UNIVERSITY OF EDUCATION, WINNEBA

HEALTH MANAGEMENT, REPRODUCTIVE HORMONES AND PRODUCTIVE PERFORMANCE OF DAIRY CATLE IN HOT-HUMID AND COASTAL ENVIRONMENTS, GHANA

ISMAIL COFFIE

DOCTOR OF PHILOSOPHY

2020

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

FEBRUARY, 2020

DECLARATION

STUDENT'S DECLARATION

I, Ismail Coffie, declare that this thesis, with the exception of quotations and references contained in published works which have all been identified and duly acknowledged, is entirely my own original work and it has not been submitted, either in part or whole, for another degree elsewhere.

SIGNATURE ……

DATE ……..

SUPERVISORS' DECLARATION

We, hereby declare that the preparation and presentation of this work was supervised in accordance with the guidance for supervision of thesis laid down by the Unversity of Education, Winneba.

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DEDICATION

This work is wholeheartedly dedicated my beloved mother, Akua Rabi and to my late father, Kofi Baah Yakubu Coffie.

ACKNOWLEDGEMENT

I am most thankful and grateful to my Principal supervisor, Prof. J. K. Kagya-Agyemang and co-supervisors, Prof. S. Y. Annor and Sir Prof. Anthony Annan-Prah (KSG) for their insightful and inspirational guidance, counselling and capacity building.

My passionate appreciation goes to the Department of Animal Science Education of the University of Education, Winneba, for providing facilities for the study, and to EDULINK II project for supporting the author in Summer Schools, capacity building and conferences in Kenya.

My heart felt appreciation goes to Dr. Zachariah Coffie and the entire Coffie family for the financial support. Many thanks go to my sweet mother, Akua Rabbi, for her wonderful prayers and love. My passionate acknowledgement goes to my wonderful wife, Mariam Amponsah, and to my sisters, especially, Zinabu Coffie, Rose Coffie, Mary Coffie, and Nancy Coffie for their kindness, support and prayers. Nathaniel Arthur, I am grateful to you for your support in typing and data management.

I am so grateful to Hudu Seidu, Thomas Quaye, Larry Tay and all staff of the Animal Farm of the University of Education, Winneba for their spectacular assistance during data collection. Amrahia Farms (Greater Accra Region), Hallelujah Farm (Greater Accra Region), Nartey Farm (Greater Accra Region), Embik Farms (Ashanti Region), Zaare Farms (Ashanti Region), Karima Farms (Ashanti Region), Penteng Komfuo Farms

(Ashanti Region), Sparkx Farms (Ashanti Region), and all others whose names are not mentioned here, you are highly acknowledged for providing facilities for this study.

All authers whose works have been cited are highly acknowledged.

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LIST OF ABBREVIATIONS AND ACRONYMS

ALP	Alkaline Phosphatase
ALT	Alanine aminotransferase
AST	Aspatate aminotransferase
AvMY	Average milk yield
BCS	Body Condition Score
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CC	Cycling Cows
CFU	Colony forming Unit
CL	Corpus Luteum
cm	centimeters
CRT	Creatinine
DBIL	Direct Bilirubin
DHIA	Dairy Herd Improvement Association
DNA	Deoxyribonucleic Acid
E_2	Oestediol
EDTA	Ethylene Diamine Tetraacetic acid
EIA	enzyme immunoassay
ELISA	Enzyme-Linked Immunosorbent Assay
EU	European Union
EVF	Erythrocyte Volume Fraction
FAO	Food and Agricultural Organization
FBC	Full blood count
fl	Fentolitre
FSH	Follicle Stimulating Hormone
G_E	Early Gestation/1st Trimester
γGT/GGT	γ-glutamyl transferase (Gamma glutamyl transferases)
G_L	Late Gestation/3rd Trimester
GLM	Generalized Linear Mode
GLOB	Globulin
GLU	Glucose
G_M	Mid Gestation/2nd Trimester
GnRH	Gonadotrophin Releasing Hormones
GOT	Glutamic oxaloacetic transaminase
GP	Gestation period
GSH	Ghana Shorthorn
GSS	Ghana Statistical Service
GTH	Gonadotrophic hormones
H&E	haematoxylin and eosin

HCT	Haematocrit
HDL	High Density Lipoprotein
HGB	Haemoglobin
HRP	Horseradish Peroxidase
HTC	Haematocrit
IBIL	Indirect Bilirubin
ILRI	International Livestock Research Institute
ITM/C	Integrated tick management/control
KFT	Kidney Function Test
KPA	Key performance areas
KPI	Key performance indicators
LB	Luria Bertani
LBSP	Level of biosecurity practices
LDL	Low Density lipoprotein
LFT	Liver Function Test
LH	Luteinizing Hormone
LL	Lactation Length
LS	Least Squares
MC	Muzzle Circumference
MCH	Mean corpuscular haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
MCV	Mean Corpuscular Volume
mL	milliliter
MoFA	Ministry of Food and Agriculture
MPV	Mean Platelet Volume
MSCC	Milk Somatic Cell Count
MSPs	Major Surface Proteins
NEB	Negative Energy Balance
NEFA	Non-esterified fatty acids
ng	Nanogram
OD	Optical Density
OIE	Office International des Épizooties/World Organization for Animal Health
P_4	Progesterone
PCR	Polymerase Chain Reaction
PCT	Plateletcrit
PCV	Packed Cell Volume
PDW	Platelet Distribution Width
pg	Picogram
pH	Potential of Hydrogen
PLT	Platelets
PM	Postmortem
PPA	Postpartum Anoestrus

PPI	Post-partum Interval/ Anoestrus
TP	Total protein
RBC	Red blood cell
RDW	Red cell Distribution Width
SCC	Somatic Cell Count
SNF	Solid-non-fat
SPSS	Statistical Package for Social Sciences
TBC	Total Bacterial Count
TBDs	Tick-Borne Diseases
TBIL	Total Bilirubin
TC	Total Cholesterol
TCC	Total Coliform Count
TG	Triglycerol
TP	Total Protein
TTC	Total tick count/load
VLDL	Very Low Density Lipoprotein
WASH	West African Shorthorn
WBC	White Blood Cells
β-HBA	Beta-hydroxybutyrate

ABSTRACT

The objectives of this study were to (1) assess prevalence of tick and tick-borne diseases (TBDs) and factors influencing their management in dairy cattle herds in hot-humid and coastal environments of Ghana, (2) find out effect of breed physiological state and feed supplementation on haemato-biochemical indices of cows, (3) assay gonadotrophic and reproductive steroid hormones using commercial enzyme immunoassay (EIA) and (4) assess factors influencing productive performance and milk quality of dairy cows in the study areas. The study was conducted in Ashanti, Eastern and Greater Accra Regions of Ghana from July, 2015 to August, 2018. Twenty eight (28) farms were purposively chosen, from which a total of 1052 dairy cattle breeds were used for the study. Tick identifications were done as described in literature whereas determination of prevalence of TBDs were done through culturing, microscopy and molecular (PCR) confirmation. Haematology of dairy cows was determined using fully automated BC 5800 haematology system. Serum biochemical indices were assessed using Mindray BS 130 fully automated blood chemistry analyser. Blood glucose level was determined using portable glucometer. Reproductive hormones were assayed using commercial EIA (ELISA). Data on milk yield were measured with 1 litre graduated beaker after hand milking. Milk composition analysis was carried out as decribed in literature. Lactometer, pH meter, haemocytometer and standard plate count were used for milk quality assessment. Data were analysed using IBM SPSS version 25 for windows. Analysis of data showed that, the most prevalent tick species was *Amblyomma variegatum*, followed by *Boophilus decoloratus*, *Rhipicephalus spp.* and *Hyalomma rufipes* in descending order. Effect of breed, farm and location on Log10 (X +1) + 0.5 total tick loads/infestations were masked by management regimes and level of biosecurity practices observed by farmers. Jersey cattle had the least ($P<0.01$) tick load. Dairy herds kept under exclusive zero grazing had the least ($P<0.01$) tick infestation, followed by partial zero gazing and range grazing in descending order. Dermatophilosis was the most prevalent (27.37 %) tick-borne disease (TBD), followed by anaplasmosis (21.40%) and heartwater/cowdriosis (7.41%). PCR assay showed a prevalent rate of 92.50 %, 33.75 % and 10.00% for *Dermatophilus congolensis*, *Anaplasma marginale* and *Ehrlichia ruminantium*, respectively. Dairy cattle herds reared under exclusive zero grazing, insect proof barns and moderate level of biosecurity practices had no prevalence of the TBDs whereas cattle kept on range grazing had the worst. In post tick infestation,

weekly application of acaricide resulted in the lowest ($P<0.01$) tick infestations and incidence of TBDs. Red blood cells, HGB and HCT were highest ($P<0.01$) in Sanga while Friesian-Sanga crossbreds and Jersey cows had similar ($P>0.05$) values. Values of MCV and MCH were highest ($P<0.01$) in Jersey, followed by the crossbreds and Sanga in descending order. Breed had little ($P>0.05$) effect on mean values of MCHC, RDW, PLT, MPV, PDW and PCT. Breed also influenced ($P<0.01$) serum concentration of hepatic enzyme ALP but had insignificant effect on ALT, AST and γGT. Sanga had the highest ($P<0.01$) concentrations of serum mean total protein (TP) and globulin. Direct bilirubin, urea/BUN, cholesterol, HDL, ketone, and chlorine, potassium, and phosphorus ions/cations concentrations were dependent on the type of breed. Nevertheless, breed was not a good determinant of serum levels of Na^{+}, Ca^{2+}, HCO_3^{-}, and Mg^{3+}. Physiological state influenced erythrocytes indices such that values were generally high during gestation. Haematocrit (HCT) was higher ($P<0.01$) in heifers and dry cycling cows than those observed in gestation periods. Higher ($P<0.01$) level of WBC, neutrophils, lymphocytes, eosinophils and basophils were noticed in first trimester of gestation (G_E). Physiological state had high ($P<0.01$) effect on serum ALT, AST, GGT GGT, triglycerol, cholesterol, and NEFA levels. Slight ($P>0.05$) elevation in serum ALP was observed in non-cycling heifer. Heifers and Cycling cows (CC) had higher ($P<0.01$) protein profile than cows in gestations. Physiological states had little ($P>0.05$) effect on most serum electrolytes but influenced ($P<0.05$) HCO_3^{-} and potassium. Cows in G_L had the lowest ($P<0.01$) serum phosphorus (P) concentration. Haemato-biological indices generally improved with regular feed supplementation. Progesterone (P_4) concentrations during pre-pubertal stages ranged from 0.21 to 0.96 ng/mL. Physiological onset of impending cyclicity marked by a P_4 rise of ≥ 1.0 ng/mL occurred at the 415^{th}, 295^{th} and 285^{th} day in Sanga, crossbred, and Jersey heifers respectively. The first overt heat in Sanga, crossbred, and Jersey heifers occurred in 435^{th}, 340^{th} and 320^{th} day respectively. Feed supplementation led to early onset of cyclical activity, and conception with increased ($P<0.01$) in P_4 levels with increasing level of feed supplementation. There were significant positive correlations of 0.92, 0.90, 0.71, and 0.93 between serum and milk concentrations of oestradiol, progesterone, testosterone, and FSH, respectively, during 45 days postpartum. Serum and raw milk concentrations of LH were moderately and negatively correlated (- 0.38). Testosterone concentration increased with increasing age of bulls from one year and peaked at age four. The highest ($P<0.01$) level of

oestradiol was observed in 1 year old bulls, whereas bulls of up to 6 months, and 2, 3, 4 and 12 years old had similar (P>0.01) oestradiol concentrations. Insect proof farm housing, regular feed supplementation, good body condition score 3 to 4, cows of parity 2 to 5, well-set big udder with medium teat size and milking twice a day had high (P<0.01) milk yield with normal composition at lengthier (P<0.01) lactation length. Quality assessment of milk and udder health through milk somatic cell count (MSCC) gave an overall mean of 134000 ±7498.7 cells/mL. Milk of Jersey cows had the highest (P<0.01) MSCC followed by Friesian-Sanga crossbred and Sanga cows in descending order. The MSCC increased (P<0.01) with increasing parity from 1 to 3. The overall total bacterial count (TBC) was $3.0\times10^6 \pm 7.4\times10^5$ CFU/mL. Cows kept in good sanitary premises had the least (P<0.01) TBC and vice versa. The overall mean for total coliform count (TCC) was $2.9\times10^4 \pm 1.4\times10^4$ CFU/mL. Jersey cows, good sanitary management and early stage of lactation (1 - 30 days) had significantly (P<0.01) reduced TCC. It is concluded that control and prevention of tick and tick-borne diseases can be enhanced for effective dairy production provided productive breed, improved farm facility, grazing management, zero grazing, enforcement of biosecurity practices, feed supplementation and weekly tick checks are given paramount attention. Commercial EIA could be used for assaying of reproductive hormones in dairy cattle.

Key words: *Dairying, Friesian-Sanga crossbreds, Jersey cows, tick control, tick-borne diseases prevention, cowdriosis, dermatophilosis, anaplasmosis, haemato-biochemical indices, enzyme immunoassay, reproductive hormones assay, productive traits, milk quality standards, milk somatic cell count, total bacterial count, coliform count.*

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Cattle production in Ghana is crucial to the improvement of smallholder farmers' livelihood and poverty alleviation (Apiiga, 2014). Meat and milk obtained from cattle production play a vital role in economic development of rural and peri-urban dwellers and uplift their living standards (FAO, 2011a). In Ghana, locally produced milk is processed into a traditional fresh soft cheese called wagashi (Annor, 2012). Dairy calcium offsets cholesterol effects on dairy fat/blood lipids, thereby reducing the risk of cardiovascular disease (Lorenzen and Astrup, 2011).

Milk production performance of indigenous breeds of dual-purpose cows in terms of milk yield, is low with seasonal variations (Coffie *et al*., 2015a). This has been the general trend in West African breeds of cows where higher milk yields are recorded during the rainy season (Millogo, 2010) and lower yields in the dry season. The low milk yield is a challenge to both producers and consumers (Millogo *et al*., 2008). This has been the reason for crossbreeding Ghana Sanga (a crossbred between Ghana Shorthorn and Zebu cattle) with a Friesian bull to incorporate the hardiness of the adapted Sanga breed with the high production capacity of *Bos taurus* (exotic breed) under Ghanaian environment (MoFA, 2004).

Friesian-Sanga crossbreds have better milk production potentials than the local pure breeds. The crossbred are mostly experimented in research stations (Obese *et al.*, 2013) and few private farms in southern Ghana. These crossbreds possessed exotic genes that are

prone to ticks and tick-borne diseases (Asafu-Adjei and Dantankwa, 2003). There is the need for management practices that can support the use of higher yielding breed, enhance survival and productive potential in the face of prevailing vectors or disease risks. Zero-grazing or intensification of dairy cattle herds is one of the potential management strategies that can be utilized in combination with tick prevention/control and selection for productive breed (Walker, 2011).

One of the most important prerequisite for sustainable dairy production system and productivity is reproductive performance which can be influenced by stress from a disease (Nuraddis and Ahmed, 2017). Reproductive performance is also influenced by a variety of factors such as different aspect of management including good feeding, breed, and disease prevention and containment. Therefore, health and reproductive management of the dairy cattle herds are crucial for sustainable development and improvement of dairying. Economically important traits such as reproductive traits and milk yield, can be enhanced through health monitoring and good management by manipulating environmental factors (Walker, 2011; Krogh and Enevoldsen, 2012; Abbas *et al*., 2014).

Approaches such as improving the resistance of animal to the causative agents of diseases, reducing/getting rid of disease pathogens, and altering the environment to prevent animals from coming into contact with disease causing agents might be influential (Stanton, 2011). Dairy herd health management for productive success, therefore, demands monitoring of animals' general status, reproductive and productive indices, housing conditions, nutrition, clinical and para-clinical examinations (Solcan, 2009).

1.2 Problem Statement

A challenge to commercial development of the dairy sector in Ghana is the low milk production potential of acclamatized indigenous dual-purpose cattle. Exotic breeds of higher milk production potentials are not well adapted due to disease outbreaks. Thus, the development of the dairy sector in the country with the exotic breeds of cattle (Friesian and Jersey) and their crosses with indigenous ones has been jeopardized by infectious and vector-borne diseases (Koney, 1996). Among the diseases that pose hindrance to the growth of dairy cattle industry and still of great concern are contagious bovine pleuropneumonia (CBPP), and tick-borne diseases (TBDs) viz: dermatophilosis, babesiosis, anaplasmosis and heartwater (Asafu-Adjei and Dantankwa, 2003). The tick infestations and TBDs are the major drivers to the slow development of the dairy sector in Ghana (Koney, 1996; Walker and Koney, 1999). These conditions of infections are worse in exotic and crossbred cows (Opiro *et al*., 2013) that favour economic dairying.

Though there are numerous acaricides to combat ticks, multi-resistant tick strains have been a challenge with the complexity and cost of new classes of chemical (Walker, 2011). For example weekly dipping of cattle still resulted in a significant infestation at Boadi cattle Research Station, Kumasi, due to acaricide resistance by ticks (Okai *et al.*, 2005). The use of tick-resistant breeds is sound despite its complexity in nature. Host resistance to ticks differs from breed to breed and within breeds such that individuals in the breed may be more or less resistant than the average for the population (Marufua *et al*., 2011). In addition, the resistant breeds of cattle are poor milkers and, therefore, not economical for dairying. According to Vetrivel *et al*. (2017), losses due to these diseases in cattle could be

prevented by application of scientific management practices and control over these predisposing factors.

Many approaches to tick control have been employed (Koney, 1992; Annan-Prah, 2011; Alim *et al.*, 2012; Manjunathachar *et al.,* 2014), yet, the control of ticks and tick-borne diseases (to uplift sustainable reproductive and productive potentials) is still unsatisfactory and requires concerted efforts (Ashour, 2017). Information on factors to consider, in view of modified/managerial approaches to prevention and control of ticks and tick-borne diseases, which should be resorted for promotion of economic dairy production in Ghana is rare. In view of the challenges associated with tick control, a strategic and integrated management approach to disease risks containment might be crucial (FAO, 2007). However, ways and levels of application are hardly tested at farm levels with the breed of dairy herds in the study area. Information on assessment of effect of different levels of biosecurity practices on tick infestations in dairy herds in the study area of this thesis is also rare. The influences of management regimes, environmental variables and their interactions on prevention, control of ticks and TBDs are hardly traced. There is also paucity of data on effect of location (geographical zones), type of housing, and feed supplementation on the tick load of dairy herds in the study area. Although information on seasonal variation of the tick vectors have been documented by Koney (1992), current situations in various environments of a given geographical location is vital for effective development of mitigation measures or control of tick burdens (Walker, 2011).

Blood indices are important indicators of the physiological, nutritional, metabolic, and clinical status of farm animals (Mirzadeh *et al*., 2010). Haemato-biochemical indices

reflect the responsiveness of an animal to its internal and external environments (Yaqub *et al*., 2013), and hence, enhance assessment of enzymes, electrolytes and other health indicators **(**Szablewski, 2011**).** These indices are required in animal health decision-making, but have been given little attention in dairy herd health assessment in the study area. A baseline assessment of Liver Function Test (LFT), Kidney Function Test (KFT) and electrolytes with respect to breed and different physiological states would be vital for subsequent reference in dairy herds in the hot-humid and coastal environment in Ghana.

