pregnancy early, mid and late 2.58±0.68, 1.97±0.52 and 2.31±0.32 respectively. The mean value of phosphorus in mid pregnancy groups 1.97± 0.52 differs significantly from then mean values of both the early and late pregnant groups 2.58±0.68 and 2.31±0.32.

In the present study the mean value of potassium in different physiological state of cows were shows in table 7. The mean values of potassium in three stages pregnancy early, mid and late were 3.36± 0.74, 3.88± 0.62 and 2.82±0.52 respectively. The mean value of potassium in mid pregnancy 3.88± 0.62 significantly ($p<0.05$) differs from the mean values of both early and late pregnancy groups 3.36±0.74 and 2.82±0.52 respectively and the mean value of potassium in late pregnant group 2.82±0.52 was significantly differs from the mean value both early and mid pregnancy 3.36±0.74 and 3.88±0.62.

The mean value of potassium is three stages lactation early mid and late were 4.45±0.63, 5.25± 0.61and 3.96±0.73 respectively. The mean values of potassium in late lactating groups 3.96±0.73 was significantly differs from the mean values of both early and mid lactating groups 4.45± 0.63and 5.25±0.61 respectively. The mean value of potassium is three stages lactating groups differs significantly differs from the mean values of both control groups.

In the present study the mean value of sodium were analyzed in different physiological states were as shown in table 7. The mean value sodium in three stages of lactation early, mid and late were 136.06±11.97, 111.53±5.65 and 126.33±12.14 respectively. The mean value of mid lactating groups 111.53± 5.65 was significantly differs from the mean values of both early and mid lactating 136.06± 11.97 and the mean value of late lactate groups 126.33±12.14. The mean values of sodium in three physiological stages of pregnancy early, mid and late 129.06±10.43, 115.8±9.39 and 123.73±10.24 respectively.

The mean value of sodium in mid pregnant groups 115.8±9.39 was significantly differs from the mean values of sodium in both early and late pregnant groups 129.06± 10.43 and 123.73±10.24 respectively. The mean values of sodium in early lactating groups 136.06±11.97 was significantly differs from the mean values of both control groups.

In the present study the mean value of chlorine in different physiological state of he study groups were analyzed in table 7. The mean value of chlorine mid lactating groups 99.74±9.54 was significantly differs from both mean value of chlorine in early lactation 82.35±9.76 and late lactation 88.61±11.19. The mean value of chlorine mid pregnant group 97.29±9.31 was significantly differs from mean value of chlorine in late pregnant group 84.95±10.05. The mean value of chlorine in different stages of physiology of experimental groups differs significantly the mean value of chlorine in control groups.

Table 8. Establishing reference values of serum biochemistry of cross Holstein Friesian non pregnant cows and heifers in Bishoftu (Reference Intervals).

Parameters	Heifers(n=15)			Non-pregnant dry cows(n=15)		
	Mean	SD	95%CI	Mean	SD	95%CI
AST(U/L)	51.64	9.2	46.53-56.75	57.91	17.3	48.32-67.5
ALT(U/L)	22.14	4.05	19.89-24.38	30.65	7.75	26.35-34.94
ALP(U/L)	36.6	5.66	33.52-39.8	28.93	8.51	24.22-33.64
GGT(U/L)	27.93	6.66	24.24-31.62	41.53	9.66	36.18-46.88
Urea(g/dl)	25.33	4.78	22.68-27.98	24.08	6.15	20.67-27.5
Creatinine(g/dl)	1.1	0.23	0.98-1.34	1.05	0.19	0.94-1.15
Glucose(mg/dl)	30.88	4.1	28.46-38.87	36.82	7.47	32.68-40.96
Total Bilirubin((mg/dl)	0.26	0.067	0.14-0.32	0.23	0.1	0.18-0.29
Total Protein(g/dl)	7.7	1.25	6.74-8.87	7.61	1.75	6.64-8.58
Calcium(mmol/l)	2.4	0.74	1.99-2.82	1.82	0.4	1.59-2.04
Magnesium(mmol/l)	0.94	0.27	0.79-1.09	0.62	0.24	0.82-1.07
Phosphorus(mmol/l)	1.76	0.54	1.23-2.04	1.41	0.35	1.09-1.93
Potasium(mmol/l)	3.54	0.43	3.3-3.78	3.23	0.81	2.78-3.68
Sodium(mmol/l)	128.33	12.99	121.13-135.53	128.26	13.06	121.03-135.5
Chlorine(mmol/l)	96.14	5.29	93.2-99.07	83.72	11.08	77.58-89.85

Table .9 Establishing reference values of serum biochemistry of cross Holstein Friesian early and mid pregnant cows and heifers in Bishoftu (Reference Intervals).

Parameters	Early(n=15) Pregnancy (up to three months)			Mid (n=15) Pregnancy(up to six months)		
	Mean	SD	95%CI	Mean	SD	95%CI
AST(U/L)	51.02	8.1	46.01-55.01	20.5	11.7	14.03-26.9
ALT(U/L)	51.02	7.8	46.67-55.38	16.31	2.71	14.81-17.81
ALP(U/L)	47.24	19.1	36.65-57.82	33.4	6.55	29.76-37.03
GGT(U/L)	24.26	2.37	22.95-25.58	33	10.39	27.24-38.75
Urea(g/dl)	32.31	7.77	28-36.62	26.35	4.39	23.92-28.78
Creatinine(g/dl)	0.72	0.18	0.62-0.82	1.06	0.23	0.93-1.19
Glucose(mg/dl)	24.92	4.12	22.63-27.2	32.36	4.87	29.66-35.05
Total Bilirubin(mg/dl)	0.072	0.06	0.03-0.1	0.24	0.098	0.18-0.29
Total Protein(g/dl)	7.98	1.76	7.01-8.96	6.04	0.86	5.57-6.52
Calcium(mmol/l)	2.16	0.18	2.06-2.26	2.01	0.35	1.82-2.21
Magnesium(mmol/l)	1.02	0.3	0.85-1.18	0.93	0.13	0.85-1.00
Phosphorus(mmol/l)	2.58	0.68	2.2-2.96	1.97	0.52	1.68-2.26
Potasium(mmol/l)	3.36	0.74	2.95-3.78	3.88	0.62	3.54-4.23
Sodium(mmol/l)	129.06	10.43	123.2-134.8	115.8	9.39	110.59-121
Chlorine(mmol/l)	83.39	9.19	78.3-88.48	97.29	9.31	92.13-102.44

Table 10. Establishing reference values of serum biochemistry of cross Holstein Friesian late pregnant and early lactating cows and heifers in Bishoftu (Reference Intervals).

parameters	Late(n=15) Pregnancy(up to nine months)			Early(n=15) lactation (3-6) week		
	Mean	SD	95%CI	Mean	SD	95%CI
AST(U/L)	38.19	10.12	32.59-43.79	78.3	22.7	66.13-91.45
ALT(U/L)	26.03	4.24	23.68-28.38	26.23	5.96	22.93-29.53
ALP(U/L)	31	8.97	26.02-35.97	49.73	18.56	39.45-60.01
GGT(U/L)	22.73	4.28	20.36-25.1	28.2	6.1	24.8-31.58
Urea(g/dl)	23.19	3.55	21-25.16	32.05	5.73	28.87-35.23
Creatinine(g/dl)	0.75	0.18	0.64-0.84	0.87	0.16	0.78-0.96
Glucose(mg/dl)	38.13	5.31	35.18-41.07	45.7	11.57	39.29-52.11
Total Bilirubin(mg/dl)	0.11	0.082	0.073-0.16	0.21	0.074	0.17-0.25
Total Protein(g/dl)	7.79	1.38	7.02-8.56	8.3	1.46	7.50-9.12
Calcium(mmol/l)	2.6	0.60	2.26-2.93	2.29	0.19	2.18-2.39
Magnesium(mmol/l)	0.85	0.12	0.78-0.91	1.19	0.39	0.97-1.41
Phosphorus(mmol/l)	2.31	0.32	2.12-2.49	2.32	0.58	2.0-2.65
Potasium(mmol/l)	2.82	0.52	2.53-3.11	4.45	0.63	4.09-4.8
Sodium(mmol/l)	123.73	10.24	118.06-129.4	136.06	11.97	129.43-142.69
Chlorine(mmol/l)	84.95	10.05	79.36-90.5	82.35	9.76	76.94-87.75

Table 11. Establishing reference values of serum biochemistry of cross Holstein Friesian mid and late lactating cows and heifers in Bishoftu (Reference Intervals)

parameters	Mid(n=15) lactation (15-20 week)			Late (n=15) Lactation (22-38 week)		
	Mean	SD	95%CI	Mean	SD	95%CI
AST(U/L)	63.12	19.87	52.11-74.12	62.2	14.4	55.91-74.35
ALT(U/L)	35.61	5.9	32.34-38.89	27.25	9.13	22.18-32.3
ALP(U/L)	36.4	9.35	31.2-41.59	34.06	18.9	23.59-44.53
GGT(U/L)	38.4	13.58	30.87-45.92	40.26	7.03	36.37-44.16
Urea(g/dl)	23.65	7.89	19.28-28.02	15.8	6.58	12.15-19.45
Creatinine(g/dl)	1.21	0.42	0.98-1.45	1.34	0.44	1.09-1.58
Glucose(mg/dl)	34.95	7.06	31.04-38.86	28.5	7.05	24.59-32.41
Total Bilirubin(mg/dl)	0.27	0.12	0.20-0.34	0.26	0.12	0.19-0.33
Total Protein(g/dl)	7.0	1.9	5.95-8.05	5.47	1.0	4.91-6.03
Calcium(mmol/l)	1.76	0.56	1.45-2.07	1.37	0.24	1.23-1.51
Magnesium(mmol/l)	0.63	0.45	0.38-0.88	0.96	0.46	0.7-1.21
Phosphorus(mmol/l)	3.27	0.58	2.95-3.59	2.11	0.98	1.56-2.65
Potasium(mmol/l	5.25	0.61	4.91-5.59	3.96	0.73	3.55-4.36
Sodium(mmol/l)	111.53	5.65	108.4-114.66	126.33	12.14	119.6-133.05
Chlorine(mmol/l)	99.74	9.54	94.45-105.03	88.61	11.19	82.41-94.81

5. DISCUSSION

All hematological indices associated with RBC differed significantly($p<0.05$)(table2).Mean RBC values during different physiological stages differs significantly from each other. During lactation stage the mean RBC values are 5.66 ±0.61, 4.75±0.98 and 5.57±0.86 respectively in early mid and late stage of lactation (table 2). The value recorded during early and late stage of lactation differed significantly ($p< 0.05$) with mid stage of lactation (table 1). During pregnancy stage the mean RBC values are 4.66±0.82 , 6.31±0.33 and 4.91±0.69 respectively in early mid and late stage of pregnancy. The values recorded during mid stage of pregnancy differed significantly ($p<0.05$) from both early and late stage of pregnancy (Table 2).

The results observed in the present study as represented in the Table 2 are accordance with findings of Mir, *et al*. (2008) who found that the total RBC count were significantly low ($p\leq0.05$) during early and late pregnancy in comparison to mid pregnancy stage where higher values were recorded in crossbred cows. To confirm the effect of lactation stage and pregnancy on mean values of RBC the mean values of the two control group (both heifers and non pregnant dry cow mean values of RBC)were recorded (Table1).The means values of RBC of heifers (6.49±0.76) was significantly ($p<0.05$) higher than the mean values of RBC of non pregnant dry cows (control).

The mean values of RBC of heifers (6.49±0.76) was significantly ($p<0.05$) higher than the mean values of RBC of early pregnant group (4.66±0.82)and the mean value of RBC of late pregnant group (4.91± 0.69) the mean value of RBC of early lactating group (5.66±0.61),the mean value of mid lactate group(4.75±0.98)and the mean value of late lactating group(5.57±0.86) respectively. Similar to our study, Sattar & Mirza, (2009) reported significantly ($p\leq0.05$) high RBCs count in heifers while the lowest values were observed in non pregnant dry cows. Neelu, *et al*. (1996) reported significantly higher values for RBCs count in heifers than other studied cows of different physiological stages.

These values are closely related to the values of the present study. Similarly Kuha (2009) show that the less than two year old cattle found to be higher RBCs count than those of the older cattle while the non-pregnant cows show high significant RBCs count than early and late pregnant cows. The results of the present study are not in agreement with that reported by Flores, *et al*. (1990) who found non significant differences in RBCs count values during gestation and early lactation in cows. Also unlike our study, Nazifi *et al.,* (2004) showed that the RBCs count in earl y and mid pregnant cows significantly higher than that of postpartum cows. The reduction in the erythrocyte count in pregnant animals may be related to the physiological anemia occurring to hemodilution (Ozegbe, 2001; Nuwayhid, 1979).

The PCV recorded in the present study were not much more differs significantly from each other during different physiological stages and in between control groups. The mean value of PCV of non -pregnant dry cows (27.11±6.07) was significantly lower than the mean value of PCV of the early lactating group (33.18±4.81) and the mean value of the PCV of the late lactating group (32.44±3.4) respectively. The mean value of PCV of early pregnant group (32.07±7.6) was significantly higher than the mean value of PCV of the late pregnant group (26.91±4.33). PCV values recorded in the present study as depicted in Table 2 disagreed with Sattar & Mirza, (2009); Neelu., *et al* (1996) who recorded high significant PCV in non pregnant heifers than pregnant cows and the low significant ($p \leq 0.05$) values were observed in non pregnant dry cows. These results not agreed with Kumar & Pachauri, (2000) who found that PCV values were highest in late pregnant cows in comparison with non pregnant dry cows. In multiparous cows the results disagree with Gonzalez & Roch (1998); Nina *et al.,* (2009) who noted that the PCV values were higher in non pregnant animals than lactating animals.

PCV values found in this study are close to the values observed by Mir *et al.,* (2008); Opara *et al.,* (2006) who reported that the values were significantly low ($p \leq 0.05$) during late pregnancy in comparison to mid pregnancy stage where higher values were recorded. However, the finding of some earlier workers indicate a rise in PCV as the pregnancy advanced and the phenomena could be increase in RBC volume causing increased volume of water during advanced pregnancy (Kataria, *et al*.2002; Abdellatif *et al*., 2009; Nazifi *et al.,* 2004).

While our results corresponded with other workers found that non significant difference in PCV value shows during pregnancy and after parturition (Balikei & Yildiz, 2005 in sheep; Kilnkon & Zadnik, 2007) in cows; Hagawane *et al.,* 2009 in buffaloes).The decline in PCV and Hb values in present study known as physiological anemia (Reece,1997) which may be attributed to using of the iron by the fetal development which may results into iron deficiency.