Physiology of reproduction is mainly controlled by gonadotrophic and reproductive steroid hormones. Determination of serum and milk hormonal levels requires separate Enzyme Linked Immuno Sorbent Assay (ELISA) kits or Radio Immunoassay (RIA) kits specific for either plasma/sera or milk. Several commercial enzyme immunoassay (EIA) kits are available for determination of hormonal profiles in human, though information on these products' application and usefulness in dairy cattle, especially prepubetal stage to conception, is rarely documented in the study area. Age at which a heifer can conceive and support pregnancy without health concerns is crucial (Akers and Denbow, 2013). This is associated with the age at first oestrus, and it is an important index for reproductive efficiency. However, the first rise in progesterone concentration ≥ 1.0 ng/mL in days and the first overt oestrus marking the onset of puberty in Sanga, Friesian-Sanga crossbred and Jersey heifers in dairy setting is unknown. Although some validation studies have been conducted elsewhere for progesterone (Bayemi *et al*., 2007), oestradiol and progesterone (Domenech *et al*., 2011) and assessment of P_4 levels postpartum in Sanga and Friesian-Sanga cows (Obese *et al*., 2015; 2018), there are paucity of data on levels of P_4 and E_2 during gestation of dairy cows in the hot-humid and Coastal environments of Ghana. The

relationship between serum and milk (skimmed) levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), oestradiol, progesterone and tostesterone postpartum in Sanga, Friesian-Sanga crossbred and Jersey cows in the given geographical location of the current study is rare.

Effect of breed and non-genetic factor on milk yield, lactation length (LL) and compositional yield in dual-purpose cattle are well documented (Aboagye, 2002; Coffie *et al*., 2015a, b and c). Also, effect of breed, and season on lactation length of Sanga and Friesian-Sanga cows on-station have been reported in Ghana (Darfour-Oduro *et al*., 2010). However, information on the effect of factors, other than breed and season on milk yield traits in Friesian-Sanga and Jersey cows or cows on smallholder dairy herd setting, is limited in the study area. Also milk composition and milk quality assessment of dairy cows in dairy herds in the study area of the work are limited.

1.3 Objectives of the Study

The purpose of this project was to determine the factors influencing prevalence of ticks and tick-borne diseases, reproductive hormone levels and productive performance of dairy cattle herds in hot-humid and coastal environments of Ghana.

The objectives of this project were to:

1. Determine the prevalence of ticks and tick-borne diseases (TBDs—dermatophilosis, anaplasmosis and heartwater) in dairy cattle and factors influencing their management in hot-humid and coastal environments of Ghana.

2. Find out effect of breed and physiological state on haemato-biochemical profile of dairy cows (Sanga, Friesian-Sanga crossbred and Jersey cows) in the selected study areas.
3. Assay gonadotrophic and reproductive steroid hormone levels in serum and milk at different physiological states in Sanga, Friesian-Sanga crossbred and Jersey cattle using commercial enzyme immunoassay (EIA) in the study areas.
4. Determine factors influencing milk yield, lactation length, milk composition and quality of milk in the dairy cows in the selected study areas.

1.4 Significance of Study

This study would provide a holistic baseline health management scheme and factors that could be considered to mitigate menace of ticks and tick borne diseases in dairy herds in Ghana. Factors that enhance sustainable management of reproductive and productive indices of dairy breeds would be highlighted. This study would promote dairy cattle production and improvement, especially those that should be reared under zero-grazing units. It would, in addition, make available information on haemato-biochemical, hormonal profiles of dairy cattle at different physiological states, which information could readily serve as reference data and enhance further research. Information from this research would play a crucial role in growth, development and improvement in management of the dairy cattle production in Ghana.

1.5 General Layout of the Study

This project is put into six chapters. Chapter one presents the introduction of which background, problem statement, and objectives are presented. Chapter two deals with the

literature review whiles Chapter three describes materials and methods for the study. This employs multi-stage sampling techniques involving nonparametric and parametric sampling. Chapter four presents findings of the study and covers incidence of tick species, and prevalence of TBDs. This chapter presents factors that could be used to mitigate the incursions of tick infestations and outbreaks of TBDs in dairy herds in the hot-humid and coastal savannah environment of Ghana. The study also seeks to determine haemato-biochemical indices of dairy cows of different breeds, physiological states and feed supplementation, as important indicators of the physiological, enzymatic, metabolic, and clinical status of Sanga, Friesian-Sanga crossbreds and Jersey cows. Gonadotrophic and reproductive steroid hormones profiles at different physiological states (including heifers, cycling cows, cows in early, mid and late gestations) were determined using commercial enzyme immunoassay (EIA) technique. The concentrations of progesterone, oestradiol, testosterone, follicle stimulating hormone (FSH) and Luteinizing hormone (LH) in the dairy cows were ascertained. Factors that influence productive performance (mean milk yield, lactation length and percencentage milk composition) and assessment of milk quality in Sanga, Friesian-Sanga crossbred and Jersey cows have been elucidated in the study. Chapter five deals with discussions of findings of the study whiles chapter six presents summary of findings, conclusions and recommendations of the study.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 History of Development of the Dairy Industry in Ghana

Several attempts have been made to promote dairying in Ghana (Otchere and Okantah, 2001; Aboagye, 2002). Exotic breeds including Holstein-Friesian, Jersey and Brown Swiss were imported from Europe and elsewhere in conjunction with the use of West African Shorthorn (WASH), in order to establish a productive (crossbreed) dairy industry in the country (Oppong-Anane, 1999). It is worth noting the time line of several efforts meant to establish dairying in the country as summarized by Kabuga (1989), Oppong-Anane (1999), and Aboagye (2002) as follows: The first attempt at establishing a dairy enterprise in the country was made by the Ministry of Agriculture at Nungua, in Accra, with WASH cattle dates back to 1941. The scheme was dropped after a few years of operation due to low milk production. In 1942, the Ministry of Agriculture imported a total of 117 Bunaji (White Fulani) cattle from Nigeria to start a dairy herd at Nungua. The Bunaji bulls were used to cross WASH cows. Most of the heifers were lost because of Contagious Bovine Pleuropneumonia (CBPP) (Aboagye, 2002).

In 1958, the first attempt at using temperate cattle for milk production in the country was initiated at Animal Research Station (ARS) and University of Ghana. This involved the use of one Jersey sire for a crossbreeding programme with WASH, N'dama and Zebu cows. The programme was suspended as a result of culling due to tuberculosis outbreaks in 1978 to 1981 and theft of the replacement herd in 1981 (Oppong-Anane, 1999).

The State Farms Corporation imported 30 black and white cattle from Russia to set up a dairy farm at Adidome in the Volta Region in 1964. In 1967, the Ministry of Agriculture imported 100 in-calf heifers and 20 Friesian bulls from the United Kingdom to form a nucleus dairy farm at Amrahia in the Accra Plains. This was followed by importation of 400 Friesian cattle from Holland, 200 each in 1974 and 1976. The initial stock of Friesians imported was said to be for investigation purposes, and no emphasis was placed on obtaining pedigreed stock. The exploratory phase was devoted to survivability and maintenance of fertility status. In addition, University of Science and Technology, Kumasi, received five Holstein-Friesian heifers and five bulls from Canada in 1974 (Aboagye, 2002).

In 1977, Sam and Sam Ltd., a private firm in Accra, imported 37 Brown Swiss cattle from Austria to set up a dairy farm. In 1978, Agro Mim Industries also established a dairy farm at Mim, in the Brong-Ahafo Region, with Jersey crosses from ARS. Thereafter, a few other individuals and companies attempted to set up dairy farms using available dairy animals in the country (Oppong-Anane, 1999).

A total of 623 cattle were imported between 1964 and 1977, and at the moment a few farmers can boast of pure Jersey cattle in the Southern Ghana (Bekoe, 2011). Most of the crossbreeds of dairy cattle available are produced through artificial insemination with imported semen. It appears that the attempts at setting up a dairy industry in Ghana using pure exotic cattle have been difficult task. Challenges associated with the sustainable management of the pure exotic breeds for effective production and efficient utilization of

their products may be attributed to a number of factors, the principal ones being housing, disease incidence, and shortage or unavailability of good quality feed all-year-round.

2.2 Dairy Cattle

Dairy cattle are bred for their ability to produce large quantities of milk from which dairy products are made (Rege, 1999). Historically, there were little distinction between dairy cattle and that of beef cattle with the same stock often being used for both meat and milk production (Aboagye *et al*., 1994; Rege, 1999). Today, dairy industries are well specialized to breed animal for milk production. Holstein-Friesian is known to be the higest milk producer compared to Jersey, Brown Swiss, and others in the world (Jorritsma *et al*., 2008). In Ghana, Sanga is mostly advocated to be used for milk production and crossbreeding programmes because it performs better than the WASH (Aboagye *et al*., 1994). Others such as N'Dama, Zebu, and selected WASH are also used at the smallholder farms (Otchere and Okantah, 2001).

2.2.1 Indigenous dairy breeds

Dual-purpose cattle are adapted for production of both milk and meat (Aboagye *et al*., 1994; Rege, 1999), in satisfactory proportions (MoFA, 2004). These animals are solely cows that have good characteristics of meat and milk production. In Ghana, all indigenous cattle incuding *Bos taurus* breeds (N'dama, WASH), and *Bos indicus* (Sokoto Gudali, and White Fulani) and their crosses (including Sangas) are reared as dual-purpose cattle by farmers, for their milk and meat (Aboagye, 2002). Dairy production and development is important in West African countries because it serves as a means of improving the

nutritional status of farmers, and as a means of employment and income generation to the poor families (Ndambi *et al*., 2007).

Sanga is a crossbred between the humpless taurine breed (e.g. N'Dama and WASH) and the Zebu (Aboagye, 2002; Oppong-Anane, 2013). The Ghana Sanga is purposely meant for meat and milk production (MoFA, 2004; Oppong-Anane, 2013). These cattle can potentially provide adequate food and income for their keepers (Otchere and Okantah, 2001). The naturally crossbred cattle are believed to have been in existence for some time now and are also common in other countries. The Ghana Sanga is believed to have been among the recent derivatives (Rege, 1999). Its main uses in order of importance are: milk, meat, draught, and manure (Rege, 1999).

Some Government stations have bred a special Sanga which is an N'Dama and Sokoto Gudali cross, used for milk production (Millogo, 2010). Amrahia farms under the Ministry of Food and Agriculture, Ghana, has been mandated to crossbreed Sanga dam and Friesian or Jersey (Sire) for milk production purposes (Oppong-Anane, 1999; MoFA, 2004; 2007). The mean number of service per conception in Sanga cows has been recorded as 2.17 on station (Obese *et al*., 2008), and 2.00 on-farm (Obese *et al*., 1999). Mean conception rate of Sanga cows is 46% (Obese *et al*., 2008). The breed's mean partial milk yield ranges from 0.87 ± 0.01 litres per day (Aboagye, 2002) to 1.9 ± 0.09 litres per day (Coffie *et al*., 2015a).

2.2.2 Friesian-Sanga crossbred

Friesian-Sanga crossbred cattle are produced specifically for milk production in Ghana when exotic taurine high milk producing breed suffered from vector-borne diseases and warm-humid environmental challenges. The Friesian-Sanga is bred from the Ghana Sanga dam and Friesian bull through artificial insemination (AI) (MoFA, 2004). The crossbred has been experimented in on-station and a few smallholder farms in the country (Obese *et al*., 2013). The crossbred cows produced higher milk than the Sanga and other indigenous cattle in Ghana. The Friesian-Sanga crossbreds are, however, more susceptible to ticks and tick-borne diseases than the indigenous breed (Aboagye, 2002).

2.2.3 Exotic dairy breed

Holstein-Friesian is known to be the highest milk producer among other milk producing breeds like Jersey, and Brown Swiss in the world (Jorritsma *et al*., 2008). Holstein-Friesian, can produce 12,000 kg or more milk per lactation (Jorritsma *et al*., 2008). Jersey cows daily milk yield performance stands at 7 kg under tropical environment (Wangdi *et al.*, 2014; Fernando *et al*., 2016). Jersey cattle given to smallholder farmers by Heifer International (an international NGO) in Eastern and coastal plains of Ghana appear to be successful and a few farmers can boast of pure Jersey cattle in the Southern Ghana (Bekoe, 2011; Oppong-Anane, 2013).

2.3 Health Management of Dairy Cattle Herd in Ghana

2.3.1 Health challenges of dairy cattle production in Ghana

A major challenge to dairy development in Ghana is diseases outbreak. Okantah *et al*. (1998) identified that the major diseases that affect cattle in southern part of the country are

linked to skin problem induced by ecto-parasites infestations. The most devastating vector-borne diseases include dermatophilosis (Koney, 1996), heartwater, anaplasmosis, theileriosis, babesiosis, and trypanosomiasis (Penrith, 2012). Among other diseases that pose hindrance to the growth of dairy cattle development and still of great concern are Foot-and-mouth disease (FMD) and the tick-borne diseases (Asafu-Adjei and Dantankwa, 2003). Effects of these diseases have dwindled the pace of dairy development in Ghana (Koney 1996; Otchere and Okantah, 2001).

2.3.2 Management of dairy herd health

Herd health and production management systems aimed at implementing an integrative approach to good health, reproductive and productive quality management in dairy cows. It ensures identification and control of hazard factors, resulting in cows' welfare and improving of milk quality (Solcan, 2009). Interventions ensuring good health of dairy cattle involve handling disease occurrence through disease preventive measures, routine prophylactic and timely therapeutic treatments (Krogh and Enevoldsen, 2012). Cattle health influences dairy reproductive and productive performances. Dairy herd health management for reproductive and productive success also demands monitoring of general animals status, reproduction and production parameters, housing conditions, nutrition, clinical, para-clinical examination (ultrasound, blood and/or urine biochemistry, bacteriological, and serological study) (Noordhuizen, 2006; Solcan, 2009).

2.3.2.1 Disease mitigation measure

Disease outbreaks occur through movement of animals, inputs/products, formites, people and equipment. Hence, understanding of animal disease spread and interventions to prevent and/or control disease outbreaks is crucial to animal production development (FAO, 2011a). Managing animal health employs prophylaxis, control and where possible eradication of diseases (Penrith, 2012). Building on the resistance of animal to the disease causing agents, inactivating or eliminating the causative factor, or manipulating the environment to prevent animal from coming into contact with infectious agents are some of the measures to mitigate disease outbreaks on farms (Penrith, 2012). A good disease control measures might acknowledge early detection and a swift response to all events that threaten spread of outbreaks (OIE, 2013). Tools available for disease control measures include information systems, communication, farmer awareness and education, laboratory diagnostics, risk assessment, contingency planning, simulation exercises, modelling, vaccination, chemotherapy, biosecurity, segregation of population at risk or posing a risk, animal identification and traceability. These tools can be broadly grouped into three categories, viz: tools meant for collecting and disseminating information about animal health; those meant for supporting strategic planning and evaluating disease prevention and control; and tools used for disease prevention and control interventions (Penrith, 2012).

2.3.2.2 Biosecurity measures in dairy farms

Biosecurity involves the various management practices that prevent the spread of disease by biological organisms including human, microorganisms, rodents, arthropods, and other vectors. In other words, it includes all practices that prevent or mitigate disease from spreading, entering within or being released from livestock/poultry operations (CFIA,

2012). Biosecurity constitutes a form of risk management in animal production operations. It highlights and reinforces recommendations applicable to the location and construction, establishments, operation of animal establishments, and prevention of dissemination of infectious disease agents in livestock/poultry and from live animal markets (OIE, 2017). This is achieved by minimizing movement of the biological organisms onto or within the production site by vehicles, visitors, personnel, and pests.

Biosecurity is centered on three major components such as isolation, traffic control and sanitation (Mathis and Hagevoort, 2010). Isolation involves preventing contact between cattle and other biotic factors or organisms within the control environment. Traffic control is achieved by controlling traffic onto farm's operational area, together with movement of workers/employees within the operational zone. Design of animal rearing facility can take into consideration traffic control checks on farm structures, loading and sales points at a periphery of the farm. Sanitation describes disinfection and hygiene of materials, equipment, and people that enter the farm operation premises (Mathis and Hagevoort, 2010). Biosecurity, therefore, broadly addresses the prevention of the introduction, transmission, spread, and/or existence of a range of pests, vectors, pathogens and other disease causing agents (including toxins from plant/animal) from production operations.

2.3.2.3 Disinfection

The principle of sanitation in biosecurity measures is achieved through disinfection, a backbone to biosecurity programme. The main purpose of disinfection is the elimination of hazard for contamination or infection. It is centred between the principle of cleaning such as removal of dirt or pathogen or reduction of microorganisms, and sterilization which

involves killing of all microorganisms on the surface of an article. Disinfection is achieved through the use of antiseptics, disinfectants and/or sterilants and these are extensively used in animal and human health care (Zahra, 2015). Antiseptics are usually applied topically to living tissue, and seek to prevent, arrest the growth or action of pathogens by inhibiting their activities or by destroying microorganisms (Rutala *et al.* 2008). Disinfectants include substances applied to inanimate objects. These are usually chemical agents that destroy pathogens but might not kill bacteria spores. Environmental protection Agency categorizes disinfectants as "limited", "hospital" or "general" disinfection (Rutala *et al.* 2008).

Some common classes of chemical disinfectants/sterilants include Alcohols (60 – 90 %); quaternary ammonium compounds; phenolics; iodophors; gluteraldehydes; hypochlorites (≥ 500 ppm free available chlorine); hydrogen proxide (≥ 3 %) ortho-phthalaldehyde (OPA) and other germicides including mercurials, sodium hydroxide, β-propiolactone, chlorhexidine gluconate, cetrimide-chlorhexidine, glycols (triethylene and propylene), the Tego disinfectants and Ultraviolet radiation (UV—328 nm – 210 nm) (Cole, 1998; Rutala *et al.*, 2008; Zahra, 2015). Mode of action, microbial activity and uses of these disinfectants are well documented (Rutala *et al.*, 2008). Agents and techniques used for sterilization of farm tools and equipment include steam sterilization, microwaves, formaldehyde steam (formaldehyde is known to be mutagen), gaseous chlorine dioxide, infrared radiation, ionizing radiation, and dry heat sterilization (Cole, 1998; Rutala *et al*., 2008).

2.3.2.4 Early detection of disease through surveillance

Early detection of a disease condition can be achieved through disease surveillance (Cirkel, 2010) which facilitates swift control of outbreaks. Early detection of a disease situation can be assessed by monitoring the determinants of the disease. The disease determinants involve an interaction of an agent (virulence, pathogenicity, and infective load), a host (genotype, age, sex, immune status, and stress), and an environment including husbandry, location, and climate (Ameri *et al.*, 2009).

Animal disease surveillance is a key to improving disease analysis, early warning and predicting disease emergence and spread (FAO, 2011b). It tracks zoonotic diseases and identifies emerging diseases and as such, to support improved animal and global public health. According to Hoinville (2011), animal disease surveillance involves a systematic, continuous or repeated measurement, collection, collation, analysis, interpretation and timely dissemination of animal health and welfare related data from defined populations, essential for describing health hazard occurrence and to contribute to the planning, implementation, and evaluation of risk mitigation measures. One of the primary sources of surveillance data is from individual livestock farmer's notification or giving out dead animal for examination (Wurtz and Popovich, 2002). Types or components of livestock disease surveillance including general, syndromic, active/proactive, passive, enhanced passive, participatory, indicator-based, hazard-specific, early warning or epidemiological watch, event-based/media-based, and risk-based surveillance are well defined and documented (Ameri *et al.*, 2009; Cirkel, 2010; Hoinville, 2011).