The MCH recorded in present study were that of the mean value of MCH of the three stages of lactation early (13.21±2.06), mid (12.95±1.65),late (13.37±1.53) were significantly lower than that of the mean value of control groups and the mean value of three stages of pregnant group like Early (30.31±3.91), mid (31.93±4.08) , late (26.01±4.26). The present results of MCV values are closely related to those of the Sattar&Meriza (2009); Kupezynski&Bozena (2002) who found that MCV, MCH and MCHC values were highest in parturient and the lowest values were recorded in pregnant lactation cows. Similarly Mbassa, (1991) observed that the MCV an MCH values increased during late stage of pregnancy in Danish Landrace goat. Moreover Kupezynski & Chudoba-Drozdowska (2002) noted that MCV and MCH increased in delivery while MCHC decreased in the same period while Nina *et al.*,(2009) showed slightly higher MCV and MCH in pregnant than non pregnant of red deer.

The mean values of WBC of both control groups (heifers and non pregnant dry cows) were higher than all pregnant experimental groups and lower than mean value of some lactating experimental groups. The mean values of total white blood cells (WBCs) and differential white blood cell count as represent in the Table1. The results of WBCs count and neutophils percentage obtained in the present study are agree with the reporters of several other researchers Saad, *et al* (1989); Cai, *et al*. (1994); Detilleux *et al*., (1995); Kimurra *et al*., (1999); Klinkon&Zadnik (1999); Kulberg *et al*., (2002); Gerardo *et al.,* (2009); Guidry *et al*., (1976); Kehrli *et al*., (1989); Meglia *et al*., (2001) who all found that the total WBCs count and neutophil s percentage were significantly higher than after calving in compared with pregnant cows. Our result disagree with Mallard *et al*., (1998) and Meglia *et al*., (2005) reported higher WBCs and lower lymphocytes in lactating cows than pregnancy period.

These results are totally opposes to the present study. Nazifi *et al*., (2004)found that the WBCs count in pregnant cows were significantly ($p \leq 0.05$) higher than in early lactating cows in 25-30 days after parturition, while Talvelkar *et al*.,(2006)show that neutrophilia occur on the day of cal ving these results are disagreed with results of the present study.However, Bozdogan & Baysal, (2003) observed a significant increase in lymphocytes during pregnancy in Tuj sheep. Mir *et al*., (2008) also found that no variation in the mean values of esonophils, basophils and monocyte. Balikei & Yildiz, (2005) show no significant decreases for lymphocytes, eosinophils and basophile during and after parturition in sheep. The higher WBCs count was reported in pregnant heifers and the lowest values were observed in parturient cows.

The difference was significant ($p \leq 0.05$), also high significant ($p \leq 0.05$) lymphocytes count was observed in non pregnant heifer compared dry cows, on the other hand non-significant differences were found in moncytes, neutrophils, esonophils and basophils between all studied group (pregnantcows, lactating cows, non pregnant dry cows and non pregnant heifers (Sattar & Mirrza, 2009). Kornmatitsuk *et al*.,(2004) reported a constant numbers of WBC and neutophils up to two weeks before parturition in Holstein dairy heifers. Then an increase of WBCs was found up to the time of parturition and there after the number decreased during the postpartum period. The same pattern was also found for the numbers of nuetrophils.The average numbers of monocyte and eosinophils maintained stable during late pregnancy.

The same results were recorded in heifers in the present study. Sattar & Mirza (2009) reported hi gh significant ($p<0.05$) WBCs count in heifers and low significant values were recorded in dry c ows, also significant increased in lymphocytes was observed in heifers compared with dry cows, on other hands non significant differences were found of monocytes, neutrophils, eosinophils and basophiles between heifers, lactating cows, non pregnant dry cows, pregnant cows most of these values are closely related to the present study. Unlike the present study Subandrio *et al*.,(2000) s howed that the peripheral WBC counts were not influenced by the reproductive state of cows.
The results of the present study of lymphocytes as represented in Table 2 are in agreement with Mallard *et al* (1998); Meglia *et al.* (2005); Klinkon & Zadnik (1999); Mir *et al.*, (2008) who all found low significant mean value of lymphocytes at late lactation. The reduction of lymphocytes around dry period mainly due to reduced lymphocytes proliferation in dairy cows. (Saad, *et al*. 1989).

As represented in the Tables 2 ,the results of the present study are in agreement with Aikhuomobhogbe & Orheruata (2006) in goats;Mir *et al*.,(2008);in cows; Balikei & Yildiz (2005) in dairy sheep who all found that no significant changes for monocytes, eosinophils and basoph iles were observed in precalving and postcalving periods.

The mean Hb values of control groups and experimental groups were recorded in the table 2. The mean value of Hb during pregnancy stages, early, mid, late stages are 9.22±1.18, 9.46±1.39 and 8.70±1.44 respectively. The mean value of Hb in early lactating groups 10.68+2.06 was significa ntly($p<0.05$) differs from the mean values of Hb of both mid lactation groups (8.16±1.06) and late lactating groups(7.78±1.08).The mean values of the Hb of the heifer (control) group (10.78± 1.88) was significantly higher than the mean value of Hb of the three pregnant experimental groups early, mid, and late pregnant group 9.22±1.18, 9.46±1.39 and 8.70±1.44 respectively.

The Hb concentrations presented in Table 1 indicate low significant ($p\leq0.05$)Hb concentrations i n late pregnant cows and heifers and the high significant Hb concentrations were recorded in control cows and heifers, and the Hb concentration in non pregnant heifers were significantly higher than that in non pregnant cows. These results agree with the findings of Mir *et al.*, (2008); Hagawane *et al.*, (2009). Who found that the Hb concentration decreased significantly was a consequences the advance of pregnancy until parturition and first month of lactation.

The decrease in the content of Hb during late pregnancy could be due to the dilution of blood which occurs as consequence of increase of plasma volumes (Singh *et al*., 1991). The results obtained in the present study are comparable with reports of several other researchers Sattar & Mirza(2009); Neelu *et al*., (1996) who found that the Hb concentration was significantly higher ($p\leq0.05$) in heifers compared with pregnant cows, non pregnant dry cows and lactating cows.

The results also agreed with Chandra *et al.*, (2008) who shows that the Hb concentration was highest in the lower age groups and there was a decrease in concentration of Hb values in buffaloes above 3 year age. This may be due to reduction in red bone marrow and decreased erythopoiesis (Sharma *et al*., 1985; Jain, 1993; Feldman, 2000; Khadjeh & Paphan, 2002).The results of the present study also not agreed with Steinhardt *et al*., (1994); Klinkon&Zadnik (1999); Abdellatif *et al*., 2009) who all found that the Hb concentration was significantly higher during last trimester of pregnancy and the highest values were found on the early lactation.

The mean value of MCV of the control groups and experimental groups of present study were recorded in table 2. The mean value of MCV of the decreases significantly when pregnancy advanced and the mean value of MCV of early pregnant cows (53.65±6.68) was significantly higher than the mean value of late pregnant cows, (27.39±3.24). The mean value of MCV of heifers (control) group (57.42±5.05) was higher than the mean values of all experimental groups and in particularly the mean value of MCV of non pregnant dry cows. The results of the present studied are given in the Tables 1 indicates that the mean values of MCV and MCH differ significantly during late pregnancy compared with non pregnant dry cows and early lactation stages these results are in agreement with Opara *et al*., (2006) and Balikei&Yildiz, (2005). The significant increase in MCV values reported in the early pregnant cows and heifers in the present study may be due to a significant decrease in RBC count or as a r esult of Hb deficiency per erythrocyte in the blood.