2.3.2.5 Emergency disease control

Quarantine, culling, and movement control, are employed to regulate spread of disease outbreaks (Penrith, 2012). Quarantine and movement control are usually considered in the principle and implementation of biosecurity practices. Its continuum of activities is dependent on whether it involves an entire country, region or a farm. It takes into consideration risk analysis involving disease risk assessment; management and communication; implementation of risk management and emergency preparedness (AVA, 2008).

2.4 Ticks and Tick-borne Diseases

2.4.1 Tick systematics

Ticks are ecto-parasites belonging the Phylum arthropoda; Class, Arachnida; Subclass Acari; Order, Parasitifomes; suborder, Ixodida/Metatastigmta; and family, Ixodidae (hard tick)/Argasidae (Soft tick) (Walker *et al.*, 2003). The Ixodidae and Argasidae families have different genera, species (spp.) and subspecies while Nuttalliellidae has only one species (*Nyttalliella nemaqua*). The argasidae has five genera with each having one or more species embracing Argas (56 spp.), Ornithodiros (100 spp.), Otobius (2 spp.), Antricola (8 spp.) and Nothoaspi (1 spp,). The Ixodidae, which is the most important in disease situation in the tropics, consist of two subdivisions, viz: Prostriata (Ixodinae) and Metastriata (Amblyomminae, Haemaphysalinae, Hyalomminae, and Rhipicephalinae). It has fourteen (14) genera including *Ixodes* (Over 245 spp.), *Amblyomma* (102 spp.), *Aponomma* (24 spp.), *Haemaphysalis* (155 spp.), *Hyalomma* (30 spp.), *Demacentor* (30 spp.), *Cosmiomma (1 spp.), Nosomma (1spp.), Rhipicephalus* (70 spp.), *Anomalohimalaya* (3 spp.), *Rhipicentor* (2 spp.), *Boophilus* (5 spp.), *Margarropus* (3 spp.) (Guglielmone *et*

al., 2010) and *Otocentor,* which resembles Demacentor but distinguished with seven festoons (Annan-Prah, 2011). Epidemiologically, important tick species include *Amblyomma variegatum* (Fabricius), *Rhipicephalus (Boophilus) microplus* (Canestrini) and *Rhipicephalus (Boophilus) decoloratus* (Koch) (Muyobela, 2015).

2.4.2 Common ticks in Ghana

Ticks (Acari: Ixodida) are important to the health of livestock in Ghana and the West African countries in general, as carrier of many pathogens and predisposing factor of cattle diseases (Walker and Koney, 1999). The economic influence of tick infestation on the dairy development in Ghana has resulted in the diversion of attentions from pursuance of dairy development agenda. This is as a result of the direct effect tick infestation on cattle (de Castro, 1997) including skin damages, coupled with the various pathogens they transmit to and fro healthy and infected cattle (Jongejan, 2007). *Rhipicephalus appendiculatus*, *Amblyomma variegatum* and *Rhipicephalus (Boophilus) microplus* are some of the important tick that infests cattle (Walker and Koney, 1999).

One of the most common tick species in Ghana is Rhipicephalus (Walker *et el*., 2000). Walker and Koney (1999) noted that the most important tick population of cattle in Ghana included *Amblyomma variegatum*, *Hyalomma marginatun rufiges*, *Boophilus decoloratus*, *Boophilus gyegyi, Boophilus annullatus, Rhipicephilus senegalensis, Hyolomma truncatim, Rhipicephilus evertsi evertsi and Rhipicephilus spp.* in descending order.

Amblyomma Tick

Ambyomma (A) ticks are three-host tick which completes its life cycle in three different cattle. The species *Ambyomma variegatum* and *A. hebreum* are of greatest economic importance in Ghana (Annan-Prah, 2011). They are vectors of transmitting rickettsia pathogen, *Ehrlichia (Cowdria) ruminantium* that cause Cowdriosis or hearrtwater disease in cattle and sheep. *Amblyomma variegatum* are distributed from the northern, middle parts and the coastal savanna zone of Ghana (Walker and Koney 1999; Walker *et al*., 2000). Adults of *A. variegatum* are present throughout the year on cattle and buffalo although infestations are heavier during the wet warm months of September to May, while nymphs of this tick are only found between June and December (Petney *et al*., 1987; Ndhlovu, 2014). *Amblyomma variegatum* has a clearly defined seasonal pattern of occurrence (Walker *et al*., 2003). Delay oviposition in the tick is responsible for this pattern of seasonal abundance due to delayed development of females (Morphogenetic diapause) usually in the dry period (Walker *et al*., 2003; Muyobela, 2015).

Hyalomma spp.

Hyalomma ticks have irregular and parallel festoons. *Hyalomma marginatum rufipes* are common in Guinea savannah woodland, moist semi-deciduous forest and coastal grass and thicket of Ghana (Koney, 1992). *Hyalomma spp*. are two-host life cycles. *Hyalomma rufipes* transmits *Anaplasma marginale* to cattle causing bovine anaplasmosis or gallsickness and causing benign babesiosis. *Hyalomma rufipes* appears to be the most efficient vector of the virus (Walker and Koney, 1999).

Rhipicephalus (Boophilus) spp.

Boophilus ticks, sub-genus of Rhipicephalus genus, are small ticks with short mouth parts that unable them to penetrate so deep into their host skin, especially cattle. However, *Boophilus spp* which preferably feed on cattle, cause itching or irritations in their host leading to anorexia and loss of weight in live animals (Annan-Prah, 2011). Boophilus are one-host ticks and eggs are laid on soil. Larvae hatch after several weeks of development and crawl onto vegetation to quest for a host. When they have completed feeding they remain attached to the host and moulting occurs there. The nymphs then feed on the same host and also remain attached to it. After another moult the adults hatch and then feed on the same host. The adults change position on the same host for mating. Thus, all three feedings of any individual one-host tick occur on the same individual host. The life cycle of one-host ticks for example Boophilus, is usually rapid, it takes three weeks for the feedings on one host and two months for egg laying and larval development (Walker *et al*., 2014).

Rhipicephalus (Boophilus) decoloratus is also known as the blue tick because of the colour of engorged females. It is the commonest, most widespread and frequent of the one-host cattle ticks in Africa. In West Africa, it occurs together with *Rhipicephalus (Boophilus) annulatus* and *Rhipicephalus (Boophilus) geigyi* (Walker *et al*., 2003). *Rhipicephalus (Boophilus) decoloratus* transmits the protozoan *Babesia bigemina*, causing bovine babesiosis in cattle. This tick transmits the *Anaplasma marginale*, that causes bovine anaplasmosis, and *Borrelia theileri* which also causes spirochaetosis in cattle, sheep, goats and horses. Heavy infestations of *Rhipicephalus (Boophilus) decoloratus* are likely to cause damage to hides and to reduce the rate of growth of cattle (Walker *et al*., 2014).

Rhipicephalus (Boophilus) annulatus is a famous tick due to early work on the biology and control of babesiosis of cattle. It is a typical one-host tick of the Boophilus sub-genus within the genus Rhipicephalus (Walker *et al*., 2014).

2.4.3 Factor influencing the distribution of ticks and on a host

Major constraints to cattle production in Ghana, particularly, dairy production systems and management regimes are the increasing risks of ticks and tick-borne diseases (Koney, 1996). The distribution of tick species in predominant zones of natural vegetation may depend on host population, season and humidity (Swai *et al*., 2006). The seasonal escalations of different developmental stages of ticks are important in the epidemiology of tick-borne diseases and the planning of appropriate tick control strategies. Recognition of tick types and numbers in pastures provides useful information on tick population dynamics, tick-borne disease transmission, and estimates of the resistance of different hosts (Norval *et al*. 1992). Tick abundance varies with respect to season, year, space (between habitats and ecological zones) due to interactions of factors, such as host diversity and climate (Norval and Lightfoot, 1982). Swai *et al*. (2006) noted seasonal variation in the abundance of *Rhipicephalus appendiculatus* such that their populations increased in rainy and hot dry periods far more than cool to dry seasons. A study in Benin showed a significant increase in the abundance of tick population with increasing rainy periods of the year (DeClercq *et al*., 2012). Field infestation levels vary across different locations and seasons, with the cool-to-dry seasons having lower tick counts (Mohammed and Hassan, 2007). In Ghana, there is a general trend for the Guinea savanna zone to show the widest variety of species and most frequent infestations. This trend is followed in order by the moist semi-deciduous forest zone and the coastal grass and thicket zones (Walker and

Koney, 1999). Generally, the seasonal activities of ticks are known to vary from species to species and country to country due to variation in photoperiod in various countries and different agro-ecological zones (Swai *et al*., 2006).

Studies have shown that whitish type colour have heavy tick infestation than Ash type sheep in Sudan (Mohammed and Hassan, 2007). Hassan (1997) also indicated that tick burdens may be correlated with host coat colour in Kenya. The author found that cattle with white coats predominantly carried significantly more ticks than those with brown coats, and while black-coated cattle carried the least number of ticks. The author suggested that ticks picked up by animals with black or brown coats die or leave the host before attachment, because of the raised temperature of the host environment generated by the dark coat colour (Mohammed and Hassan, 2007).

Management and husbandry practices may affect tick infestation load and rate. Swai *et al*. (2006) alluded that most infected ticks are obtained from traditionally managed pastoral and grazed smallholder dairy cattle, while the zero-grazed smallholder cattle have almost no ticks on the hosts. Estrada-Pena and Salman (2013) noted that the poor management of farms, uncontrolled movements of domestic animals, abundance of wild animals, and absence of an adequate framework to capture the ecological plasticity of certain ticks may be attributed to complexity of the control measures.

Multifaceted factors embracing the levels of resistance of hosts (breed factor), absence of control measures, and management practices that affect host behaviour may have an impact on the tick infestation (Punyua and Hassan, 1992). Strategic and tactical management of

ticks should include pasture rotation, good barn management for zero-grazed animals, and separation of infested cattle from the healthy ones (Annan-Prah, 2011).

2.4.4 Ticks control measures in Cattle herds

Sustained and intensive tick control measures may be required for preventing outbreaks of tick-borne diseases in endemic areas (CABI, 2018). Tick control of infestation can be achieved through the wise use of acaricides, good sanitation, pasture management (rotational grazing, pasture spelling), burning (when necessary) of natural enemies and adoption of quarantine measures (Annan-Prah, 2011). The control methods can be grouped into chemical (using acarcides) and non-chemical methods (grooming, pasture spelling—leaving pastures unstocked to break the tick's life-cycle, endosymbiotic approach, biological control, genetic manipulation, and use of biopesticide) (Manjunathachar *et al.*, 2014).

2.4.4.1 Chemical/Acaricide use

Considering chemical control method of ticks, the interval of treatment is dependent on the life cycle of the tick (Annan-Prah, 2011). Accurate recommended dosage must be adhered to in order to minimize development of resistance by the tick vectors. According to Ashour (2017), chemicals available for tick control include:

- Organophosphates: (e.g. parathion, Malathion, diazinon, chlorpyrifos and coumaphos). They irreversibly inactivate acetylcholinesterase, thus influencing cholinergic nerve transmission in insects, humans, and many other animals. Products with these chemicals are no longer used for tick control because of their toxicicity to vertebrates.

- Carbamates (carbaryl and promacyl) are broad-spectrum compound used for a wide variety of pests on the grass, on pets, and in the home.
- Pyrethrins and Pyrethroids, extracted from *Chrysanthemum cinerariaefolium* plants, have a little residual effect and highly unstable in light and air. Natural pyrethrins are considered knockdown agents because they rapidly paralyse insects. Chemically modified synthetic pyrethroids, derivatives of the natural compounds, are less volatile and stability.
- Macrocyclic lactones (avermectins—Ivermectin and doramectin) and milbemycins (moxidectin)). Macrocyclic lactones are active in very low doses for the control of ticks.
- New Acaricides: (1) Benzoyl phenyl urea acts as acarine growth regulator. (2) Spinosad is a fermentation metabolite of the actinomycete *Saccharopolyspora spinosa*. It acts by disruption of the binding of acetylcholine in nicotinic acetylcholine receptors at the postsynaptic cell (Ashour, 2017).

2.4.4.2 Control of tick species on the basis of their life cycles

The interval of tick control using acaricides treatment is governed by the length of the different stages of life cycle of the tick species. While one host tick can be controlled bi-weekly interval, two host and three host ticks must be tackled in less than one week (Annan-Prah, 2011).

2.4.4.3 Integrated tick management/control (ITM/C)

Tick infestation prevention and control may involve a complete exclusion of vectors through environmental modification or a control of the herd from getting into contact with

tick vector using an integrated pest management practices. This involves the combination of a series of multi-disciplinary control measures to make the best use of each without placing too much reliance on any single component (de la Fuente and Contreras, 2015; Ashour, 2017). This type of vector management dwells much into non-chemical methods of ticks prevention and control measures such as grooming, pasture spelling (leaving pastures without stock to break the tick's life-cycle), endosymbiotic approach, biological control, genetic manipulation, use of biopesticides, herbal acaricides and vaccination with tick antigens (Manjunathachar *et al.*, 2014). The ITM embraces a judicious use of chemicals, thus, only when it is necessary and usually at the last resort. It has been noticed that some of the macrocyclic lactone acaricides, when applied on vaccinated cattle, showed greater increase in efficacy. This method effectively controls tick infestations while reducing the number of chemical acaricide treatments and consequently reduce their cost and disrupt the induction of acaricide resistance (Ghosh, Azhahianambi and de la Fuente, 2006; de la Fuente and Contreras, 2015).

It has been realized that appropriate tick control using acaricide, when combined with other measures to improve the husbandry of the cattle, will reduce the incidence of ticks and severity tick-borne diseases (Walker, 1996). Strategic and integrated methods of tick control will play a vital role in livestock production system by making tick infestation treatment cost effective. This brings about reducing the chemical residual effect on animals and environment for a sustainable management (Manjunathachar *et al*., 2014)**.**

2.4.5 Tick-borne diseases of cattle

Control of ticks and tick-borne diseases is still unsatisfactory and requires concerted efforts (Ashour, 2017). Tick and tick-borne diseases are prevalent in 80 % of cattle population around the globe (Manjunathachar *et al*., 2014). Major tick-borne diseases of cattle including Heartwater, Dermatophilosis, and Anaplasmosis have jeopardized the dairy development in Ghana (Koney, 1996; Walker and Koney, 1999).

2.4.5.1 Bovine dermatophilosis

Dermatophilosis also called cutaneous streptotricosis is an important contagious, zoonotic, skin disease (dermatitis) that affects cattle, sheep and humans. It is characterized by generalized or localized exudative and pustular skin lesions in different body parts, such as the dorsal region, the feet, the external genital area, mammary skin, and the head area (Chitra *et al.,* 2017). Dermatophilosis is caused by a pleomorphic, Gram-positive non-acid fast facultative actinomycete, *Dematophilus congolensis,* with filamentous hyphae and zoospores.

Dermatophilosis is transmitted by ticks and has worldwide distribution. The disease is reported most frequently in relatively low altitude areas with tropical and subtropical climates with high ambient temperature and torrential rain patterns (Gebreyohannes and Gebresselassie, 2013). It was first reported in the Democratic Republic of Congo in 1915 and has been reported in most countries in the African continent (Chitra *et al.,* 2017).

Survey of large number of cattle in Africa revealed prevalence rates approaching 15% with a 100% infection rate in some herds at the time of peak seasonal prevalence. In

temperate climates the disease is usually sporadic but can still have considerable economic importance where predisposing factors pertain (Radostits *et al.*, 1994; 2007). *Ambylomma varigatum, Hyaloma asticum*, and *Boophilus microplus*, are greatly associated with the occurrence of extensive lesions of Dermatophilosis. Climate is the most important risk factor in tropical and subtropical regions. It has been noticed that rain fall can act indirectly to increase the range and activity of potential arthropod vectors (Gebreyohannes and Gebresselassie, 2013). The disease has highest incidence and severity during the humid and high rainfall season. The seasonal occurrence is associated with concomitant increase in tick and insect infestation (Radostits *et al*., 2007).

The lesion commences as a circumscribed moist patch, often with raised or matted hairs, giving a characteristic Paint-brush like tufts (OIE, 2008c). Discrete lesions occur in the initial stages which coalesce to form large areas of hyperkeratotic scab and crust (Radostits *et al*., 1994). Distribution of the gross lesion usually correlates with the predisposing factors that reduce or permeate the natural barrier of the integument. Typical lesions consist of circular, dome shaped scab 2-9cm in diameter. Scab may be of variable thickness and on removal show a concave underside coated in thick, yellowish exudates, leaving a row, bleeding epidermis (Andrew *et al*., 2003). Death usually occurs particularly in calves because of generalized disease with or without secondary bacterial infection and secondary fly or screw worm infestation (Kahn, 2005; Gebreyohannes and Gebresselassie, 2013).

Various diagnostic methods including microscopic examination for appearance of the organism, isolation, cultural procedures and characteristics and molecular diagnostic

methods are well documented (Radostits *et al*., 2.007; OIE, 2008c; Dalis *et al.*, 2010; Gebreyohannes and Gebresselassie, 2013). Dermatophilosis can be confused with ring worm, staphylococcal dermatitis or folliculitis, scabies, pediculosis, and fleece rot (Sheep) (Radostits *et al*., 2007) hence the need for differential diagnosis (Radostits *et al*., 2007; OIE, 2008c).

Treatment of bovine dermatophilosis involves removal of factors predisposing cattle to infection. Most conditions that result in cutaneous maceration must avoid giving the skin an opportunity to dry out (Smith, 2009). The disease that occurs in temperate areas, Tetracycline (5mg/kg body weight) repeated weekly as required is recommended and long acting oxytetracycline (20mg/kg bw) in one injection is recommended (Radostits *et al*., 2007). In tick infested areas, the treatment is combined with acaricide application (Gebreyohannes and Gebresselassie, 2013).

Dermatophilosis prevention and Control therefore embrace avoidance of skin trauma and enguage in management practices that promote transmission (Radostits *et al.*, 2007); Treatment with antibiotics (Kahn, 2005); grooming of infected animals to remove crusts that contain the organism (Smith, 2009); proper disposal of crust to prevent further contamination of the environment (Awad *et al*., 2008); Establishment of breeds resistant to *Dermatophilus congolensis* (Jubb *et al*., 1992); and use of protective clothing, gloves and observation of personal hygiene (Krauss *et al*., 2003).

2.4.5.2 Anaplasmosis

Anaplasmosis, also called gall sickness, is a tick-borne disease of cattle and other wild ruminants of tropical and sub-tropical regions of the world, causing significant economic losses including mortalities, weight loss and reduction in milk yield (Annan-Prah, 2011). The disease is very severe in late pregnancy, and young animals are more resistant than old one. Chronic malnutrition predisposes cattle to the infection.

Anaplasmosis is caused by an obligate intraerythrocytic rickettsial microorganism, *Anaplasma species* (Rickettsiales: Anaplasmataceae) (Kumar *et al.*, 2015). Five *Anaplasma* genera including *A. marginale*, *A. centrale*, *A. phagocytophilium*, *A. bovis* and *A. ovis* are usually identified in cattle and sheep (Noaman and Bastani., 2016). Outbreaks of bovine anaplasmosis are attributed to infection with *Anaplasma marginale* though, *Anaplasma centrale* is capable of producing a moderate degree of anaemia, but clinical outbreaks in the field are extremely rare (OIE, 2015). Current classification of *Anaplasma species* are well documented (Brayton *et al*., 2009).

Ticks and biting flies are the agents of transmission of Anaplasmas. Ticks enable the organisms to undergo cyclic development. In the tropics, the one-host ticks *Boophilus annulatus*, *B. microplus* and *Amblyomma* ticks are primary vectors. Transovarial tranamission occurs with *Dermacentor andersoni and D. occidentalis. Anaplasma* can be transmitted through the egg of the tick from one generation to the nest and may remain in it for as long as four to five years from the time the initial tick fed on an infected animal. Biting flies transmit that organism within a few minute after feeding by the direct transfer

of infected blood to susceptible animal. Man can also be a major vector through the use of veterinary instruments contaminated with blood of infected animals (Annan-Prah, 2011).

Incubation period of anaplasmosis is about 3 – 4 weeks in cattle with tick-borne infections and 2 – 5 weeks with blood inoculum, though it varies with the infectious dose (Radostits *et al*., 1994). Anaplasmosis is characterized by progressive haemolytic anemia associated with fever, jaundice and decreased milk production, weight, abortions, hyperexcitability and, in some cases, sudden death (OIE, 2015). The clinical disease can only be confirmed by identifying the organism (OIE, 2008b).

Identification of *Anaplasma marginale* depends on the detection of specific antibodies using serological tests, or of rickettsial DNA using molecular amplification techniques (OIE, 2015). The recommended methods for identifying agent in individual freedom from infection prior to movement and confirmation of clinical case are microscopic examination and PCR techniques (OIE, 2015). The various methods of identification of agents including microscopic examination, and PCR; Serological tests such as competitive enzyme-linked immunosorbent assay (C-ELISA), indirect ELISA (I-ELISA) or card agglutination test (CAT) have been fully described (OIE, 2015). According to Kumar *et al.* (2015), classical Giemsa stained thin blood smear (GSTBS) parasitological method is a gold standard test for early, easy and economic detection of parasite.

Therapeutic management involving administration of tetracycline along with supportive medication including vitamin C, parenteral haematinics (iron, vitamin B_{12}, and folate) and liver extract ensures complete and smooth recovery (Kumar *et al.*, 2015).

2.4.5.3 Heartwater (Cowdriosis)

Heartwater (also known as cowdriosis) is an acute, fatal, infectious, and non-contagious tick-borne rickettsial disease of ruminants caused by *Ehrlichia ruminantium* (formerly *Cowdria ruminantium*) and transmitted by *Amblyomma* ticks (OIE, 2008a). The course of the disease varies from peracute, acute, sub-acute to mild, depending on age, immune status, breed, and virulent of the strain (Allsopp *et al*., 2005; Annan-Prah, 2011). Although, the precise role of wildlife in the epidemiology of heartwater remains to be fully investigated, they transfer the infection to *Amblyomma* ticks, which feed on these hosts in nature (Peter *et al.*, 2002). Domestic cattle, sheep and goats are most susceptible to heartwater disease (Camus, *et al*., 1996). The disease is transmitted by various spp, of *Amblyomma* ticks through bites and the use of contaminated or insufficiently sterilized needle and fomites (Annan-Prah, 2011).

The average incubation period in natural infections is 2 – 3 weeks, but can vary from 10 days to 1 month (OIE, 2008a). Cowdriosis is characterized by high fever, nervous signs, hydropericardium, hydrothorax and oedema of the lungs and brain, and death (Allsopp *et al*., 2005), in the peracute form fever of 40.5 – 41.0 ℃, diarrhoea, convulsion and death occur. Gastrointestinal disturbances, oedematous swellings and nervous symptoms including unsteady gait, chewing movement can be seen. Diseased animal sometimes goes down in convulsion, paddling with the legs in the air and the neck thrown backwards (Annan-Prah, 2011).

The percentage of *E. ruminantium* infected Amblyomma ticks at different sites ranges from 1.6 to 15.1% depending on the site of sampling (Faburay *et al.*, 2007a). A study done in

2011 on 7 sites in south of Nigeria shows a 9.6% of *E. ruminantium* tick prevalence (CABI, 2018).

In the peracute form, heartwater can be confused with anthrax. The acute form may resemble rabies, tetanus, bacterial meningitis or encephalitis, babesiosis, anaplasmosis, cerebral trypanosomiasis, or theileriosis. It must in additionally, be differentiated from poisoning with strychnine, lead, ionophores and other myocardial toxins, organophosphates, arsenic, chlorinated hydrocarbons, or some poisonous plants (OIE, 2008a; Annan-Prah, 2011).

Detection of *Ehrlichia ruminantium* infection in *Amblyomma* ticks is essential for developing an understanding of the epidemiology of heartwater and devising effective control measures (Peter *et al.*, 1995). Different ticks can act as a vector for *Ehrlichia spp.* and hence understanding of the distribution of *E. ruminantium* in a given location is an important prerequisite for effective control of cowdriosis (Kifle and Sori, 2014). Whole blood or plasma collected on the second or third day of febrile reaction > 41°C can be used for isolation of the organism as described by OIE (2008a). Scrutinizing leucocytosis together with blood culture in live animals, though it extremely difficult (Kifle and Sori, 2014), and hydro-pericardium at postmortem (pm) examination or brain culture in dead animal can also be used to identify organism (Radostits *et al*., 1994; 2007).

2.4.6 Polymerase chain reaction (PCR) detection of causative agents of the tick-borne diseases

2.4.6.1 PCR detection of Dermatophilus congolensis

Polymerase chain reaction can be carried out for the detection of the *Dermatophilus congolensis* genome isolated from the suspected samples. The major steps for amplification process have been outlined by Samon *et al.* (2010) and Shaibu *et al.* (2010). Shaibu *et al.* (2010) run PCR condition in 33 cycles while Oladunni *et al*. (2016) considered a total of 39 cycles, and a final extension at 72°C for 10 min.

2.4.6.2 PCR detection of Anaplasma marginale

One of the most useful methods for *Anaplasma spp.* identification and confirmation of clinical cases is the PCR techniques (OIE, 2015). The PCR assay is considered to be more sensitive than the peripheral blood smear examination method in the detection of *A. marginale*, because of the higher possibility of false-negative results of the latter (Tamamoto *et al.*, 2007).

There are six membrane proteins of the *Anaplasma.* These membrane proteins are named major surface proteins (MSPs) which are involved in host-pathogen and tick-pathogen interactions (Corona *et al.*, 2009). The MSPs are identified as 1α, 1β, 2, 3, 4 and 5. These proteins are recognized by neutralizing antibodies and they have a strong intermolecular relationship in the membrane of the initial bodies, performing important functions (Palmer and McElwain, 1995). These have been used as markers for the genetic characterization of *A. marginale* strains and phylogenetic studies (Corona *et al*., 2009). The MSPs is

represented in the genome by a single copy gene or forming part of multigenic families (Barbet *et al*., 1999).

The MSP5 protein is of little structural complexity, equally conserved and it induces high antibody titres (Knowles *et al*., 1996), thus, it is a strong candidate for bovine anaplasmosis diagnosis. Major surface protein 5, as comparable to msp1 and msp4, is present in the genome as a single copy gene which makes the gene an easy useful candidate for diagnosis of bovine anaplasmosis by PCR (Corona *et al*., 2009). The MSP5 differs from msp1β, MSP2 and MSP3 genes, which are present in multiple copies in *A. marginale* genome (McGuire *et al*., 1991). MSP5 is a good tool for molecular detection due to its high conservation in *A. marginale* species. The MSP5 is a highly conserved 19-kDa protein, which is encoded by a single-copy 633-bp gene on the genome of *A. marginale.* It has been used in several detection studies utilizing different primer sets (Torioni *et al.*, 1998; Visser *et al.*, 1992). The band size of *A. marginale* is dependent on the primer used (Corona *et al*., 2009; Singh *et al.*, 2012; Bacanelli *et al*., 2014). An amplicon of 458 bp specific for MSP5 of *A. marginale* has been reported in 45.2% of surveyed animals (Singh *et al.*, 2012).

2.4.6.3 PCR detection of Ehrlichia ruminantium

Polymerase chain reaction (PCR) techniques are available to detect the presence of *Ehrlichia ruminantium (Cowdria ruminatium)* in the blood of animals with clinical signs, in the tick vector, and to a lesser extent in the blood or bone marrow of carrier animals (OIE, 2008a; 2018). PCR can be used to amplify the agent in the blood and other target organs, such as brain, lungs, kidneys, and thoracic fluids from just before the onset of fever to a few days after recovery but detection in carrier animals is inconsistent (OIE, 2008a).

PCR is widely used for research on the *E. ruminantium* genome and for epidemiological studies (OIE, 2008a). Less sensitive DNA probes are also available. Since serological diagnosis is subjective and should be used only as a tool of investigation rather than definitive diagnosis. Definitive diagnosis should be by demonstration of the organism on a smear, or by PCR amplification using the pSC20 nested PCR assay and corroborated by isolation of *E. ruminantium* in endothelial cell culture (OIE, 2018).

Oligonucleotide primers, AB 128 (5'-ACTAGTAGAAATTG CACAATCTAT-3') and AB 129 (5'-TGATAACTTGGTGCGGGAAATCCTT-3') (Peters *et al.*, 1995; OIE, 2008a), which amplify a 279-bp region of open reading frame 2 of the 1,306-bp pCS20 sequence of *E. ruminantium,* have been as useful as primers for PCR amplification (Waghela *et al*., 1991; Mahan *et al.*, 1992; Faburay *et al*,. 2007b). These primers do not specifically amplify bovine *Anaplasma marginale*, *Babesia bigemina*, *Trypanosoma brucei brucei*, or *Escherichia coli* DNA (Mahan *et al.*, 1992).

Molecular techniques for *E. ruminantium* specific DNA PCR and the probe pCS20 have proven to be useful screening techniques for identification of heartwater in both infected animals and ticks (Suliman, 2011). Molecular diagnostic tools allow a better estimation of the prevalence of heartwater than to detection both in organs from suspected dead ruminants and in ticks. In Burkina Faso, detection of *E. ruminantium* prevalence in ticks by pCS20, nested PCR has been evaluated from 3 to 10 % depending on the year of tick samplings (Adakal *et al.*, 2010; CABI, 2018).

2.5 Haemato-biochemical Indices of Cattle

2.5.1 Haematology

Blood indices are importanct in veterinary medicine as indicators of the physiological, metabolic, and clinical status of farm animals (Mirzadeh *et al*., 2010; Adeyemi *et al*., 2015). Physiological equilibrium is maintained mainly by the blood in the body but many physiological conditions may alter this equilibrium (Mirzadeh *et al.*, 2010). Hence, the haematological values during different physiological situations should be known for diagnosis of various pathological and metabolic disorders which can adversely affect the productive and reproductive performance of cows, leading to heavy economic losses (Sattar and Mirza, 2009; Mirzadeh *et al.*, 2010). The results of complete blood count (CBC) indices are often helpful in the diagnosis, monitoring, and prognosis of a disease (Tibbo *et al*., 2004; Cetin *et al*., 2009; Roland, Drillich and Iwersen, 2014). Haematological indices reflect the responsiveness of an animal to its internal and external environments (Yaqub *et al*., 2013).

2.5.1.1 Red Blood Cells (Erythrocytes) as a haematological index

Erythrocytes are generally considered to be discocytes, with some degree of concavity. In adult ruminants (cattle, sheep, and goats) erythrocyte lifespan varies from 125 to 160 days (Reece *et al*., 2015, p. 123). The erythrocyte indices, including mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) (Kosshak *et al*., 2014), are calculated from determinations of erythrocytes (RBCs), haematocrit (HCT) and haemoglobin (HGB) concentration. These three indices are important and each relates to a value for a single RBC (Reece *et al*., 2015).

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

Merck Manual (2012) reported the range of normal number (count) of RBC value for cows as 5.0 – 10.0 X 10^6/mm^3. Higher than normal numbers of RBC can be due to congenital heart disease, dehydration resulting from severe diarrhoea, low blood oxygen levels (hypoxia), polycythemia vera among other. When an animal moves to a higher attitude, the RBC count increases for several weeks (Gernsten, 2009; Bunn, 2011).

Lower RBCs may be attributed to anaemia, bone marrow failure resulting from radiation, toxins or tumour, erythropoietin deficiency (secondary to kidney disease), haemolysis (RBC destruction) due to blood vessel injury or other causes, haemorrhage (bleeding), malnutrition, nutritional deficiencies or iron, copper, folate, vitamine B_{12}, vitamin B_6, overdehydration, pregnancy among others (Etim *et al*., 2014). Drugs also decrease the RBC count (Gernsten, 2009; Bunn, 2011). Bloody diarrhoea, bleeding, and blood sucking parasites are specific causes of chronic blood loss and abnormalities (Johnston and Morris, 1996; Chineke *et al*., 2006).

2.5.1.2 Haematocrit/Packed cell volume

The cellular components of blood (erythrocytes, leukocytes, and platelets, also known as thrombocytes) occupy the lower portion (a capillary column) and, taken together, are known as the haematocrit (HTC). When a column of blood is centrifuged, the components are separated according to their relative specific gravities (Reece *et al*., 2015). Packed cell volume (PCV) which is also known as HTC or erythrocyte volume fraction (EVF) (Etim *et al*., 2014), represents the percentage (%) of red blood cells in blood (Purves *et al*., 2003).

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

High PCV reading may indicate an increase in the number of circulating RBCs (Patel *et al.,* 2016). Isaac *et al*. (2013) stated the RBCs of PCV are involved in the transport of oxygen. Increased HTC shows a better transportation and thus results in an increased primary and secondary polycythemia. A low haematocrit with a low MCV and with a high RDW suggests a chronic-iron-deficient anaemia resulting in abnormal haemoglobin synthesis during erythropoiesis (Etim *et al*., 2013). An elevated haematocrit is most often associated with dehydration, which is a decrease in amount of water in the tissues. These conditions reduce the volume of plasma causing a relative increase in RBCs concentration, usually termed haemo-concentration (Etim *et al*., 2013). Low haematocrit requires

increased production of red blood cells, so dietary modifications may include increased protein and iron. Elevated glucose levels cause RBCs to swell and may cause a falsely elevated haematocrit (Lockwood, 2015).

2.5.1.3 Haemoglobin

Haemoglobin is the iron-containing oxygen-transport metalloprotein in the red blood cells of all vertebrates (Maton *et al.*, 1993; Etim *et al.*, 2013). Haemoglobin carries oxygen from the respiratory organs (lungs) to the rest of the body tissues where it releases the oxygen to burn nutrients to provide energy for the correct functions of the organism, and takes away carbon dioxide to bring it back to the respiratory organs to be dispensed from the organisms. Bovine haemoglobin has also been used extensively as oxygen - carrying substitute in humans and in small animals (Wood and Quiroz-Rocha, 2010). Cattle HGB ranges of 8.0 – 15.0 g/dL (Radostits *et al*., 1994; Etim *et al*., 2014), 7.0 – 13.9 g/dL (Kalaitzakis *et al*., 2011), 8.4 – 12.0, 9.0 – 14.0 g/dL (Roland *et al.*, 2014) have reported. Increased hemoglobin occurs when there are burns, heart failure, dehydration, erythrocytosis, haemoconcentration, high altitudes, and polycythemia vera (Lockwood, 2015).

Decrease in circulating RBCs affects the hemoglobin level. Conditions with abnormal types of hemoglobin often result in lower total hemoglobin because the red blood cells with abnormal hemoglobin are readily damaged. According to Lockwood (2015), specific disorders that result in decreased hemoglobin include thalassaemia (an autosomal recessive hemoglobinopathy resulting in insufficient globulin in either the alpha or beta subunits), sickle cell disease—an abnormally-shaped hemoglobin known as sickle hemoglobin

(hgbS), iron deficiency anaemia (hypochromic anaemia), physiological anaemia of pregnancy, haemolytic disorders, malignancies (such as leukaemia, carcinoma, lymphomas), Hodgkin's disease, pregnancy, nutritional deficit, fluid retention and intra venous overload. In chronic haemorrhage, RBC count, HCT, and HGB are decreased while reticulocytes as well as MCV are increased. In ruminants, only a moderate rise in reticulocytes is observed in responding anemia (Roland *et al*., 2014).

Merck Manual (2012) reported the normal range of cattle MCV value as 39 – 55(fl). Radostits *et al*. (1994) also documented the normal range of MCV (fl) values in sheep as 23 – 48 and in cows as 40 – 60.

Mean Corpuscular Haemoglobin

Mean corpuscular haemoglobin (MCH) is the average amount of haemoglobin (Hb) per red blood cell and is calculated by dividing the haemoglobin by the red blood cell count (Gernsten 2009). That is, MCH = $\frac{HGB}{RBC}$, measured in picogram (pg). The reference ranges of 11 – 17 pg (Radostits *et al*., 2007), 13 – 17 pg (Merck Manual, 2012) and 14 – 19 pg (Roland *et al*., 2014) have reported in cattle. Also the normal values of MCH for sheep fall within the range of 8 – 12 (Radostits *et al*., 1994).

Mean Corpuscular Haemoglobin Concentration

Mean corpuscular haemoglobin concentration (MCHC) is the average concentration of haemoglobin per unit volume of red blood cells and is calculated by dividing the haemoglobin by the hematocrit. This is the most sensitive test for iron-deficiency anaemia. Merck Manual (2012) reported that the normal range of values for MCHC and platelet for

cow are 30 – 36(g/dl). Radostits *et al*. (1994) also documented that the normal values for MCHC (g/dl) for sheep, 31 – 38, respectively; cow, 11 – 17, 30 – 36. Reduction in mean MCH and MCHC can be an indication of direct reduction in the level of haemoglobin concentration which is due to iron deficiency (Singh *et al.*, 2014). The MCHC is diminished ("hypochromic") in microcytic anaemia and normal (normochromic) in macrocytic anaemia (due to larger cell size, though the haemoglobin amount or MCH is high, the concentration remains normal. The MCHC is elevated/hyperchromic in some cases (Etim *et al*., 2013).

The MCHC is also used to determine if red blood cells are normochromic, hypochromic, or hyperchromic. Value of MCHC is increased in spherocytosis and thalassemia whiles it is decreased in iron-deficiency anaemia. Anaemias can be classified using erythrocyte indices in the following way (Lockwood, 2015): (1) Decreased MCV, MCH, and MCHC is Microcytic, hypochromic anaemia, usually related to iron deficiency anaemia. (2) Increased MCV, variable MCH and MCHC is Macrocytic anaemia, usually related to vitamin B_{12} deficiency or folic acid deficiency.

2.5.1.4 Platelets as a haematological index

Platelets are anuclear cytoplasmic fragments of megakaryocytes, and play an essential role in haemostasis (Russell, 2010; Boudreaux *et al*., 2011). Bovine platelets are small compared to those of other species and have an average range of mean platelet volume (MPV) of 4.0–4.8 femtoliters (Boudreaux and Ebbe, 1998). The normal range of platelet count in cows is 300 – 800 (Merck Manual, 2012).

Platelet parameters that can be estimated include total number of platelets, MPV, thrombocrit or plateletcrit (PCV), and platelet distribution width (PDW) (Russell, 2010; Boudreaux *et al*., 2011; Sembulingam and Sembulingam, 2012). In sepsis however the platelet count increases (Fouzas *et al*., 2010). The PDW and MPV increase in sepsis with appearance of large and heavy platelets in circulation, PDW increased with sepsis (Golwala *et al*., 2016).