In the present findings, the mean values of ESR during different physiological state and control groups were recorded in the table1. The mean values of ESR of the three stages of pregnancy early, mid and late pregnancy 10.86±2.72, 10.66±2.52 and 11.45±1.79 respectively recorded. These mean values of ESR of experimental groups were significantly higher than the mean values control groups of heifers and dry cows 6.11±1.39 and 7.19±0.52 respectively.The mean values of ESR of three stages of pregnancy early, mid and late stages 10.68±2.72, 10.66±2.52 and 11.45±1.79 values were significantly higher than the mean values of the three stages of lactation early, mid and late stages 6.70±0.52, 6.49±0.46 and 6.97± 1.78 respectively.

The results of ESR as represented in the Table 1 were accordance with Pampori, (2003); Sattar & Mirza, (2009) who reported high significant values of ESR in pregnant cows compared with the dry non pregnant cows. Similarly ESR values begin to decrease after the third month of the pregnancy and significant increase of ESR witnessed as pregnancy advanced. On the other hand Mir *et al.,* (2008) observed that no significant difference in the ESR values during early, mid and late stage of pregnancy in crossbred cows.

The present finding shows that the mean value of basophils both experimental and control groups show no significantly ($p<0.05$) difference b/n all studied groups. The mean value of neutrophils in both experimental and control groups present study is recorded as shown in table 1. The mean value of neutrophils of late pregnant cows (40.50±2.66) was significantly ($p<0.05$) higher than the mean values of neutrophils of late lactating groups (34.00±3.68). The mean value of monocytes of both experimental and control groups were shown Table 2 in the present findings there were not significant ($p<0.05$) differences were observed in the percentage of monocytes in all studied groups.

The mean values of Lymphocytes were show in table 1. The mean value of Lymphocytes in both early lactation and mid lactation 58.33±4.32 and 58.50±4.50 respectively were significantly ($p<0.05$) higher than other studied groups. The mean value of Lymphocytes in late lactation (48.83±2.93) was significantly ($p<0.05$) lower than other studied groups. The mean value of Eosinophils we shown in Table 1. The present findings shows non significant differences were recorded in eosinophils in between all studied groups. However,unlike present study Bozdogan & Baysal, (2003) observed a significant increase in lymphocytes during pregnancy in Tuj sheep.

Corresponds to our results,Mir *et al*., (2008) also found that no variation in the mean values of esonophils, basophils and monocyte.The same to previous report Balikei & Yildiz, (2005) show no significant decreases for, eosinophils and basophile before and after parturition in sheep. The higher WBCs count was reported in heifers and the lowest values were observed in dry cows.

The difference was non significant ($p>0.05$), also highly significant ($p<0.05$) lymphocytes count was observed in heifer compared dry cows, on the other hand non-significant differences were found in moncytes, neutrophils, esonophils and basophils between all studied group (pregnantcows, lactating cows, dry cows and heifers (Sattar & Mirrza, 2009).This is completely corresponds with our results.

The high significant AST concentrations were recorded in the lactating cows and non pregnant dry cows than other studied groups while the low significant concentration were recorded in pregnant groups. These results are accordance with the findings of El-Ghous, *et al*. (2000) who reported that AST showed high activity in the first week of pregnancy compared with late pregnancy. The results in the lactating cows and dry cows in the present study were accordance with the Broniki & Demhinski (1994) which observed increased activity of AST before parturition Khan *et al*., (2002) determined that AST activities was non significant differences at the prepartum and postpartum but ALT was significantly higher at prepartum stage than the postpartum stage which not agreed with the results of the present study, while the same researcher recorded a high AST and ALT values in the pregnant animals than non pregnant anim als which are disagreement with the findings of the present study.

The activities of AST and ALT in the blood are associated with implantation, embryo survival, growth, uterine carbohydrate metabolism amino acid metabolism and glycogen deposition (Milinkovic-Tur *et al.,* 2005). Consistent with our findings, increasing pattern of serum AST and ALT were also found after parturition by (Jacob *et al.,* 2001). The decreased in AST and ALT values at postpartum recoded by (Sevinc *et al.,* 1999) and an increased in AST and ALT levels obtained during pregnancy by (Jovanovic *et al*., 1997) and the findings are dis agreement with the present results. However, results of the present study are not agreement with the findings of Khan *et al*.,(2002) and Yokus *et al*.,(2006) who reported that serum enzymes levels do not chang ed during pregnancy and postpartum.

The results of serum ALP concentration are presented in Table 7. The ALP concentrations found in blood serum of lactating cows are disagreement with those previously reported by Doornenbal *et al.,* (1988) who recorded that after parturition ALT levels dropped during lactation and increased again toward weaning. Similar observations were recorded during milking period by (Masek *et al.*, 2007; Sato, *et al.*, 2005). The high significant values of serum ALP concentration observed in first month lactating cows compared with dry cows in the present study accordance with Roussel (1982) who found a negative relationship between ALP activity and age. The serum ALP originates mainly from the liver, however, in growing animals much of ALP originat es from bone tissues.Therefore the higher serum levels in young animals are indicative of rapid skeletal growth (Doornenbal, *et al.,* 1988). The higher levels of serum ALP in lactation periods than in dry periods and it was explained by increased bone specific ALP and liver ALP activities lactation period and by the suggestion that ALP originating from mammary gland could influence serum ALP values (Sato *et al.,* 2005).High level of ALP reported in mid and late lactat ion compared with pregnancy period (Khan *et al.,* 2002;Yokus&Cakir,2006).

The mean values of GGT in non pregnant dry cows 41.53±9.66 was significantly higher than mean values of pregnancy stages and mean values of heifers (control) 27.93±6.66. The present result is the range of normal animal value correspondence with report of Malikides *et al.,(*2001) If your lab report indicates significantly increased levels a follow up examination by your regular veterinarian is strongly recommended. This is a very accurate enzyme which to measure liver tissue, and has a normal reading between 20-60 in the enzyme is sensitive to mild liver damage and levels increase from 60-150 often after intake of drugs or some nutrient changes. A bleeding reduction supplement and summer weeds will both push GGT over 100 and if acute liver disease is present, GGT reading will rise even more than this but these cause (which include biliary obstruction, cancer, liver fluke, bacteria (viral hepatitis) are those reflected in severe liver disease and are reasonably rate in all animals. Gamma Gluatamyi tranferase increase in longer term liver damage, it is particularly useful in ruminants (Malikides *et al.,* 2001)

The mean value of urea significantly decreases towards the end of lactation. The mean value of urea in early lactation 32.05±5.73 was significantly higher than both in mid lactation 23.65±7.89 and late lactation 15.8±6.58.The mean value of urea in early pregnancy stage 32.31±7.77 was significantly ($p<0.05$) higher than the mean values of mid pregnant groups, late pregnant group, heifers (control) and non pregnancy dry cows (control) 26.35±4.39,23.19±3.55, 25.33±4.78 and 24.08±6.15 respectively. The results of serum urea concentrations are given in the Table 7.Urea shows a significant ($p\leq0.05$) decreases with advance pregnancy reached high significant values in early pregnant cows and early lactating cows compared with control and other studied groups and the low significant urea concentration were recorded in late lactating and late pregnant cows and heifers. These results are incomparable to that reported by Piccione *et al*., (2009) in ewes who found that the serum urea concentrations were significantly increased during late gestation which may occurs due the high thyroid activity in pregnant females which induce an increased protein metabolism.