Erythrocyte mean corpuscular volume (MCV) and mean platelet volume (MPV) have been reported to be indirect signs of disturbance of erythrocyte and platelet production and bone marrow response to infections (Wiwanitkit, 2004). A blood platelet count is indicated with severe haemorrhage or increased bleeding tendency. This includes clinical signs such as petechia, ecchymosis, haematurian (blood in urine), epistaxis (nosebleeding), melena (passage of dark, tarry stool containing blood), haematemesis (bloody vomititus), and hyphema—blood in the anterior chamber of the eye (Russell, 2010; Thomas, 2010).

2.5.2. Leucocyte indices

White blood cells, leukocytes, are classified into two primary groups. (1) Granulocytes (granules in leucocyte cell cytoplasm) which include neutrophils, eosinophils, and basophils (Lockwood, 2015). The three types of granulocytes are named according to which component of the haematoxylin and eosin (H&E) stain (haematoxylin, basic and coloured blue; eosin, acidic and coloured red) is taken up by the granules (Reece, 2015a). Since these cells have a multilobed nucleus, they are also called polymorphonuclear leukocytes or "polys." Neutrophils have segmented nuclei, and are sometimes referred to as segmented neutrophils or "segs" (Lockwood, 2015). Neutrophils are neither markedly

acidophilic nor basophilic and incorporate both basic and acidic components into their granules. Basophils only accept the basic (haematoxylin) component, and eosinophils only accept the acidic (eosin) component (Reece, 2015a). (2) Agranulocytes (no granules and non-lobular nuclei) are of two types, including lymphocytes and monocytes which are sometimes referred to as mononuclear leukocytes (Lockwood, 2015). The determination of the percentage distribution of WBCs is known as a differential white blood cell count (Reece, 2015a).

The different types of leukocytes of humans have many similarities with those in animals (Reece, 2015a). In some respects ruminant WBC responses are similar to those in other species, but they also have some distinct features (Tornquist and Rigas, 2010). Cattle normally have the lowest neutrophil to lymphocyte ratio (N:L) of the domestic species with a ratio of about 0.5 in adult cattle (Taylor, 2000). Sheep have a similar N:L to cattle (Tornquist and Rigas, 2010). The total leucocyte count ranges from 7000 to 10000/µL. Radostits *et al*. (1994) recorded a range of 4.6 to 12.0 X 10^9/L (4000 – 12000//µL).

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

Cattle, sheep and goats typically have a lower peak level of leukocytosis in the face of acute inflammation than do other domestic animals (Valli, 2007). For cattle, and goats

WBC count of 20,000 – 30,000/µL and 22,000 – 27,000/µL, respectively, are considered extreme leukocytosis (Jain, 1993).

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

A stress leukogram is frequently present in ruminants that have been exposed to endogenous or exogenous glucocorticoids. Leukogram changes include a mild mature neutrophilia, lymphopaenia, eosinopaenia, and variable monocytosis (Anderson *et al.*, 1999). In cattle, the N:L ratio is frequently reversed to greater than 1.0 (Tornquist and Rigas, 2010).

Blood parasites are known to cause leukocytosis. Among them is *Anaplasma phagocytophilum* which is an obligate intracellular bacterium formerly known as *Ehrlichia phagocytophila* (Whist *et al*., 2003; Woldehiwet, 2006). It infects neutrophils, eosinophils, and monocytes of cattle, sheep, and goats as well as other mammals (Stuen, 2007). The organism appears as small dark – blue to purple cocci in the cytoplasm of infected WBCs in blood smears. Microcolonies form morulae that may also be seen in the cytoplasm of WBCs (Rikihisa, 2006).

2.5.2.1 Neutrophils

Neutrophils are the most common white blood cells which phagocytize and engulf pathogens or debris in the tissues; in addition to releasing cytotoxic enzymes and chemicals to kill pathogens. The ingestion of particulate matter (bacteria, cells, degenerating tissue) is known as phagocytosis, while the ingestion of extracellular fluid is pinocytosis, and both are forms of endocytosis (Reece, 2015a). Azurophilic granules (one of the two types of neutrophil's granules) are the lysosomes of the neutrophil and supply enzymes to digest the ingested bacteria, viruses, and cellular debris. The other granules produce hydrogen peroxide, a bactericidal substance, which is potentiated (made more active) by peroxidase, one of the lysosomal enzymes (Reece, 2015a).

Neutropaenia is frequently seen in ruminants with peracute and acute severe inflammatory diseases including Gram - negative sepsis, mastitis, peritonitis, metritis, pneumonia, and gastrointestinal disease (Santos *et al*., 2002). The neutropaenia usually begins to resolve after 48 hours as bone marrow releases immature and eventually mature neutrophils (Tornquist and Rigas, 2010).

2.5.2.2 Monocytes

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

inflammatory conditions (Weiss and Perman, 1992). Monocytopaenia may be associated with endotoxaemia and other peracute and acute inflammation (Jain, 1993).

2.5.2.3 Lymphocytes

Lymphocytes originate from a lymphoid stem cell, known as a lymphoblast, in lymph tissue, such as lymph nodes, spleen, tonsils, and various lymphoid clusters in the intestine and elsewhere (Reece, 2015a). A range of 19.0 to 92.0 %; 40.0 to 70.0 % and 60 to 65 % of leucocytes have been reported by Kalaitzakis *et al*. (2011), Etim *et al*. (2014) and (Reece, 2015a), respectively. Radostits *et al*. (1994) reported on a lymphocyte number range of 2.7 to 7.5 X 10^9/L. A gradual decrease in lymphocyte numbers is then seen as cattle ages (Mohri *et al*, 2007; Tornquist and Rigas, 2010).

Lymphocytosis can occur in the healing phase of infectious diseases, during chronic antigenic stimulation due to infectious agents, neoplasia, and hypoadrenocorticism (Fowler, 1998; Keller *et al*., 2006). According to Dore *et al.* (2007), reactive lymphocytosis is observed during chronic purulent diseases, such as hepatitis, peritonitis, pericarditis, nephritis, mastitis, or bronchopneumonia.

Studies have shown that certain subpopulations of circulating lymphocytes are disproportionately reduced in experimentally induced stress. The percentage of γ, δ and T cells have been shown to decrease in cattle given immunosuppressive doses of dexamethasone with more variable effects on B cells and other T cell populations (Menge and Dean–Nystrom, 2008). Decreased proliferative capability of lymphocytes is observed in cattle treated with dexamethasone (Anderson *et al*,. 1999; Menge and Dean–Nystrom, 2008).

2.5.2.4 Basophil

This white blood cell enters damaged tissues and releases a histamine and other chemicals that promote inflammation in the body to fight pathogens. Basophils constitute < 1 % of WBC (Reece, 2015a). The mast cells and basophils play an exceedingly important role in some types of allergic reactions because the type of antibody that causes allergic reactions, the immunoglobulin E (IgE) type, has a special propensity to become attached to mast cells and basophils (Guyton and Hall, 2006; Tornquist and Rigas, 2010). Basophilia has been reported in cattle with tick infestations (Tornquist and Rigas, 2010) and in goats experimentally infected with nematodes (Richard and Cabaret, 1993). Cattle infected with flukes showed no increase in basophil numbers (Conboy and Stromberg, 1991). Sheep appear to be less likely to display basophilia than some other species (Rothwell *et al*., 1994).

2.5.2.5 Eosinophil

Eosinophil stains with the red dye "eosin" hence its name. Its count ranges from 2 to 5 % of WBC (Reece, 2015a). Eosinophils are crucial cells in host response to parasitic infections and in allergic reactions. Eosinophilia has been reported in a variety of endoparasitic infections in cattle (Conboy and Stromberg, 1991) and small ruminants (Tornquist and Rigas, 2010).

Ectoparasites have also been reported to elicit an eosinophilia (Jacquiet *et al*., 2005). However, one study showed that cattle experimentally infested with ticks did not have increased peripheral eosinophil numbers when compared to controls (Tornquist and Rigas, 2010). Eosinopaenia may be a component of a stress response in ruminants. Extreme

eosinopenia has also been reported in *Theileria parva* and *Theileria annulata* infections in cattle (Mbassa and Poulsen, 1991; Omer *et al*., 2002).

2.5.3 Factors influencing Haematological indices

Physiological and pathophysiological variables such as recent activity and stress have been reported to influence haematological values in cattle. Despite the range and sensitivity of technology used, cattle haematology reference intervals are uniformly broad (Tornquist and Rigas, 2010).Variables that contribute to the thresholds and width of reference intervals include age, sex, stress, diet, body condition, reproductive status, recent activity, hydration, ambient temperature, and altitude (Wood and Quiroz-Rocha,2010; Krimer, 2011). Reports of reference intervals, however, seldom include consideration of such variables as age, sex, physiologic state, history, form of restraint, ambient temperature, hydration status, BLV status or parasite burdens (Tornquist and Rigas, 2010).

2.5.3.1 Effect of genetic factors on haematological indices

Influence of breed on haematology of domestic animals including goats, rabbits amd chicken have been documented (Isaac *et al.,* 2013; Etim *et al*., 2014). Ekiz and Yalcintan (2013) observed significant variations in PCV in different breed of goats kids. Some authors, however, reported that no significant breed effect on the blood parameters (Ologunowa *et al*., 2000). Erythrocyte osmotic fragility of Zebu Nelore cattle differed in breed lines, in NaCl concentration g/dl (Ayres *et al*., 2014).

Beef cattle breeds have higher RBC counts than dairy cattle (Tornquist and Rigas, 2010). Bulls have greater RBC counts than cows. Non-lactating cows have higher RBC counts

than lactating cows **(**Wood and Quiroz-Rocha, 2010**).** Al-Bulushi *et al.* (2017) observed that RBC, HGB, PCV, MCV, MCH and MCHC were lower in Shrawi breed than the Omani breed of goats. Haemoglobin and HCT/PCV differed among breeds and ranged from 11.58 – 15.14 and 34.00 – 39.67 %, respectively. The RBC indices are reported higher in Tharparkar cattle than Sahiwal breed (Aggarwal *et al*., 2016).

2.5.3.2 Physiological status and haematological indices

Physiological variability in haematological profiles of cattle is observed in all species (Roland *et al*., 2014). Ijaz *et al.* (2003) reported an increase in HGB concentration, erythrocyte sedimentation rate, MCH, MCHC in cyclic as compared to non-cyclic cows. It has been observed that, many of the globulin fractions decreased during the last month of gestation (Yaqub *et al.*, 2013). There were differences in total protein, albumin and globulin fractions between pregnant and non-pregnant cows (Zvorc *et al*., 2000).

Pregnant buffaloes are reported to have higher RBC, HGB and PCV values than non – pregnant lactating and non-pregnant dry cows (Kopp and Hetesa, 2000; Chineke *et al*., 2006). The elevated erythrocytes indices during gestation have been observed to be due to maternal adaptation to pregnancy in order to meet the requirements of growing foetus Foetal growth that occurs during pregnancy produces greater oxygen demands. This greater need for oxygen is compensated by the endocrine system that stimulates the release of erythropoietin by renal tissue (Patel *et al*., 2016). Mirzadeh *et al.*, (2010) found in Iranian cattle at various physiological states that total erythrocyte count differed among dry cows, non-pregnant cows, pregnant heifers, suckling and non-suckling calves and the

values were 5.09±0.23, 5.28±0.26, 5.37±0.31, 5.91±0.15, 4.59±0.12 and 5.65±0.16 $X10^6/\mu L$, respectively.

The reduction in RBC count, haematocrit and haemoglobin, occurs in the third period of gestation, which represents the main cause of "pregnant physiological anaemia" a clinical condition described in various species (Meliani *et al.*, 2015). Physiologically, higher RBC, haematocrit and haemoglobin levels can play a role in better physical activity and some parasitic diseases. It can also provide significant condition, such as in sports activities (Meliani *et al.*, 2015). The highest HGB concentration has been recorded in nonpregnant heifers while the lowest values are observed in non-pregnant lactating cows. Similarly, highest RBCs count and PCV have been observed in non-pregnant heifers whereas the lowest values are noticed in pregnant dry cows (Sattar and Mirza, 2009). Erythrocyte numbers increase slightly as the pregnancy advances (Jain, 1993). Percentage of neutrophils has been reported to rise during gestation to 60% of the total leukocyte count and then decline to 45% by day 14 of lactation (Nazifi *et al*., 2008).

The highest MCV, MCH and MCHC have been observed in parturient cows and the lowest values are observed in pregnant lactating cows. The highest WBCs count is recorded in pregnant heifers whereas the lowest values are observed in parturient cows. Lymphocytes decrease around parturition mainly due to reduced lymphocyte proliferation (Sattar and Mirza, 2009).

2.5.3.3 Effect of post postpartum on haematological indices

Differences in haematological indices including RBC, HGB, PCV, and WBC in postpartum period have been reported but similar values in the differentials been recorded (Nazifi, Ahmadi and Gheisari, 2008). The leukocyte count and haemoglobin concentration in pregnant cows have been reported higher than in the postpartum cows in 25 – 30 days after parturition (Nazifi *et al*., 2008). Neutrophils constitute the first line of an increase in postpartum defense against the invading pathogenic organisms, resulting in large neutrophil populations within the uterine lumen (Butt *et al.* 1991). Differences in segmented neutrophils in the clinically healthy and cows experiencing subclinical endometritis in 25–30 days after parturition have been reported (Nazifi *et al*., 2008).

2.5.4 Serum biochemical parameters of dairy cows

2.5.4.1 Enzymes

The transferases are a large group of enzymes that catalyse the transfer of groups such as acetyl, amino and phosphate from one molecule to another (McDonald *et al.,* 2011, p. 142). These enzymes include Alanine aminotransferase (ALT), aspatate aminotransferase (AST), alkaline phosphatase (ALP), γ-glutamyl transferase (GGT/γGT) lactate dehydrogenase (LDH).

Alanine aminotransferase (ALT) and aspatate aminotransferase (AST)

Alanine aminotransferase (ALT) is the most sensitive target in the diagnosis of acute liver damage, whereas aspartate aminotransferase (AST), also known as glutamic oxaloacetic transaminase—GOT) is more sensitive in reflecting the degree of damage (Ioannou *et al*., 2006). The tissue activities of the transaminase enzymes are markers for the functions and

integrity of the heart and liver **(**Shanhjahan *et al*., 2004). Increased activity of AST in blood serum is a very sensitive indicator in assessing damage liver cells, especially in the infiltration mass and degeneration of hepatocytes (fatty liver) (Meyer and Harvey, 2004; Lubojacka *et al*., 2005).

The ALT and AST rearrange the building blocks of proteins. These are released from damaged liver cells (Nelson and Cox, 2003). Elevation of these enzymes in the serum have been reported to indicate cellular damage, tissue necrosis as well as a calculated risk for cardiovascular diseases and elevated myocardial infarction (Ioannou *et al*., 2006)**.** Though high ambient temperature enhances ALT and AST activities, Ronchi *et al.* (1999) observed a decrease in hepatic enzyme activities during heat exposure. A decrease in enzymes activities has been reported to due to the reduction in the function and not resulting from liver damage (Aggarwal *et al*., 2016).

Gamma-glutamyl transferase

Gamma-glutamyl transferase/γ-glutamyl transferase (GGT or γGT) is a microsomal membrane-bound enzyme as a biomarker of stress and metabolic dysfunction. The presence of the enzyme in the serum/plasma is significant as a sign of hepatobiliary system diseases connected with cholestasis and is used in diagnosing liver disease. Its activity is relatively high in livers of cows (Stojević *et al*., 2005). Increased activity of this enzyme in the blood can indicate damage to the cellular structure of hepatocytes (Lubojacka *et al*., 2005; Krsmanović *et al*., 2016). It has been indicated that the activities of AST and γGT enzymes showed irregular change during pregnancy and early lactation (Stojević *et al*., 2005). γ-glutamyl transferase is more specific to liver tissue, but the correlation of these

serum activities with hepatic lipidosis is not as high (Sevinc *et al*., 2002). Sevinc *et al*. (2001) have indicated that cows with severe fatty liver have higher levels of γGT and AST. Stojević *et al*. (2005) reiterated that γGT is an essential indicator of hepatic lesions and function.

Alkaline phosphatase (ALP)

Alkaline phosphatase is involved in bone growth and excreted in the bile. It may be elevated if bile excretion is inhibited by liver damage (Ophardt, 2003). The mammalian organs having very high ALP activities are those involved in active transport mechanism (Ophardt, 2003). ALP is a marker enzyme for the plasma membrane and endoplasmic reticulum (Muhammad, 2007; Adeyemi and Muhammad, 2010**).** It is therefore an ectoenzyme of the plasma membrane (Shanhjahan *et al*., 2004).

Alkaline phosphatase is used to assess the integrity of the plasma membrane (Akanji, 1993) and in the tissue and serum indicates likely damage to the external cell boundaries or plasma membrane (Adeyemi *et al.*, 2015). An increase in ALP level may quarry the possibility of membrane damage, because ALP is a membrane bound enzyme (Rao, 2006; Ruothalo, 2008). High levels of serum ALP activity is usually observed in liver damage, cancer and heart infections (Jaroslaw *et al*., 2009). A decrease in serum ALP may be an indication of the healthier state of the plasma membranes (Adeyemi *et al.*, 2015). According to Fauci *et al*. (2008), fatty liver disease is the most likely explanation. Striking elevations > 1000 U/L—occur almost exclusively in disorders associated with extensive hepatocellular injury such as (1) viral hepatitis (2) ischemic liver injury (prolonged hypotension or acute heart failure), or (3) toxin- or drug-induced liver injury.

2.5.4.2 Metabolites of dairy cattle

Protein profile (Total protein, Albumin and Globulin)

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

Serum globulins are a group of proteins made up of globulins (immunoglobulins) produced by B lymphocytes and globulins are produced primarily in hepatocytes. Globulins are increased in chronic liver disease, such as chronic hepatitis and cirrhosis. In cirrhosis, the increased serum gamma globulin concentration is due to the increased synthesis of antibodies, some of which are directed against intestinal bacteria (Fauci *et al.*, 2008). Serum globulin concentration in cow is 30 – 35 g/L (3.0 – 3.5 g/dL) (Weiss and Wardrop, 2010; Latimer, 2011; Merck Manual, 2012). Radostits *et al*. (1994) recorded 7 – 12, 6 – 12, and 16 – 32 g/L for α, β, and γ globulin concentrations, respectively, in serum. Globulin increased in response to an inflammatory process (Kaneko, 2008). Total protein gives information about kidney damage, liver damage, and nutritional health (Stojević *et al*., 2005).

Urea

The essential blood analytes for assessing the protein profile is blood urea nitrogen (BUN). It is a good indicator of the energy intake of the cow, and in particular, as an indication of the synchronization between fermentable carbohydrates and rumen degradable protein

(RDP) (Van Saun, 2010). Concentrations of urea have been noted to have effect on reproductive disorders including repeat breeding. It helps understand the mechanisms impairing fertility in repeated breeder cows (Mimoune *et al.*, 2017).

Blood urea concentration depending on nutrition is diagnostically vital for assessment of diseases of kidney (Klinkon and Ježek, 2012). Serum/plasma urea concentration ranges from 2.5 to 7.8 mmol/L (Higgins, 2016). Increased serum concentration of urea in calves indicates increased catabolism of proteins and appears at long lasting diarrhoea. Serum urea concentration varies with age and has been observed to be 2.7 mmol/L in 60 days calf (Klinkon and Ježek, 2012). Garcia *et al*. (2017) observed 5.75 mmol/L in high producing cows whereas Cozzi *et al*. (2011) reported on urea concentration of 4.6 mmol/L (range, 2.5 to 6.7 mmol/L). Measurement of serum urea concentration is very useful for assessment of dehydration and disturbances of acid-base balance in calves with diarrhoea Calves experiencing diarrhoea can have twice as high mean plasma urea as compared to healthy calve of similar age (Klinkon and Ježek, 2012).