Unlike to the results of the present study the serum urea concentration significantly lower during postpartum compared with prepartum in normal parturition dairy cows (Yokus *et al*., 2007). The values recorded in the first month pregnant cows and lactating cows were significantly high ($p\leq0.05$) when compared with the control non-pregnant groups, which corresponded to the finding of Hagawane *et al.*, (2009) in buffaloes found that the mean urea values in early lactation was significantly higher as compared with the normal healthy control. Unlike to the present study El-Sharif & Asaad (2001) reported that the plasma urea concentration start to increasing in pregnancy ewes from 10th weeks of pregnancy reaching a maximum level at parturition. The high significant urea concentration in non- pregnant control multiparous cows compared with control (non-pregnant) heifers obtained in the present study incoordinated with Doornenbal *et al*., (1988) who concluded that the urea serum concentration increased significantly with age.

The increased of urea concentration in the present study during early stage of pregnancy may be results due to the increase in the total volume of blood which induces an increase in glomerular filtration, which also responsible for the increase volume of albumin and urea during early gestation (Yokus *et al.*, 2006).The other reason for those highest serum urea values could be the increased cortisol level affecting the catabolism of protein in the body (Silanikove,2000). It is possible that the protein catabolism and high demand for energy by the pregnancy during the gestation associated with increase in the formation of embryo tissue led to an increase in urea level (Rodriguez *et al.*,1996).

The mean values of creatinine in lactation stages increases towards the end of lactation. Peterson &Waldern, (1981) reported that the mean value of creatinine concentration increased significantly with advanced pregnancies which are close to the values reported in the present study. A significant decrease in creatinine concentration observed in first month of lactation in the present study are agreement with Piccione *et al*., (2009) who found that the protein mobilization start during gestation reaching its peak during late gestation, during which the lower creatinine values was observed and after calving protein mobilization probably is reduced and creatinine values reached its peak during dry period. The creatinine concentration and muscle diameter decreased through the period from 1 week before calving to 4 week after calving in dairy cows (Blum *et al.*, 1983).

Similar to the value obtained in parturient cows of the present study, Yokus *et al*., (2007) demon strated that serum creatinine concentration was higher in postpartum dairy cows compared with p repartum period. The lower creatinine concentrations in the late gestation are important indicators of the higher glomerular filtration rate that occurs due to increased total blood volume (Fischbach, 2000). The results of serum creatinine concentration obtained in first month lactating cows are comparable with Gurgoze *et al*., (2009) who reported that the low significant serum creatinine concentration on day 14 postpartum than on days 21 and 120 of pregnancy in ewes. Also the high significant serum creatinine concentration observed in the present study in control and non lactating dry cows compared with other groups are in agreement with Kuha (2009) who reported that creatinine concentration were higher of dry cattle than the early lactating and pregnant one. Unlike the present study no significant differences were found in the creatinine levels with reproductive status in cattle (Yokus& Cakir, 2006).

The results of serum glucose concentration in this study which indicated to the gradual significant decrease in the serum concentration of glucose with advanced pregnancy with low significant values were recorded in the late pregnant cows and and the low significant values were recorded in the early lactating groups compared with other studied groups. These results are in agreement with Abdellatif *et al.,* (2009) in ewes who found a relative decrease in plasma glucose level during the advanced stages of pregnancy which indicates that the size of the fetus induces change on maternal carbohydrate metabolism.

Similarly Basoglu *et al.,* (1998) observed that there was a significant higher level of glucose in periparturient cows than in early and late lactation. The decrease in glucose level during the advanced stage of pregnancy may be attributed to the reduction in insulin secretion which minimizes peripheral glucose utilization and maximizes glucose extraction of the uterus,(Connolly *et al*.,2004). The results of serum glucose concentration in early lactating groups of the present study accordant to that reported by Onita & Colibar *et al*., (2009) who found that hypoglycemia occurs in the first 21 days postpartum, it's possible that cows with milk production have an energy and protein-deficiency especially in the first day of lactation. In this condition, metabolism is based on mobilization and usage of the lipid and usage of glucose in other organ than mammary gland is low.

The hypoglycemia after parturition was attributed to heavy drain of glucose for lactose synthesis (Nale, 2003). The results of the present study of serum glucose concentration also corresponded to the finding of Mujalli, (2008) who reported that the values of glucose in cows sampled 1 week before calving were significantly lower than the dry cows and two groups of cows sampled at 1 week and at 4 week after calving. The decrease in glucose level in late pregnancy could be associated with increase in the rate of secretion of glucocorticoides by adrenal cortex and stimulation of glucogenolysis in the liver (Swenson, 1993). Similar results were presented by Radostits *et al.*, (2000) and Djokovic *et al.*, (2007) who also observed low significant values of glucose concentration in the blood of puerperal healthy cows compared with the groups of late pr egnancy.

The mean TP concentration in the present work are in agreement with Mir *et al.,* (2008) who all found that serum TP increased in late pregnancy compared with other stage of pregnancy and early lactation. The observed TP concentration during first month of lactation in the present study confirms the findings of Yokus *et al.,* (2007) in dairy cows who found that serum total protein was significantly higher in postpartum compared with peripartum period. The results of the TP are comparable to the values obtained by Mir *et al*., (2008) who found a significant ($p \leq 0.05$) increase in the concentration of TP with advanced pregnancy reached higher values at p arturition.

Unlike the results of the present study, Yildiz *et al.,* (2005) showed that TP was not affected in the gestating period. The decreased serum TP was observed at parturition in sheep (Vihan &Rai, 1983), similarly as recorded in the present study. These changes in serum TP might represent an adaptive response to the higher need of water mobilization by blood to mammary glands for milk production. The significant reduction of the serum TP at parturition suggest to be occurred due to rapid leave of immunoglobins from the plasma during the last month of gestation when colostrums is being found in the mammary gland (Kaneko & Cornelius, 1980).On the other hand the significant depression in the total protein concentration in the present study may be ascribed to the fact that the fetus synthesis all its proteins from the amino acids derived from the mother, and maximum levels, especially in muscles, during late pregnancy (Jainudee & Hafez, 1994; Yokus&Cakir, 2006). The high levels of TP in the late trimester of pregnancy confirm the fact that high level of protein are needed for optimum secretion of ganadotropin release factors and n umber of other hormones needed in the culmination of the pregnancy (Mir *et al.*,2008).

The mean total concentrations of serum electrolyte parameters of cows in their stages of pregnancy and of lactation obtained in this study were shown on table 7.These values were generally within the normal range reported for cross Holistein Fresien cows (Piccione *et al*.,2010) except for chloride and phosphorus whose mean values were slightly below the lowest values of the range. This study showed a significant differences in the mean serum electrolyte values of pregnant and lactating cows in between all groups of study were due to irregular and imbalanced supplement of minerals in deit. This result agrees with the results of other workers (FAO,1994) for serum inorganic phosphorus, sodium and chloride that these electrolyte values remain significant difference in between individual cows and groups, but in contrast with others (Kaneko *et al.*,2008) who reported no differences for prepartum and postpartum calcium, magnesium and potassium levels respectively.