According to Higgins (2016), causes of elevated serum urea concentration include: Renal disease/failure (i.e. Acute kidney infiltration—AKI or chronic kidney disease—CKD); Dehydration due to low fluid intake, excessive fluid loss (sweating, vomiting, diarrhoea, and diuretic drugs); Decreased renal perfusion due to heart failure, hypovolemic shock, and severe hypotension; Gastrointestinal bleeding; High-protein diet; Ageing; and Catabolic state, such as trauma, severe infection, starvation, and some drugs with catabolic effect, example, use of corticosteroids).

Decreased plasma/serum urea concentration of <2.5 mmol/L or blood urea nitrogen of <7.0 mg/dL might be associated with pregnancy, low-protein diet, overhydration, advanced liver disease (cirrhosis, liver failure), and inherited defect in "urea cycle" enzymes (reduced urea synthesis) (Higgins, 2016)

Blood urea nitrogen (BUN)

Whereas BUN reflects only the nitrogen content of urea, urea measurement reflects the whole of the molecule. One can therefore convert BUN (mg/dL) to urea (mmol/L) by multiplying by 10 to convert from /dL to /L and dividing by 28 to convert from mg BUN to mmol urea; i.e. 10/28 = 0.357. Hence, BUN (mg/dL) multiplied by 0.357 = urea (mmol/L) (Higgins, 2016).

Total bilirubin

Bilirubin is a breakdown product of the porphyrin ring of haeme-containing proteins, which is found in the blood in two fractions—conjugated and unconjugated (Fauci *et al*., 2008). The unconjugated fraction, also termed the indirect (IBIL) fraction, is insoluble in water and is bound to albumin in the blood. The conjugated (direct) bilirubin (DBIL) fraction is water soluble and can, therefore, be excreted by the kidney. Hyperbilirubinaemia due to increase in DBIL almost always implies liver or biliary tract disease. In such condition, both direct and indirect fractions of the bilirubin tend to be elevated (Fauci *et al*., 2008).

The increment in total and indirect bilirubin may be related to fat infiltration of the liver (Sevinc *et al.,* 2002). Isolated unconjugated hyperbilirubinaemia (bilirubin elevated but

<15% direct) should prompt a workup for haemolysis (Fauci *et al*., 2008). In the absence of haemolysis, an isolated unconjugated hyperbilirubinaemia in an otherwise healthy patient can be attributed to Gilbert's syndrome (a hereditary condition characterized by hyperbilirubinaemia due to reduced activity of enzyme glucuronyltransferase) and no further evaluation is required (Fauci *et al*., 2008).

Creatinine

Creatinine is synthesized at endogen metabolism in muscles. Creatinine is excreted with urine. Its concentration in serum does not depend on the nutrition. Creatinine concentrations in cows' sera are reported to range from 44 – 194 µmol/L (Merck Manual, 2012), 67 – 175 µmol/L (Radostits *et al*., 1994) and 88 – 177 µmol/L (Mayer *et al*., 1992). Diagnostically, creatinine is important for the assessment of functioning of the glomerular system in the kidneys, but its concentration increase only at serious damage (Klinkon and Ježek, 2012). It has been observed that serum creatinine concentration in Holstein calves decreased from 1st to the 70th day of age (Mohri *et al.*, 2007).

Triglyceride

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

Total cholesterol

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

High density lipoprotein (HDL)

High density lipoprotein (HDL) or good cholesterol concentration in blood serum of cows ranged from 2.55 to 3.45mmol/L (Petkova *et al.*, 2008). Latimer (2011) related that, in ruminants and horses, the majority of cholesterol circulates as HDL. The HDLs contain cholesterol, protein, and phospholipid with very little triglyceride accounting for their "high" density. They are formed in the intestine and liver (Evans, 2011).

Low density lipoprotein (LDL)

The most prominent indication of severe fatty liver in cows is the dramatic decrease in plasma or serum LDL-cholesterol, particularly very low density lipoprotein (VLDL) cholesterol (Sevinc *et al*., 2002). The LDLs are rich in protein with small amounts of cholesterol and triglyceride. They are formed in the vasculature when hepatic lipoprotein lipase removes additional lipid from intermediate density lipoproteins (IDLs—formed from the degradation of very low-density lipoproteins as well as high-density lipoproteins). The LDLs distribute cholesterol to peripheral tissues (Latimer, 2011).

Very-low-density lipoproteins (VLDL)

The lipid component of Very-low-density lipoproteins (VLDL) consists of triglyceride, cholesterol, and phospholipid in an approximate ratio of 4:1:1 (Evans, 2011). The VLDLs are synthesized primarily in the liver. These VLDLs export hepatic triglyceride and cholesterol and distribute triglyceride to adipose tissue and striated muscle (Latimer, 2011).

Glucose

Prepartum and postpartum differences in serum glucose concentrations have been reported (Pal and Bhatta, 2013). Blood glucose concentration ranges from 1.9 to 3.9 mmol/L in cattle (Radostits *et al*., 1994). Glucose concentration in mg/dL can be converted to mmol/L by multiplying by a factor 0.0555 (Szablewski, 2011). It has been reported that there is a peak in the blood glucose concentration during parturition followed by a decrease after parturition (Studer *et al*., 1993; Vazquez-Añon *et al*., 1994). Increase in the blood glucose concentration is due to the gluconeogenesis and the glycogenolysis (Szablewski, 2011) stimulated by glucocorticoids and cathecolamins during parturition (Fonseca *et al*., 2004). Low glucose concentration up to the 7^{th} week of lactation has been reported (Fonseca *et al*., 2004; Studer *et al.* (1993). Vazquez-Añon *et al.* (1994) however, reported an increase in blood glucose concentration after the second week of lactation. Increase in dry matter intake and energy status leads to an improvement in blood glucose level.

Ketone/Beta-hydroxybutyrate (β-HBA)

Ketone bodies are fat-derived, water-soluble metabolites that serve as glucose substitutes, and in ruminants ketone body β-hydroxybutyate is formed from butyrate in the rumen

epitheium (Cunningham and Klein, 2007, p. 406). Hyperketonaemia is manifested clinically with reduced appetite, rapid weight loss and reduced milk yield. In some cases, animals exhibit nervous signs as pica, biting and licking unusual object, and blindness (Hungerford. 1990; Marutsova Binev and Marutsoy 2015). The faeces is usually hard, dry and scanty. The β-HBA is used as early marker for detection of ketosis in ruminant (Duffield, 2004; Oetzel, 2007). Low body condition score (BCS), abomasum displacement, disturbance of the reproductive performance and early embryonic death (López-Gatius *et al.*, 2002) are other predisposing factors for high blood ketone bodies concentrations**.** Blood β-HBA concentration increased with decreasing BCS. Blood β-HBA concentration is more suitable for diagnosis of ketosis than other ketone bodies including acetone and acetoacetate (Oetzel, 2007).

Limits of blood β-HBA concentrations set to be characterized by clinical signs of subclinical ketosis are >1.0 mmol/L (Goldhawk *et al.*, 2009; Kinoshita *et al*., 2010), > 1.4 mmol/L (Geishauser *et al*., 2000; Duffield *et al*., 2009; Ospina *et al*., 2010) and >1.2 mmol/L (Seifi., *et al*., 2011; McArt *et al*., 2013). Marutsova *et al*. (2015) observed β-HBA concentration of 1.2 mmol/L and 2.6 mmol/L in cows having subclinical and clinical ketosis, respectively. Beta-hydroxybutyrate concentrations of 0.30±0.27, 1.57±0.55, and 4.75±1.36 mmol/L have been reported for healthy, subclinical ketosis and clinical ketosis, respectively in cows (Marutsova *et al*., 2015). Sometimes, in very high blood β-HBA concentrations, clear clinical signs of ketosis could be absent (Oetzel, 2007).

Blood β-HBA is an indicator of inappropriate oxidation of non-esterified fatty acids in the liver (LeBlanc, 2010). It has been reported that decreased blood glucose level and low

insulin secretion are triggers for enhanced mobilization of lipids from the adipose tissue and deposition of triglycerides in the liver parenchyma and stimulation of ketogenesis (Grummer, 1993). It is reported that, excessive fat deposition in the dry season correlates with increased occurrence of clinical ketosis in cows after calving (Markusfeld *et al*., 1997). Ruegg and Milton (1995) observed no relationship between the weight loss during lactation and the incidence of metabolic diseases.

Nonesterified fatty acids (NEFA)

Serum nonesterified fatty acids (NEFA) concentration is an indicator of fat mobilization (Whitaker, *et al*., 1999; 2000; Seifi *et al*., 2007). Increase in blood concentration of NEFA and (β-HBA) are triggered by lipid mobilization from adipose tissues. (Kovacevic *et al*., 2016). NEFA concentration reflects the mobilization of lipid reserves to compensate for the imbalance between nutrients consumed by the cow and nutrients secreted in milk (van Knegsel *et al.*, 2007; Grummer, 2008; Cozzi *et al*., 2011). NEFA concentrations are relatively low because energy balance becomes positive and cows replete/restore the mobilized tissue reserves (Walters *et al*., 2002).

It has been reported that plasma nonesterified fatty acids (NEFA) increase more than a two-fold between 2 to 3 weeks prepartum and 2 to 3 days prepartum, at which time concentration increases dramatically until completion of parturition (Vazquez-Añon *et al*., 1994; Grum *et al*., 1996; Bačić *et al.*, 2007). Plasma NEFA concentration decrease rapidly after calving but concentration remain higher than they were before calving (Bačić *et al.*, 2007).

The use of NEFA is a better indicator of NEB compared to β-HBA, although β-HBA is a more useful indicator during postpartum (Garcia *et al*., 2017). Normal NEFA concentration value in a healthy cow of positive energy balance is usually around 0.25 mM which is characterized by a low lipid mobilization (Fonseca *et al*., 2004). A higher concentration of more than 0.40 mM indicates negative energy balance and an increased lipid mobilization (Oetzel, 2004).

The increased NEFA or β-HBA correlate positively with disturbances in dairy cows' health, reproduction and milk yield during the postpartum period (Duffield *et al*., 2009; Ospina *et al*., 2010). Relationship between β-HBA and negative energy balance can be inferred by the negative correlation coefficient found between β-HBA and GLU (r = -0.41) (Garcia *et al.*, 2017). The effect of negative energy balance on reproduction is the prolonged postpartum anoestrus (Obese *et al.*, 1999; 2008). It is important to note that the cows identified with the highest concentration of β-HBA also recorded the lowest concentrations of TG which fall between 0.04 and 0.07 mM. Cows affected by ketosis the TG concentration decreases because this biochemical analyte is deposited in cells of the liver and other organs (Garcia *et al.*, 2017).

2.5.4.3 Electrolytes

Calcium

Extracellular calcium is very important for formation of skeletal tissues, transmission of nervous tissue impulses, excitation of skeletal and cardiac, blood clotting, and as an important component in milk (Goff, 2015b). Whiles 98 % of calcium is located within the skeleton, only about 2 % of the calcium in the body is found primarily in the extracellular

fluid (Goff, 2015b). The serum Ca^{2+} concentration in cattle ranges from 2.0 to 2.75 mmol/L (8.0 – 11.0 mg/dL) (Radostits *et al.*, 1994; Merck Manual, 2012). Serum Ca^{2+} level of 2.4 – 3.1 mmol/L has been reported by Meyer *et al.* (1992). Inadequate levels of Ca^{2+} increases the risk of developing postpartum diseases such as hypocalcaemia, especially if an electrolyte imbalance also occurs (Van Saun *et al*., 2006). The Na^{+}/Ca^{2+} exchange located in the plasma membrane of the osteocytes is essential for maintaining Ca^{2+} homeostasis, in that, a decrease in the Na^{+} concentration could lead to the presence of low serum Ca^{2+} levels (Michel *et al.*, 2014). This explains the positive correlation ($P<0.05$; $r = 0.38$) found in the study between Na and Ca^{2+} by Garcia *et al*. (2017). According to Goff (2015b) an animal that is hypocalcaemic and hypermagnesaemic will be able to initiate only very weak muscle contractions, a condition is known as paresis. Extracellular calcium concentration influences the secretion of other substances by nerves and endocrine glands. Thus, the hypocalcaemic cow is unable to secrete insulin from the pancreas and, therefore, becomes hyperglycemic. In addition, the parathyroid glands monitor carotid artery blood calcium concentration and secrete parathyroid hormone (PTH) when they sense a decrease in blood calcium. Bone osteocytes are embedded within the bone matrix. They are surrounded by lacunae and these are all interconnected by a series of canals called canaliculi**.** The fluid within the canaliculi and lacunae is relatively rich in calcium. PTH can stimulate the osteocytes to pump this calcium back into the extracellular fluid, known as osteocytic osteolysis. This returns a modest amount of calcium to the blood very quickly. When larger amounts of calcium are needed because a diet is not supplying adequate calcium, the animal can use osteoclasts to resorb solid bone. Dietary calcium must enter the extracellular fluid to permit optimal performance of the animal. Calcium absorption can occur by passive transport between epithelial cells across any portion of the

digestive tract whenever ionized calcium in the digestive fluids directly over the mucosa exceeds about 1.5 mmol/L though for practical reasons it likely must exceed 3–4 mmol/L to contribute greatly. Such concentrations are commonly reached when young animals are fed milk (Reece, 2015b).

Metastatic calcification is observed when plasma calcium increases above normal concentration, and begins to be deposited into the soft tissues of the body. Calcitonin is a hormone produced by the thyroid gland in response to hypercalcaemia. Calcitonin inhibits renal reabsorption of calcium from the glomerular filtrate, resulting in increased calcium excretion. It also inhibits bone calcium resorption, slowing entry of calcium into the extracellular fluid. Calcitonin is not often called on to restore calcium homeostasis unless a very high calcium diet such as milk is fed in a short period of time (Goff, 2015b).

In animals, dietary calcium deficiency forces the animal to withdraw calcium from bone for homeostasis of the extracellular fluid, leading to osteoporosis and osteomalacia which makes the bone prone to spontaneous fractures. When it occurs in cows experiencing negative energy balance (NEB) in early lactation, it is a lactational osteoporosis (Radostits *et al*., 1994; Goff, 2015b). Oral calcium drenches at calving reduces the incidence of milk fever (Bačić *et al*., 2007).

Sodium

The essential blood analyte of the mineral profile is sodium (Na), which is the main extracellular fluid cation and an important determinant of body water homeostasis (Kume *et al.*, 2011). Sodium plays a key role in acid–base balance of the body. Sodium is

important extracellular mineral determining the electrical potential of nervous tissue and plays crucial role in transmission of nerve impulses. Efficient absorption of monosaccharides and some amino acids is dependent on sodium-coupled transport processes (Goff, 2015b). Feeding sodium without chloride (for example, sodium bicarbonate or sodium propionate) to an animal alkalinizes the blood. Extracellular sodium concentration is tightly regulated and it is generally maintained at 135–155 mmol/L, depending on the species of animal. Sodium-deficient animals develop an intense craving for salt leading to pica, with licking and chewing of various objects. Prolonged sodium deficiency leads to an unthrifty animal with rough hair coat, haggard appearance, and poor growth and productivity. Severe deficiency leads to shivering, incoordination, weakness, and cardiac arrhythmia. In cows, low milk production is evident (Goff, 2015b, p.576).

Potassium (K)

Potassium is the principal intracellular cation in mammals (Van Saun *et al*., 2006). The major intracellular cation is K+ and, accordingly, it is primarily responsible for maintenance of electrical neutrality. Other intracellular cations in smaller amounts involved in maintaining electrical neutrality are Na^+ and H^+ (Reece *et al*., 2015). Normokalaemia, hyperkalaemia, and hypokalaemia refer to normal, increased, and decreased concentrations of plasma potassium cations respectively. The normal range of potassium is 3.9 to 5.8 mmol/L (Radostits *et al.*, 1994; Reece *et al*., 2015).

Hypokalaemia is a decreased concentration of K^+ ions in the extracellular fluid (ECF). This has an effect on nerve and muscle fibre membranes that prevent transmission of normal action potentials. Severe muscle weakness often develops. According to Radostits *et al.*

(1994), the common occurrence of hypokalaemia is in diseases of the abomasum which cause stasis and accumulation of fluid in the abomasum. Potassium becomes sequestered in the abomasum along with hydrogen and chloride resulting in hypokalaemia, hypochloraemia and metabolic alkalosis. Hypokalaemia and metabolic alkalosis are often accompanied by muscular weakness and paradoxic aciduria. Hypokalaemia causes muscle weakness by lowering the resting potential of membranes resulting in decrease excitability of neuromuscular tissue. Hence, hypokalaemia is indicated in differential diagnosis of animal with muscle weakness (Radostits *et al*., 1994).

Hyperkalaemia is an increased concentration of K^+ ions in the extracellular fluid (ECF). High concentrations of potassium interfere with membrane potentials that may lead to cardiac toxicity, including weakness of contraction and arrhythmia. Potassium is important in acid–base balance. In alkalaemia, hydrogen ions leave the cells, entering the ECF in exchange for potassium ions that become intracellular. The hydrogen ion exchange for potassium ions leads to hypokalaemia. The intracellular loss of hydrogen ions also occurs in the renal tubular epithelial cells whereby hydrogen ion secretion is decreased, permitting correction of alkalaemia. In acidemia, hydrogen ions enter the intracellular compartment in exchange for potassium ions that leave the cells to maintain electrical neutrality. The hydrogen ion exchange with potassium ions increases potassium ion concentration in the ECF that leads to hyperkalemia. The intracellular increase in hydrogen ions also occurs in the renal tubular epithelial cells. As a result, hydrogen ion secretion from the renal tubular epithelial cells increases and it is associated with increasing bicarbonate return to the ECF, assisting correction of the acalemia (Reece *et al.*, 2015).

Chloride

Chloride is the most abundant anion in extracellular fluid (Soetan *et al*., 2010). Chloride (Cl^-) is an important component of many secretions (e.g., gastric fluid, sweat, and saliva in horses), as either NaCl, KCl, or HCl (Latimer, 2011). Acute gastric dilatation, impaction or torsion of abomasum, and acute intestinal obstruction will lead to failure of gastic H^+ and Cl^-) ions to be reabsorbed by small intestines resulting in hypochloraemia. This condition leads to alkalosis when buffer mechanism is retention of H^+ compensated by excess K^+ excretion. This also results in hypokalaemia (Radostits *et al.*, 1994). These conditions result in muscle weakness, recumbency, depression, muscle tremor, cardiac arrhythmia and coma (Radostits *et al.*, 2007).

Bicarbonate

Excessive loss of bicarbonate ion (HCO_3^-) in acute enteritis usually results in acidosis. Acid-base balance is evaluated by measuring components of the bicarbonate buffer system instead of the phosphate buffer system because the latter is predominately an intercellular ion (Latimer, 2011).

A slight decrease in anion gap occurs when unmeasured cations increase (e.g., hypercalcemia or hypermagnesemia), thereby reducing Na^+ or when unmeasured anions decrease (e.g. in hypoproteinemia). In hypoproteinemic alkalosis, both $HCO3^-$ and Cl^- will increase in order to fill the protein gap. Thus, a decrease in anion gap is best associated with hypoproteinemic alkalosis, whereas an increase is usually associated with some form of metabolic acidosis due to retention of acid as seen in ketone acids, acetoacetic and β-hydroxybutyric acids (Reece *et al*., 2015).