Serum calcium has been reported to show a tendency to decrease shortly after calving during mid lactation while plasma potassium levels decrease in late prepartum and increase in early postpartum,corresponds with report Ahmad et al.,(2005). There was however differences in the prepartum and postpartum levels of some of the electrolytes, thus indicating the role of herd as a factor affecting electrolyte values (Zumbo *et al*.,2007). The low levels of chloride and phosphorus in early lactation could not be attributed to a particular factor. Studies by Ramin *et al* (2007)and Ling *et al*.,(2003) did not show herd differences in chloride levels.However,incorrect f eeding regime has been reported to be associated with low levels of chloride and phosphorus (Gerardo *et al*.,2009).

The low serum levels of sodium, potassium, chloride, phosphorus in some groups our experimental cows may be associated with the occasional supply of salt licks to the animals in addition to the possibility of inadequate levels of these parameters in the feed. Low blood phosphorus levels are commonly found in grazing cattle (Jacob *et al.*,2001), and therefore dietary supplement may be essential.

Cows suffered with the low level of phosphorus suffered a musculoskeletal problem (inability to stand on the legs) which could be attributed to this phosphorus deficiency. The serum electrolyte values obtained for cows in this study were not associated with clinical disease. Therefore, these values may represent baseline data considered compatible with normal health during pregnancy and lactation.

The serum concentrations of calcium and phosphorus of the present study are presented in Table 7. The results of the present study are in agreement with the finding of Meglia *et al.,* (2001) who reported that the blood levels of Ca & P is expected to decreased after calving due to large demand of colostrums and milk production in agreement with Hagawane *et al.,* (2009). The serum Ca and P concentration were low significantly during late of lactation compared with healthy group, the depression in Ca level during stage of lactation could be result of the impaired absorption of food metabolites from the gastrointestinal precursor, excessive losses through urine, colostrums as it was much more drained in the colostrums during excessive milking and due to insufficient mobilization from the skeleton. The depression in the levels of P might be due to necessary of it for colostrums synthesis (Rook & Thomas, 1983).

Unlike the results of the present study Kadzere *et al.,* (1997) determined that the Ca concentratio n in plasma increased as gestation progressed in goat. The same researcher observed that the Ca concentration decreased after Kidding which is resemble the results of the present study in parturient and first month lactating groups. In contrast of the results of the present study, Tanritanir *et al.,* (2009) found that no statistical differences between before and after parturition in Ca levels in blood serum of goat. Similarly the present findings of Ca concentration Abdelrahman *et al.,* (2002); Krajnickora *et al.,* (2003) demonstrated that Ca levels were lower at postpartum that gestational period. The present results also accordance with Gurogoze *et al.,* (2009) who showed that level of Ca were below reference range during advanced pregnancy and within reference range at lactation period, while P levels were below reference range at other period.

Unlike these results Kaushik & Bugalia (1999) reported that no significant differences were observed at the P levels at different stages of growth reproduction, pregnancy and lactation in goat. While Tanritanir *et al., (*2009) informed that P levels during late gestation and postpartum significantly increased in ewes and goat. In contrast the results of P concentration obtained in the present study, Jainudee & Hafez, (1994) showed that the P levels were fluctuated in gestation periods and lactation in cattle.

The P levels were below the reference range on day 120 gestation and within the reference range at other periods Kaushik & Bugalia (1999) reported that no significant differences were observed at the P levels at the different stages of growth, reproduction, pregnancy and lactation in goat, while Ozyurtlu *et al.*,(2007); Tanritanir *et al.,* (2009) found that P levels during the late gestation and postpartum significantly increased in ewes and goats. Masek *et al*., (2007) reported that the concentration of Ca varied significantly during the milking period with the highest value in the late and the lowest in the mid milking period. However, they were always within the reference va lues while P values were significantly higher in the early milking periods with respect to the mid or late milking periods. Alacam *et al*.,(2008) show that serum Ca values were significantly ($p<0.05$) lowered at day 20 postpartum and were significantly higher on days 30, 60 and 90 postpartu m while P values were significantly higher ($p<0.05$) at 90 days postpartum compared with 0, 20, postpartum in dairy cows.

The interval value of RBC, PCV, MCH, WBC, Hb, MCHC, ESR, Basophils, neutrophils and eosinophils reported by RAR (2009) were higher than the interval value of present study of heifers and the interval ranges between the present study of heifers were narrower than the RAR(2009).The highest value of all parameters of intervals of RAR is higher than the highest value of the intervals of heifers of present study except MCV, Monocytes, lymphocytes and eosinophils. The lowest values of all RAR were lower than all lowest value of reference interval of present study of heifers and the ranges of RAR were wider than the present study of heifers. When we come to non pregnant dry cows, the highest value of all intervals of RAR were higher than the highest value of interval value of non pregnant dry cows except monocytes and eosinophils. The same to heifers the interval ranges between the interval values of RAR were higher than the interval ranges of interval values of non pregnant dry cows (Table 3) and the highest and lowest value of non pregnant dry cows in comparison with (RAR,2009).

The interval value of PCV of Merck manual were slightly the same to the interval value of present study of early pregnant cows even if their highest and lowest values differs from each other and the interval ranges between the MCH present study of early pregnant cows were completely different from Merck manual. The highest value of some parameters of intervals of Merck manual is higher than the highest value of the intervals of early pregnant cow of present study except MCV, PCV,MCH,ESR, lymphocytes and neutrophils. The lowest values of some Merck manual were lower than some lowest value of reference interval of present study of early pregnant cows and the ranges of Merch manual were some extent wider than the present study of early pregnant cows(table 4) . When we come to mid pregnant cows, the highest value of all intervals of Merck manual were higher than the highest value of interval value of mid pregnant cows except monocytes,ESR,MCH and eosinophils Table 4. The same to above table 3 the interval ranges between the interval values of Merck manual were higher than the interval ranges of interval values of mid pregnant cows(Table 4) and the highest and lowest value of mid pregnant cows in comparison with Merck manual were clearly identified different Physiological states for the assessment of animal welfare and adaptability to environment in on table 4 below.

The reference interval value of MCH of present study of late pregnant cows were slightly the same to interval value of Jean (1993) and the wider range in between interval values of Jean(1993) were observed on table 5.The highest values of reference interval of Jean (1993) were almost higher than the highest value the present study of late pregnant cows. When we come to the reference interval values of early lactating cows, the highest values of almost all interval values of Jean(1993) were higher than the highest value of present study of early lactating cows. The wider ranges were seen in between reference values of neutrophils and monocytes of Jean(1993).

The reference values for Hb, PCV and RBC for mid lactating cows(Table 6) were lower than those for temperate breeds reported by ADVIA and the reference interval of ADVIA was wider than the present result and the highest value was higher than all the highest value of our present findings except the highest values of basophilis, monocytes and eosinophils. The lowest value of PCV,WBC, MCV,ESR, Basophils, monocytes, lymphocytes and eosinophils were higher than the lowest value of temperate breeds reported by ADVIA.

When we look at highest and lowest value of late lactating cows the highest value of RBC,PCV, MCH,Hb,MCV,MCHC of ADVIA were higher than the highest value of reference interval of late lactating cows. Almost all references ranges reported by ADVIA were wider than the reference range of late lactating cows.This may be expected as the cows in the present study were all lactating and lower Hb and PCV values have been reported for lactating animal in different Physiological states for the assessment of animal welfare and health by interpreting the following data (table 6 below).