In cow ketosis, the use of body tissues in response to negative energy balance leading the build-up ketone bodies (by its acidic nature) decrease the natural buffering capacity of bicarbonate (HCO_3^-) which in turns increase the anion gap in the blood. This causes changes in pH by movements of electrolytes, water, and carbon dioxide (CO_2) (Herdt *et al*., 2000).

Magnesium

Magnesium (Mg^{2+}) is a major intracellular cation that is a necessary cofactor for enzymatic reactions vital to every major metabolic pathway. Magnesium cation interacts with the negatively charged adenosine triphosphate (ATP) to form Mg-ATP, a substrate for most kinase-catalyzed reactions. Adenylate cyclase, responsible for producing the second messenger cyclic AMP; acyl-CoA synthetase, plays a role in β-oxidation of fatty acids. Succinyl-CoA synthetases, a key enzyme in the citrate cycle are all magnesium-dependent enzymes. Glycolysis involves seven key enzymes that require magnesium alone or in asssociation with ATP or AMP. The intracellular magnesium concentration is about 13 mmol/L, making it is the second most abundant cation found inside of cells. Magnesium is vital to normal nerve conduction. Plasma magnesium concentration is normally 0.75–1.0 mmol/L or 1.8–2.4 mg/dL (Meyer *et al*., 1992; Golf, 2015b). Serum Mg^{2+} level of 0.5 - 1.1 and 1.5 – 2.9 mmol/L, have also be reported by Radostits *et al*. (1994) and Merck Manual (2012), respectively.

Hypomagnesaemic tetany is most often associated with beef cows and ewes in early lactation grazing pre-bloomed and succulent pastures high in potassium and nitrogen and low in magnesium and sodium. This is the most common situation and is often referred to

as grass tetany, spring tetany, grass staggers or lactation tetany. Magnesium deficiency occurs most often in spring or fall when pastures are growing at maximal rates, and is most common in grazing lactating ruminants as milk production removes 0.15 g magnesium from the blood for each liter of milk produced. Ewes suckling more than one lamb and higher-producing cows are at greatest risk. Magnesium must be constantly ingested as it cannot be mobilized from body tissues to maintain normal plasma magnesium concentrations (Goff, 2015a).

Serum phosphorus

Phosphorus is an essential mineral for animal growth and reproduction (Cheng, 2001) Serum phosphorus (P**)** concentration ranges from 5.6 – 8.0 mg/dL or 1.61 – 2.60 mmol/L (Weiss and Wardrop, 2010; Latimer.2011). Phosphorus, in its various anionic forms, functions with phosphoric acid as a buffer system in body fluids (Latimer, 2011). According to Cheng (2001), eighty-five percent (85 %) of P is stored in the skeleton in the form of hydroxyapatite with Ca while 15% is present in cell cytosol, cell membrane, and body fluid. The organic form of P is primarily present as phospholipid in cell membranes and as an energy-carrying molecule, adenosine triphosphate (ATP), in cells. Of less than 1% of total body P is in blood, a large amount of blood P is within the RBC as 2-3-diphosphoglyceric acid which involves the binding of oxygen to haemoglobin. Total P in blood is about 4.32 mmol/L (14mg/dL) and only about 1.29 – 1.94 mmol/L (4-6 mg/dL) is in the form of inorganic phosphates (Cheng, 2001). The inorganic phosphates in serum exist as phosphate salts with Ca, Mg and Na or as dibasic (HPO_4^{2-}) and monobasic (H_2PO_4) ions. Serum phosphates serve as a buffer system for the maintenance of acid/base balance.

In clinical medicine, serum inorganic phosphate is also measured for diagnostic and therapeutic hypophosphataemic cows should be treated with phosphate solution to prevent muscle and nerve damage (Cheng, 2001). At last trimester of pregnancy, serum P has been observed to be lower (4.03±0.18 mg/dL) than the values for normal healthy dairy cattle (Pal and Bhatta, 2013). Calcitonin's effects are to decrease serum calcium and phosphorus. It produces these changes through the mechanisms of inhibition of PTH-stimulated bone resorption and increased phosphorus excretion by the kidney (Latimer, 2011).

2.5.5 Factors influencing serum biochemistry in dairy cattle

2.5.5.1 Breed

Total protein, Cholesterol, AST, ALP, Ca, and Mg concentration have been documented to be influenced by breed (Prisacaru, 2014). Albumin, triglycerides, glucose, uric acid, urea, amylase, alanine amino transferase and phosphorus levels of are not different in the Fleckvieh, Pinzgauer, and Black Spotted Romanian cattle (Prisacaru, 2014). Differences in AST, ALT, ALP have been observed in Tharparkar cattle and Sahiwal breed in India (Aggarwal *et al*., 2016).

2.5.2.2 Season

Increase in ALP and ALT values have been reported in Sahiwal during different seasons with the great increase in ALT higher during summer season among different breeds of cattle and Murrah buffaloes (Aggarwal *et al*., 2006).

Total protein levels are lower in young animals and higher in mature animals whilst albumin levels are lower at youth and increased in adult. Urea and albumin also increased

with age (Otto *et al*., 2000). One of the main functions of calcium and inorganic phosphorus is their involvement in skeletal growth in young animals. In older animals there is a decreased need for calcium and organic phosphorus. Aspartate aminotransferase levels increased with age (Doornenbal *et al.*, 1998; Prisacaru, 2014).

2.5.5.3 Nutrition

Serum albumin is a very sensitive and early nutritional indicator of protein status (Agenas *et al.*, 2006). Malnutrition is the prime cause of nutrient deficiencies which lead to productive disorders such as glycaemia, hypocalcaemia, hypomagnesaemia, ketosis, and acidosis (McDonald *et al*., 2011). Poor nutrition produces a fall in haematological and biochemical indices in cattle (Kaur *et al*., 2017). Obese *et al*. (2018) observed that total protein, and albumin levels increased in cows supplemented with 2.5 kg of feed a day, while NEFA reduced with the feed supplementation.

2.5.5.4 Physiological state

Grass tetany or grass staggers occurs in early lactation following grazing on pre-bloomed and succulent pastures high in potassium and nitrogen and low in magnesium and sodium (Goff, 2015b). Gestational diabetes mellitus is often seen in pregnant woman as a result of carbohydrate intolerance at onset or first recognition during pregnancy (Szablewski, 2011). Multiparous women have a very high prevalence of gestational diabetes mellitus (Wagaarachchi *et al*., 2001). During pregnancy, women with gestational diabetes do have high serum triacylglycerol concentrations but lower LDL-cholesterol concentrations than do healthy pregnant women (Koukkou *et al*., 1996).

Hypoglycaemia induces neuronal death (Lacherade *et al.*, 2009). The neuronal death resulting from hypoglycaemia is not a straightforward result of energy failure but instead results from a sequence of events initiated by hypoglycaemia (Suh *et al*., 2007). These events include activation of neuronal glutamate receptors (Nellgard and Wieloch, 1992), production of mitochondrial reactive oxygen species (Singh *et al*., 2004), neuronal zinc release and extracellular release of excitatory amino acids (glutamate and aspartate) (Szablewski, 2011).

2.5.5.5 Stress

Elevation in glucose concentrations in serum has been reported to be correlated positively with stressful condition. Increased glucose concentrations have been obtained in cattle in a disturbed condition (Prisacaru, 2014). The rapid rise in NEFA on the calving day is due to the calving stress (Bačić *et al.,* 2007).

2.5.5.5 Postpartum period

Changes in endocrine status and a decrease in dry matter intake (DMI) during late pregnancy influence metabolism and lead to fat mobilisation from the adipose tissue and glycogen from the liver (Holtenius, 2003). Plasma nonesterified fatty acids (NEFA) increase more than a two-fold between 2 to 3 weeks prepartum and 2 to 3 days prepartum, at which time concentration increases dramatically until completion of parturition (Vasquez-Anon *et al.*, 1994; Grum *et al.,* 1996; Bačić *et al.,* 2007). Plasma NEFA concentration decrease rapidly after calving but concentration remain higher than they were before calving (Bačić *et al.,* 2007).

2.5.5.6 Disease/Disorder

Higher concentrations of NEFA and BHBA have been reported in cows with retained placenta postpartum (Radostits *et al*., 1994; Civelek *et al.*, 2011). Subclinical milk fever (MF) or hypocalcaemia contributes to appetence in the fresh cow and predisposes the cow to develop other diseases. Cows that have had MF are more susceptible to other disorders such as retained placenta, ketosis, displaced abomasum and mastitis (Bačić *et al*., 2007). Heifers almost never develop MF. The risk increases with age (Horst *et al.*, 1990).

2.5.5.7 Hormonal influence

An indication that at least part of the prepartum NEFA increase is hormonally induced has been noticed (Bačić *et al.,* 2007). The parathyroid glands monitor carotid artery blood calcium concentration and secrete parathyroid hormone (PTH) when they sense a decrease in blood calcium (Goff, 2015a).

2.6 Reproductive Hormones of Dairy Cattle

2.6.1 Endocrine control of reproduction, puberty and oestrous cycle in dairy cows

The major hormones involved in the control of reproduction include follicle stimulating hormone (FSH), luteinizing hormone (LH) which are glycoproteins; oestradiol, progesterone, and testosterone (male) which are steroid hormones and are produced by the steriodogenic tissues of the gonads, and to lesser extent, zona reticularis tissues of the adrenal gland (Cunningham and Klein, 2007). Hypothalamic hormones, Gonadotrophin releasing hormone (GnRH) stimulates the release of FSH and LH (collectively called gonadotrophic hormones or gonadotrophins) and binds to receptor cells of the anterior pituitary to facilitate secretion of LH and FSH (Reece *et al*., 2015).

2.6.1.1 Gonadotrophic hormones

The FSH and LH, stimulated by GnRH via hypothalamo-hypohyseal portal system, are secreted by gonadotrophs within the pas distalis of the anterior pituitary cells (Reece *et al.,* 2015). The GnRHs are produced in the hypothalamus and are released through hypothalamus – pituitary complex coordination which functions as a command centre of the endocrine system. Perpetuation of species survival and reproductive competence is, therefore, governed by complex physiological processes regulated by structural framework through hypothalamus-pituitary-gonadal axis that regulate the release of gonadotrophic hormones—GTH (Norris and Lopez, 2011). The relationship between the hypothalamic hormones (GnRHs) and adenohypophysial hormones and in particular, the effect of GnRH on secretion of FSH and LH cannot be overlooked. It is worth noting that, several factors influence the release of GnRH viz: day length, age, plane nutrition, and other hormones interactions (negative and positive feedback mechanisms) (Reece *et al.*, 2015). It has been documented in prepubertal animals that exogenous GnRH is capable of stimulating secretion of FSH and LH which in turn stimulate follicular development in the ovary (Akers and Denbow, 2013). In Sheep, puberty is controlled by an increase in the synthesis and release of GnRH from the hypothalamus which drives GTH secretion in pulsatile form and follicular growth (Cunningham and Klein, 2007).

2.6.1.2 Follicle stimulating hormone

Follicle stimulating hormone (FSH) is a glycoprotein produced by the pituitary gland and consists of two subunits (α- and β-subunits) with an approximate total molecular mass of 35 kDa. The β-subunit is unique to FSH and confers its specific biological activity of the molecule. FSH has a major effect on the growth and development of ovarian follicles, thus,

the capacity of the hormone to induce estrogen secretion by the theca cells surrounding developing oocytes. As the waves of follicles develop during the oestrous cycle of the cow, for example, only one of a cohort of follicles becomes dominant. This follicle proceeds to enlarge and develop so that it becomes the follicle destined for ovulation (Akers and Denbow, 2013).

Follicle stimulating hormone stimulates gamete production in both male and female, and causes accelerated growth of primary follicles each month resulting in rapid proliferation of the granulosa cells, giving rise to many more layers of these cells (Guyton and Hall, 2006). FSH stimulates the granulosa cells in the wall of the developing follicle to secrete oestrogen (Reece *et al.*, 2015). FSH primarily regulates gametogenesis, in both sexes (Norris and Lopez, 2011).

The FSH control of oestrogen secretion and changing sensitivity of the hypothalamus is tied to an acute increase in secretion of LH, which promotes ovulation of the dominant follicle. This is the ovulatory surge in LH. This not only causes rupture and release of the egg, but it also promotes remaining follicular cells to differentiate into luteal cells and create a corpus luteum (yellow body) (Akers and Denbow, 2013).

2.6.1.3 Luteinizing hormone

Luteinizing hormone (LH) derives its name from luteinization process. LH also called interstitial cell hormone has a molecular weight of approximately 30 kD. This hormone is secreted by the pars distalis (anterior lobe of pituitary) of the adenohypophysis (Akers and Denbow, 2013; Reece *et al*., 2015). At puberty or at the start of the breeding season there is

associated increased pulse frequency because of tonic gonadotrophin secretion. There is increased frequency of pulsatile LH release, which stimulates the onset of cyclical activity. Progesterone appears to play a critically important role in the inhibition of the tonic mode of LH secretion in the ewe (Noakes *et al.*, 2001). Progesterone is thus the main regulatory hormone which controls the estrous cycle of the sheep and other ruminants. Thus, when the concentration of progesterone in the circulation falls in association with the regression of the corpus luteum, there is a release of LH from the anterior pituitary. The rise in LH triggers the secretion of oestradiol. This sudden rise stimulates the surge centre for the LH release and, as a result of this sudden increase, ovulation of the mature follicle occurs. In cows, there is also a concomitant surge in FSH, though its significance is unclear, it may be part of the ovulation-inducing hormone complex (Noakes *et al.*, 2001). Ovarian follicles require a 2 – 3 day period of increasing levels of LH concentrations in circulation in order to mature fully prior to ovulation. Pre-ovulatory LH surge induces ovulation only if a follicle at the appropriate stage of development is already present. The beginning of LH surge has been used to predict the time of ovulation. Thus, 27 hours from the beginning of the surge to ovulation has been noticed with accuracy, due to the association between LH surge and ovulation (Ball and Petters, 2004). Luteinizing hormone has been considered to be the main, if not the sole, luteotrophic hormone in the non-pregnant cow (Ball and Petters, 2004). The basal secretion of LH in men is episodic and has the primary function of stimulating the interstitial cells (Leydig Cells) to produced testosterone. For ovulation to occur at puberty or beyond, a marked surge in the LH concentration is required, a condition also called preovulatory surge (Akers and Denbow, 2013). Luteinizing hormone induces the ovulated follicle to form a corpus luteum (CL), and the luteinized granulosa cells now produce progesterone (Goff, 2015b).

2.6.1.4 Synthesis of reproductive steroid hormones

The innermost zone of the adrenal cortex, zona reticularis, in addition to the production of some glucocorticoids, uniquely also secretes androgens which are produced from cholesterol through cleaving off its side chain to form pregnenolone. This results in the production of progesterone, testosterone and oestradiol (Reece *et al.*, 2015). Luteinizing hormones is major regulator of testosterone secretion by the Leydig cells found in the interstitial tissue surrounding the seminiferous tubules of the testes. The synthetic pathway for the steroid hormones is shown in Fig. 2.1.

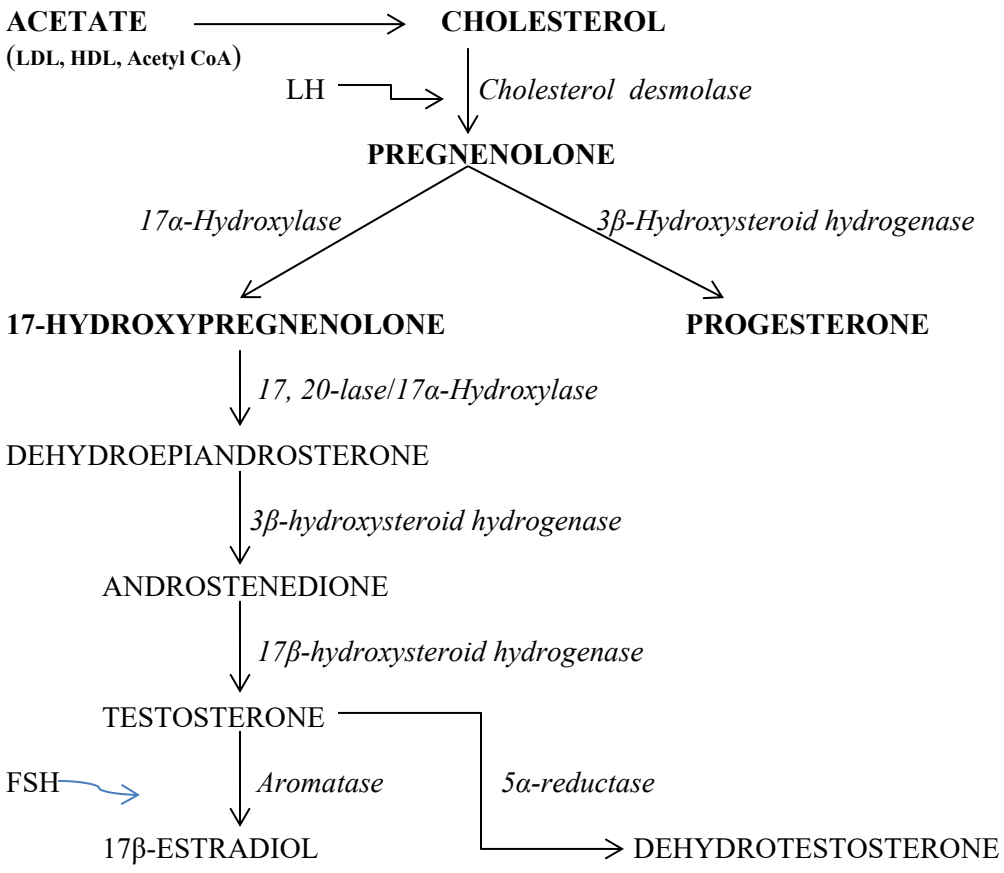

Fig. 2.1. Flow diagram showing synthesis of reproductive steroid hormones (Adapted from Ball and Petters, 2004).

Reproductive steroid hormones are synthesized from cholesterol which is in turn produced from acetate inside the cell or alternatively taken up from the blood. Principal biologically active oestrogen is oestradiol-17. The others, oestriol and oestrone, may be considered as metabolites of oestradiol. Both the theca interna and granulosa layers of the follicle are involved in the synthesis of oestrogen (Ball and Petters, 2004).

2.6.1.5 Serum and milk hormonal levels

2.6.1.5.1 Progesterone

Progesterone (P_4) is a C_{21} steroid which is synthesized from both tissue and circulating cholesterol. Cholesterol is transformed to pregnenolone which is then converted via a combined dehydrogenase and isomerase to progesterone (Reece *et al.*, 2015). Progesterone concentration in milk is dependent upon the fat content. Different threshold values of P_4 have been reported for assessment of the onset of oestrus and luteal activity (Garmo, 2010). Occurrence of two or more consecutive milk P_4 concentrations of ≥ 3ng/mL in whole milk in commencement of luteal function or activity have been documented (Royal *et al*., 2000; Petersson *et al.*, 2007).