The reference range of AST reported by Blood et al was 28-50 for late pregnant cows were wider than reference range the present results. When we look at the upper limit and lower limits of reference ranges of results of present study in 95% confidence intervals, the values are reference values of serum biochemical profiles of late pregnant cows. The highest and lowest values in this reference range were clearly observed on the table 9.When we look at early lactating cows, the reference ranges reported by Radiostits *et al*., 2006 were wider than the reference ranges of results of present study of early lactating cows. The reference values of late lactating cows with their upper and lower limits in 95% confidense interval was clearly observed on table 10.

Almost all the highest values of reference value of Merck manual were higher than reference value of mid lactating cows and wider reference range were observed in reference values of all reports by Merck manual. The lowest value of reference range of mid lactating groups compared with reports by Merck manual were shown on table 11.When we look at the reference values of late lactating cows, the reference range of ALT reported by Merck manual and reference value ALT of late lactating cows were almost the same and the reference range of ALP reported by Merck manual was wider than reference value of late lactating cows. The highest and lowest values of reference ranges shown on the table 11.

The reference values for all parameters of all study groups of present study (table 4-11) on both hematological and serum biochemical parameters were lower than those for temperate breeds reported by different researchers on Merch (2012);RAR(2009);Jean(1993);Radiostatis et al.,2006);Schalm (1975);Duck (1970);Sastry(1986);Stockham and Scot(2002)and ADVIA the reference interval of all parameters reported by different researchers were wider than the present result and the highest value was higher than all the highest value of our present findings except t he highest values of some parameters due to different internal and external factors.

This corresponds to the report of CLSI, 2008 says that Textbook reference values reported by European or United States Veterinary Laboratories are often based on animals living under good husbandry conditions in temperate climates. However, those reference sample groups may differ from those of the developing countries. Differences may be attributed to the environmental temperature, the type and quantity of the ration and t he management system**.** Published data propose erratic normal values that are often obtained from a relatively small number of animals, which kept under different nutritional and climatic conditions, it makes difficult to depend on such published data to interpret results for Holistien Fresien live in tropical countries (IFCC,1979,1987a and 1987b).

In this study, we determined the reference intervals of key hematological and biochemical variables in the blood of 120 Cross breed Holistien Fresien cows and heifers during lactation, pregnancy and dry period. The results are shown in Table 4-11. Reference intervals were determined as recommended by the CLSI (2008) document and IFCC (1979). Selection of females as the reference population places a limit on the use of the obtained reference intervals. Care should be taken when extrapolating the results to males, as they were not included in the reference population. Along the same lines, seasonal changes might affect the analyte values (Krajnicakova *et al.,* 1997; Tibbo *et al*., 2004), which means that the estimated reference intervals are most reliable for the season when the reference population was sampled. Other authors suggest that hematological variables in sheep may be affected by various internal and external factors such as age (Alonso *et al.*, 1997; Tibbo *et al*., 2004)

Despite the many influences on the values of hematological variables in the blood of cow, in most cases the values are within relatively narrow limits which usually correspond to the wide reference ranges published in the relevant textbooks (Feldman *et al*., 2000; Kaneko *et al*., 2008; Pugh and Baird, 2012). The results of this research are in agreement with our findings if we compare only the mean value obtained by descriptive statistics. However, considering that they were in their research they engaged in the study of various effects on hematological variables in the blood of cow, rather than establishing reference intervals, the results cannot be directly compared with the reference intervals obtained in our study.

The hematological reference intervals of cross breed Holistien Fresien cows can be compared with those reported by Šimpraga *et al.* (2013), who determined the reference blood intervals for another temperate breed .For comparison, the reference intervals are shown in Table 4-11. The same to our present results they can also be compared with the results of Lepherd *et al*., (2009) who published reference blood intervals for Holistien cross breed in other books , using new, more rigorous (sample selection, sample collection techniques, statistical method selection) rules and methods for reference interval determination. More differences can be seen if our data is compared with the reference intervals from relevant textbooks (Kaneko *et al*., 2008; Pugh and Baird, 2012).

6. CONCLUSIONS AND RECOMMENDATIONS

The hematological and serum biochemical profiles of cross bred Holistien Fresien cows were influenced by different stages of pregnancy and lactation.Pregnancy and lactation had significant effects on parameters of hematology and serum biochemistry Holistien Fresien cows in bishoftu. The changes in biochemical and haematological constituents are important indicators of the physiological or pathological state of the animal. During the dairy cow production cycle, the transition period is critical due to the tumultuous endocrine and metabolic changes that accompany parturition and the initiation of lactation.During occurance of this physiological changes there were significant differences in number of WBC,RBC,Lymphocytes, PCV,ESR,Hb and red cell indices during lactation and pregnancy of cross bred Holistein Fresien cows.During these physiological stages there were also significant fluctuation the amount of Liver enzymes,urea,total protein,glucose,total bilirubin,creatinine and serum enzymes from stages to stage on this values of serum biochemistry of cross bred Holistien Fresien cows.The significant flactution of the values of these parameters in between one stages to another stages during this period;this shows that this period is very sensitive and open for the onset of different metabolic dis orders and other diseases in the live of cross bred Holistien dairy cows.Even if this changes occurs during and in the way of normal physiological conditions,identification of normal change from abnormal change needs special attention on this parameters during transition period of live of dairy cows.Inorder to identify normal change from abnormal and assess the health and welfare of this animals prior to the onset of any clinical symptoms during and inbetween this transition period establishing normal reference values for all hematological and serum biochemical parameters were used for identification of normal changes from abnormal and interpretation of the causes of normal changes and abnormal in different physiological stages during transion period of dairy cows. The diagnosis of diseases is mainly dependent upon deviations from the normal range of physiological values. However, in the absence of established reference values for any blood parameters, one can be exposed to misinterpretations in diagnosing and treating different disease conditions. This again entails standardizing blood serum biochemistry profiles to once environmental conditions for use and application of research and educational practices.

Based on the above conclusions the following recommendations are forwarded:

- There should be determination of normal values of hematological and blood biochemical value are important for the clinical interpretation of laboratory data during transition period.
- should be known for the haematological values during different physiological situations for the diagnosis of various pathological and metabolic disorders .
- should be understand how normal values turn into abnormal values due to many factors, such as management, feeding and illness on this animals identify the basic causes of abnormal values.
- Should have strength the trend of laboratory analysis of blood of cows during transition period to establish reference ranges for each physiological stages during lactation and pregnancy to interprate laboratory data.
- Should be important to perform clinical and paraclinical exams in order to guarantee sanitary strategies control, prevention or treatment of diseases and to assure good management practices.
- Any stress causing factors should be avoided and evaluate of the management practice, nutrition and diagnosis of health condition as well as in determining the physiological status of animals
- Early detection of nutrient deficiency or metabolic disease can contribute to successful diagnosis and faster recovery of animal.
- health and welfare should be assessed by continous and regular examination of hematological and serum biochemical parameters of cows those which are found in different physiological conditions.
- Further research and study should be done on the effects lactation and pregnancy on hematological and serum biochemistry of highly productive cross breed Holstein cows to establish reference value for each stage of transition period.

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8. ANNEXES

Annex 1. Principles and procedures of biochemical tests

Specimen collection:

3ml of blood was collected from the individual's using a sterile vacutainer plain tube. Shaking was avoided and the blood was standing for 15-20 minutes at room temperature to allow for clot formation. Serum separated from the clot by centrifugation at 3000rpm for 5-10 minutes and transfers to tubes packed and transported on cold chain and stored at -20^{o}c prior to analysis.

Procedure for instrument set up:

Set the program on the instrument, type the individual's code no. glucose, total cholesterol total protein, albumin, AST, ALT, and ALP tests are selected from test menu. After calibration adequate controls and serum was placed in sample cup by appropriate order and enough working reagent bottles were added. The instrument by itself pipettes programmed sample volume and working reagent and after incubation, the formed color absorbance read at appropriate wavelength and the results were displayed on screen.

Glucose determination

Principle: glucose is oxidized by glucose oxidase (GOD) to produce gluconate and hydrogen peroxide (H_2O_2) H_2O_2 is the oxidatively coupled with 4 amino antipyrene (4-AA) and phenol in the presence of peroxidase (POD) to yield a red quinoeimine dye that is measured at wavelength of 546 nm proportional to concentration of glucose in the sample.

Alanineamino Transferase

Principle:

Alanine amino transferase catalyzes (ALT/GPT) catalyzes the transfer of the amino group from Alanine to oxoglutarate with the formation of glutamate and pyruvate. Pyruvate is the reduced to lactate by lactate dehydrogenase (LDH) in the presence of reduces nicotinamide adenine dineuclotide (NADH). The reaction is monitored kinetically at 340 nm by he rate of the decrease

in absorbance resulting from the oxidation of NADH to NAD, proportional to the activity of ALT present in the sample.

Aspartate AminoTransferase

Principle:

Aspartate amino transferase (AST/GOT) catalyzes the transfer of the amino group from aspartate to oxglutarate with the formation glutamate and oxalacetate. The latter is reduced to malate by malate dehydrogenase (MDH) in the presence of reduced nicotinamide adenine dinucleotide (NADH). The reaction is monitored kinetically at 340 nm by the tare of decrease in absorbance resulting from the oxidation of NADH to NAD^+, proportional to the activity of AST present in the sample.

Alkaline phosphatase

Principle:

Alkaline phosphatase catalyzes the hydrolysis of 4-nitopheniylphosphate (4-NPP) with the formation of free 4- nitrophenol and in organic phosphate, acting as the alanine buffer as a phosphafe gtoup acceptor. The reaction is monitored kinetically at 405 nm by the rate of the formation of 4-nitrophenol, proportional to the activity of ALP present in the sample.

Total protein

Principle:

Peptide bonds of protein react with cu ions in alkaline medium to form colored complex (heated) whose color intensity is directly proportional to the total protein concentration and measured at 540nm.

Annex 2. The packed cell volume determination

Materials:

- Un-coagulated whole blood in EDTAQ coated vacutainer tubes
- Microhematocrit capillary tubes

- Sealing clay
- Gauze
- Microhematocrit centrifuge
- Microhematocrit reader

Procedure:

- Capillary tubes are fied approximately ¾ of the tube with the well- mixed blood sample.
- The top of the capillary tube is occluded with index finger to prevent spillage of the blood and excess blood is wiped is wiped carefully outside the tube by using cotton.
- The vacant end of the tube is sealed with sealing clay.
- The capillary tubes are placed in the microhematocrit centrifuge with the sealed end toward the periphery. duplicate tubes are placed opposite each other for balance.
- It is centrifuged for five minutes at full speed of 12,000pm.
- Using Hawksley microhematocrit reader the reading is made in percent.
    - The bottom sealed end of the capillary tube is put at 0% and the top plasma end is adjusted at 100% mark. Then the PCV value is read at the junction space between the buffy layer and the packed RBC's. results are recorded to the nearest whole number.

Annexes 3. Hemoglobin concentration determination

Materials and reagents:

- Un-coagulated whole blood in EDTA coated vacutainer tubes
- Micropipette with disposable tips
- Graduated hemoglobin meter tube
- Hemoglobin meter (color matching chamber).
- Glass rod
- Dropper
- 1% HCI
- Distilled water

- Piece of gauze
- Hemoglobin meter

Procedure:

- The graduated tube of the hemoglobin meter is filled to 20 mark with 1% HCI.
- The micropipette tip was attached to the eppendorf micropipette and 20 ul of blood was measured and the blood was wiped from outside of the tip with a piece of Gauze.
- The blood was added into the graduated tube.
- One drop of distilled water is added to the graduated tube and missed with glass stirring rop.
- The above process of adding one drop of distilled water and stirring is continued until the color of the solution in the graduated tube matches with the color of the glass standard on the hemoglobin meter.
- The hemoglobin is read in g/dl.

Annex 4. Total red blood cell (RBC) count

Materials and reagents:

- Un-coagulated whole blood in EDTA coated vacutainer tubes
- Micropipette calibrated to dispense 1 to 1000 ul with disposable tips vial capable of holding 2ml fluid
- Hemocyto meter (improved neubauer Chamber)
- A poece of gauze
- RBC diluting fluids (hayem's solution)
- Light microscope
- Cover slip.

Procedure:

- About 995 ul of diluting fluid is measured with a micro pipette into a vial.

- 5 ul of blood is measured; excess blood outside the micro pipette tip is wiped carefully by using gauze and then the blood is added in to a diluting fluid that gives a 1:200 dilution. The solution is the mixed slowly by shaking for 2-3 minute.
- Some of the solution is taken by using a micropipette. Holding the pipette slightly inclined, small volume of the fluid was introduced under the cover slip which is placed on the counting chamber. The fluid the space by capillarity.
- The cells are allowed to settle for 2 to 3 minutes.
- The counting chamber is placed on the stage of the microscope.
- Low power (10 x) objective is switched. Light is adjusted and the large scales in the center with 25 small squares are located.
- High power (40 x) objective is switched.
- The red blood cells in the four corner squares and in the center square are counted.
- The total number of red blood cells per cubic mm (ul) is calculated by multiplying the number of red cells counted by 10,000 (correction factor).
- The following formula is used for the calculation of red blood cells:

  Total red blood cells/cubic mm

  = [number of red cells counted x dilution]/[area counted x depth of fluid]

  Where

  Dilution = 1:200 (i.e. 200)

  Area counted =80/400=1/5 sq.mm

  Since cells will be counted in 5 bigger squares and such square is further divided into 16 small squares.

  Each small square = 1/400 sq.mm

  Hence area of (5*16) =80 such areas = 80/400 sq.mm = 1/5 sq. mm

  Depth of fluid = 1/10mm

  Number of red cells counted = N

Hence total red blood cells/cubic mm = [(N*200)]/[(1/5)*(1/10)] =N*200*50 =N*10,000

Annex 5. Determination of erythrocyte indices

Erythrocyte indices are calculated from the above hematological profiles. Each of the indices is calculated as follows.

Mean corpuscular volume (MCV) is determined indirectly by dividing the PCV by the total erythrocyte count (in millions per micro liter) and multiplying by 10.

$$MCV = \frac{PCV\ (\%)\ X\ 10}{RBC\ count}$$

Mean cell hemoglobin is calculated by dividing the hemoglobin value (in gram per deciliter of blood by the erythrocyte count (in millions per micro liter) and multiplying by 10.MCH= Hg con. X 10

RBC count in millions per micro liter