Whereas onset of luteal function has been observed in the first day with P_4 concentration ≥ 5 ng/mL in pooled quarter milk samples (Samarütel *et al*., 2008), Opsomer *et al.* (2000) notice the first rise in milk progesterone of ≥ 15 ng/mL in milk fat. Shrestha *et al*. (2004) also observed two consecutive concentration of P_4 in skimmed milk samples of ≥ 1ng/mL.

The concentration of P_4 in fat is 50 – 100 times higher than in the aqueous phase of whole milk. To obtain more precise physiological progesterone values, it is preferable to estimate

it's concentration in fat-free skim milk. The range of P_4 in skim milk is 0.2-12.5 ng/mL. For routine classification purposes, P_4 values < 0.4 ng/mL correspond to the early follicular phase, 0.4 – 1.0 ng/mL to the early or ending luteal phase and > 1.0 ng/mL in skim milk indicates full luteal activity (Prakash *et al.*, 1988). The lowest P_4 assayed from pregnant cows is reported to be 7 ng/mL (Garmo, 2010).

Plasma and milk P_4 concentrations rise during the first few days of pregnancy in a similar manner to that occurring in the early luteal phase of the non-pregnant animal (Ball and Peters, 2004). It has been recently and consistently reported that, cows in which pregnancy fails tend to have lower P_4 concentrations in early pregnancy (Mann and Lamming, 1999; Mann *et al*., 1999). It is, therefore, apparent that cows with lower P_4 levels of ≤ 1 ng/mL as soon as day 5 after insemination are likely to have lower pregnancy rates (10 %) whereas P_4 level of 1, 2, 2-3 and ≥3 ng/mL have pregnancy rate of 30, 40, and 50 % respectively (Starbuck *et al*., 2001; Ball and Peters, 2004). It is also noted that low and excessive levels of P_4 5 to 7 days post artificial insemination (AI) or service are associated with low probability of embryo survival (Petersson, 2007) which is an indication that optimum P_4 levels is required for embryo survival (Stronge *et al*., 2005).

Hormonal levels in blood and/or milk are also influenced by the physiological state of the animals and may vary in blood and milk. It has been observed by Gorecki *et al*. (2004) in nanny goats/does that progesterone and oestradiol levels in blood are higher than the levels in milk in that blood P_4 levels reached 13.5 – 16.7 ng/mL in day 6 and 40 of gestation as against levels in milk. Oestradiol level followed a similar trend in blood and milk.

2.6.1.5.2 Oestrogen

Oestrogen occurs naturally and synthetically (diethylstilbestrol). The important oestrogens in mammals are steroids, produced by the ovary (granulosa cells of follicles), placenta, and in the zona reticularis of the adrenal cortex (Reece *et al.*, 2015). Oestradiol (E_2) is a steroid hormone with a molecular weight of 272.4. Oestradiol is part of the oestrogens group of hormones and it is the principal oestrogen in females. Oestrone and oestriol are chemically similar to oestradiol but are found in lower concentrations and have a lower estrogenic activity. Concentrations of oestrogen during the preovulatory period influence the establishment and maintenance of pregnancy by altering the uterine environment. Oestradiol has been proposed to induce FSH/ LH receptor expression in granulosa cells and increase the stimulatory action of FSH on aromatase activity (Zhuang *et al*., 1982; Mekonnin *et al*., 2017).

Oestrogen can influence the growth of the uterus and the mammary gland. Oestrogen has a stimulatory effect on hypothalamic secretion of GnRH. This positive feedback eventually reaches an end point where sufficient GnRH secretion has been stimulated to cause a spike in LH secretion resulting in ovulation of the mature follicle usually at puberty and beyond (Reece *et al*., 2015). Oestrogens circulate in blood loosely bound to albumin and tightly bound to the testosterone–estradiol-binding globulin which is also called the sex-hormone binding globulin. Plasma concentrations of estrogens are considerably lower than those of other gonadal steroids and vary over an almost 20-fold range during oestrous cycle (Johnson, 2003). At other times in an animal's reproductive life, estrogen inhibits LH and FSH secretion by the adenohypophysis (Reece *et al*., 2015).

Oestrogen performs very important roles in development and reproduction. According to Reece *et al*. (2015), the following functions played by oestrogen are crucial:

1. Stimulation of endometrial gland growth;
2. Stimulation of duct growth in the mammary gland and also contributes to the growth and development of mammary tissue and prepare the uterus for parturition;
3. Increases in secretory activity of uterine ducts;
4. Initiation of sexual receptivity;
5. Regulation of secretion of LH by the anterior pituitary gland;
6. Possible regulation of $PGF_2\alpha$ release from the non-gravid and gravid uterus;
7. Early union of the epiphysis with the shafts of long bones, where growth of long bones ceases;
8. Protein anabolism; and
9. Epitheliotropic activity.

2.6.1.5.3 Androgens/Testosterone

Testosterone (17β-hydroxyandrost-4-ene -3-one) is a C-19 steroid with an unseated bond between C-4 and C-5, a ketone group in C-3 and hydroxyl group in the β position at C-17. Testosterone has a molecular weight of 288.4 Dalton (Da). Luteinizing hormones is major regulator of testosterone secretion by the Leydig cells found in the interstitial tissue surrounding the seminiferous tubules of the testes, while FSH stimulates development and function of the Sertoli cells that nourish and regulate developing spermatozoa (Akers and Denbow, 2013; Reece *et al.*, 2015). Testosterone is the most important androgen secreted into the blood. In females 50% of circulating testosterone is derived from peripheral conversion of androstenedione, 25% from the ovary and 25 % from the adrenal glands.

Testosterone is responsible for the development of secondary male sex characteristics and its measurements are helpful in evaluating the hypogonadal states. In adult female, high levels of testosterone are generally found in hirsutism and virilization, polycystic ovaries, ovarian tumours, adrenal tumours and adrenal hyperplasia. In male, high levels of testosterone are associated to the hypothalamic pituitary unit diseases, testicular tumours, congenital adrenal hyperplasia and prostate cancer. Low levels of testosterone can be found in association with diseases such as hypopituitarism, Klinefelter's syndrome, testicular feminization, orchidectomy and cryptorchidism, enzymatic defects and some autoimmune diseases (Guyton and Hall, 2006; Akers and Denbow, 2013).

According to Guyton and Hall (2006), testosterone has a specific effect on the pelvis to (1) narrow the pelvic outlet, (2) lengthen it, (3) cause a funnel-like shape instead of the broad ovoid shape of the female pelvis, and (4) greatly increase the strength of the entire pelvis for load-bearing. In the absence of testosterone, the male pelvis develops into a pelvis that is similar to that of the female.

2.6.1.5.4 Hormonal changes in the oestrus

Puberty is marked by the oestrus which is accompanied by a characteristic behaviour that aids its detection. It is also defined as the ability of cow to reproduce as a result of the development of hypothalamic neurons that secrete GnRH at the appropriate frequency and in sufficient quantities to release gonadotropins and promote gametogenesis (Sartori *et al.*, 2010). Oestrus/heat is a period of acceptance for mating (sexual receptivity) that normally occurs in non-pregnant, and/or pubescent cows (Wattiaux, 2011). The first ovulatory

oestrus is the main marker of puberty (Rawlings *et al*. 2003). The estrous cycle is defined as the interval from the day of onset of sexual receptivity to mating in oestrus to the onset of the next period of sexual receptivity (Norris and Lopez, 2011)

Oestrus in cow is classically divided into cyclical phases (Oestrous cycle) which encompasses Oestrus—the period of sexual receptivity usually considered day 0, metoestrus—the postovulatory phase (i.e. day 1 – 4), dioestrus occurring day 5 – 18 when an active corpus luteum persists, and pro-oestrus—the period just before oestrus occurring at day 18 – 20 (Ball and Petters, 2004). The length of oestrous cycle ranges from 18 – 24 days in cows (Goff, 2015b). It is imperative to know that, there are significant number of cows which experience silent heat during the oestrus (Mukasa-Mugerwa, 1989; Ball and Petters, 2004). The most reliable behavioural sign of oestrus is standing to be mounted (Garcia *et al*., 2017). Other reliable signs that aid oestrus detection include licking and rubbing each other; sniffing the vagina of another cow; mutual chin resting; and lining up to mount another cow.

Behavioural signs of oestrus can be observed in cattle and used for oestrus detection for the purposes of artificial insemination (AI), bearing in mind that heifers mostly reach maximum fertility on their third oestrus (Byerley *et al*. 1987; Ball and Petters, 2004). However, these characteristics are influenced by age, milk yield, environment and hierarchy. Additionally, differences can be noted among breeds and even among genetic groups (*Bos indicus* vs *Bos taurus*). Breed differences regarding puberty, oestrous cycle patterns, oestrous behaviour, acquisition of ovulatory capacity, ovarian structures and reproductive hormones have been reviewed by Sartori *et al*. (2010).

2.6.1.5.5 Hormonal changes in the oestrous cycle

The normal oestrous cycle is regulated by a complex interplay among hormonal secretions of the hypothalamous, pituitary, ovaries and uterus. Progesterone is the major hormone regulating the oestrous cycle. During dioestrus the corpus leutium (CL), formed through luteinization of granulosa tissues (Goff, 2015b), produces high amount of progesterone and this act as a negative feedback control of the hypothalamus and pituitary, inhibiting GnRH-FSH-LH release. The presence of high progesterone level in circulation also inhibits behavioural oestrus—a similar condition occurs in pregnancy. The elevated progesterone level usually occur for 8 – 12 days before the first oestrus occur (Ball and Peters, 2004; Cunninghan and Klein, 2007). Resumption of cyclicity is usually considered as two consecutive samples with concentrations of progesterone ≥ 1.5 mg/ml (Martins *et al.*, 2012). A small surge in FSH and LH occur in dioestrus that help to maintain the CL.

Waves of follicle development seen during metoestrus (wave 1) and dioestrus (wave 2) stimulated by FSH surge, results in development of a large dominant follicle in each wave. These waves follow an oestrogen surge. In the absence of pregnancy the uterus secretes $PGF_{2\alpha}$ which induces luteolysis and drop progesterone levels (Reece *et al*., 2015). The decline in progesterone removes the block to hypothalamo-pituitary activity and it is followed by pulsatile release of GnRH, FSH and LH, leading to follicular growth and increased oestrogen release. Two or three days later, a surge of estrogen release exerts a positive feedback effect on hypothalamus followed by a preovulatory surge in GnRH, FSH and LH. This oestrogen surge also induces the characteristic oestrous behaviours. An increased release of inhibin by the ovaries at the same time prevents over-production of FSH which might cause follicular atresia. The preovulatory LH surge causes ovulation of

the dominant follicle at the time of CL regression. The LH surge lasts for about 6 – 10 hours. Ovulation occurs 24-30 hr in cows after the surge. During oestrous cycles, oestrogen levels of 20.24 and 12.36 ng/ml on the day of oestrus and day 15 of the oestrous cycle, respectively, have been observed (Naik *et al.*, 2013).

2.6.2 Age at puberty

Puberty is the time at which oestrus first occurs or the age at which reproductive competence is achieved (Ball and Peters, 2004; Akers and Denbow, 2013). Ganong (2003) strictly defined puberty in first class animals as the period when the endocrine and gametogenic functions of the gonads have first developed to the point in which reproduction is possible. Indeed, the age at which the heifer can conceive and support pregnancy without health concerns is crucial (Akers and Denbow, 2013). This is associated with the age at first oestrus, and it is an important index for reproductive efficiency. Changes that occur at puberty also depend on the production of the female gametes and the synthesis of hormones (Noakes *et al*., 2001). Taurine breeds of temperate origin attain puberty fairly earlier and may vary within the same breed (Mukasa-Mugerwa, 1989).

2.6.3 Pregnancy and gestation period

Pregnancy or gestation occurs after successful service resulting in a condition in which unborn foetus is being carried in the uterus. The foetus is carried for a length of period (gestation period) ranging from 274 to 291 days, averaging 282 days in cows (Goff, 2015b). In other words, it is a period of conception to parturition/calving. Gestation length in beef cows average 287.7 ± 9.33 days compared with 281.5 ± 7.25 in dairy cows (Rodriguez *et al*., 2013). Gestation period for Sahiwal in India, zebu cattle in Ethiopia,

Cuba ranges from 278.1 – 289.5, 279.8 – 281.0 and 281.5 – 291 days respectively. Whereas, West African Shorthorn (WASH), N'dama, Sokoto Gudali of West Africa and Africader of Zambia record 285.3, 288.5, 290.0 and 297.5 days, respectively (Mukasa-Mugerwa, 1989). Mean gestation period for Sanga cows in Accra plains of Ghana is 282 days (277 – 285 days) and 292 at smallholders' level (Obese *et al*., 1999; 2008).

2.6.4 Post-partum anoestrus or interval

Anoestrus, the primary factor reducing reproductive efficiency in dual-purpose cow-calf operations is characterized by lack or absence of the expression of oestrus. Anoestrus occurs annually in anoestrus cows after each calving (post-partum anoestrus) whiles heifers experience anoestrus prior to puberty (pre-pubertal anoestrus) (Whittier *et al*., 2008). Post-partum anoestrus is a biological protection for dam and offspring (Whittier *et al*., 2008).

Physiological mechanisms associated with anoestrus involve blockage of the GnRH "pulse generator" in the hypothalamus, but other pathways also must be involved because bypassing the pulse generator is not an effective treatment for all cows (Short *et al*., 1990). The underlying cause of anoestrus may differ from different stages of anoestrus. Lactational (year of calving in harsh environments with negative energy feedback), seasonal (as seen in seasonal breeders—ewe, and mare) and nutritional anoestrus occur when there is inadequate feed availability to support the dam bringing a competing calf into this environment (Whittier *et al*., 2008).

Management and control of the resumption of oestrous cycles in the post-partum cow are accomplished by a complex relationship among the hypothalamus, pituitary and ovary

which, in addition, is influenced by a variety of external and internal signal (Short *et al*., 1990). Factors affecting the duration of post-partum anoestrus, includes age, breed, pre- and post-partum nutrition, body condition at calving, milk yield, suckling, calving season, presence or absence of the bull, delayed uterine involution, dystocia and the general health status of the cow have been reviewed by Ahmadzadeh *et al.* (2011).

2.7 Productive Performance of Dairy Cattle

2.7.1 Milk yield

Holstein-Friesian is capable of producing 12,000 kg of milk in one lactation (Jorritsma *et al*., 2008). Jersey cows are noted to be hardier, however, their daily milk yield performance stands at 7 kg under tropical environment (Wangdi *et al*., 2014; Fernando *et al*., 2016). On the other hand, milk yield of local cattle for both taurine breeds and Zebu are low, though the latter surpasses the former in milk yield (Otchere and Okantah, 2001; Aboagye, 2002; Oppong-Anane, 2013). In Burkina Faso, lactation yields in Zebu cows have been reported to vary between 500 and 1,000 kg per lactation under practical farming conditions (Millogo, 2010). In Ghana, researchers have found out that cows of 6 to 9 years can give a maximum daily yield, ranging from 4 to 9 kg, which is attainable between 3 and 7 weeks after calving in the on-station WASH (Aboagye, 2002).

2.7.1.1 Factors affecting milk yield of cows

Many factors can be attributed to the low milk production in West African countries. The production systems are mainly extensive (Millogo, 2010), characterized by low investment with resultant low output (Ngongoni *et al*., 2006). Factors limiting milk production in West Africa and the tropics as a whole, include genetic/breed factors, and non-genetic

components embracing feed resources, climatic, sociological and pathological factors (Ngongoni *et al.,* 2006).

2.7.1.1.1 Genetic factors influencing milk yield

Milk yield is largely determined by genetic factors that depict low genotypic potential of the indigenous animals (Millogo, 2010), although milking frequency and efficiency of stripping can significantly affect milk yield per cow (De-Peters *et al*., 1985; Gisi *et al*., 1986). According to Jonhson (1991), milk yields are product of animal genetic and environmental interactions. Pagot (1992) contended that cattle breeds which originate from the tropics generally have limited genetic potential for milk production and remain mediocre producers (500-1500 kg per lactation) even when the best possible husbandry condition have been provided for them. However, these breeds are acclimatized to the prevailing climatic condition.

2.7.1.1.2 Non-genetic factors influencing milk yield

Management and environmental factors

Management of dairy animals can have effects on cows' productive performance (Bee *et al*., 2005). It has been found that local dairy cows and exotic breeds (Holstein-Friesian and Jersey) kept under different management in a given environment equally perform differently (McDonald *et al.*, 2011). Ngongoni *et al.* (2006) noted that Friesian and Jersey managed at commercial farms in Zimbabwe produced 21.8 and 15.4 litres of milk/day respectively, while those on smallholder farms yielded 7.0 and 6.1 litres/day. Different management system perhaps good housing, routine health checks and feed supplementation may account for higher yield at the commercial farms. Additionally, Lateef *et al.* (2008)

observed that in Pakistan, imported Friesian cows performed better than the farm born ones, resulting from differences in management prior to productive stages.

In addition, environment and location also play a critical role in both local cattle and exotic dairying (Ngongoni *et al.*, 2006). Poor environmental adaptation hindered survival of the exotic dairy breeds (e.g. Holstein–Friesian and Jersey) and their crosses in Ghana (Otchere and Okantah, 2001). Exotic cattle introduced to the country were found to require high levels of nutrition and management in the humid Ashanti region (Kabuga and Agyemang, 1983; Aboagye, 2002) and were also adversely affected by heat stress on the Accra Plains (Okantah, 1992). The local breeds kept at on-station perform better than those maintained at the smallholders level (Annor, 1996).

Diseases

Diseases such as trypanosomiasis, dermatophilosis and heartwater jeopardize the dream of keeping the high yielding breeds (Koney, 1996; Otchere and Okantah, 2001). This is because of high tsetseflies (*Glosina spp*) bites, ticks infestation and poor adaptation to high temperatures and humid environment (Pagot, 1992; Otchere and Okantah, 2001).

Nutrition/supplements

Major limitations to milk production in peri-urban areas of West Africa are: low availability of feed and water, and poor infrastructure (Millogo *et al*., 2008; Millogo, 2010). Annor (1996) noticed that local cows (WASH, N'dama and Zebu) supplemented in addition to natural grazing regimes produced higher quantity of milk. FAO (2013) stated that, adequate nourishment through supplementation and management early in life

improves performance, health, milk production and longevity. Adequate nutrition is required to meet cows' metabolizable energy for lactation (McDonald *et al.,* 2011). This provides benefits in twofold: less health issues, and more milk production at optimum lactation period combined with equal or even lower raising costs (FAO, 2013).

Parity of cow

Parity indicates the number of births an animal has experienced. Daily average milk yield is affected by parity **(**Darfour-Oduro *et al.,* 2010). According to Epaphras *et al.* (2004), Hatungumukama *et al.* (2006), and Afzal *et al.* (2007) milk yield of cows are low at parity one and increase with increasing parity and decline after fifth to sixth parities. On the contrary, Hatungumukama *et al.* (2008) and Darfour-Oduro *et al*. (2010) found different observations when Friesian cattle were used for milk production in Burundi and Ghana, supposedly as a result of humid environmental effect.

Season of lactation

Seasons influence feed and forage availability as well as quality and quantity of the feed resources (Ngongoni *et al*., 2006). As forages grow they tend to accumulate more fibre at the expense of the nutritive components (McDonald *et al*., 2011). The highest milk yield is usually recorded in major rains (Epaphras *et al.,* 2004; Bee *et al.,* 2005) where feed and forages are in abundance.

Many authors have reported low daily milk yield during the dry and hot periods (Ageeb and Hayes, 2000; Epaphras *et al*., 2004; Hatungumukama *et al.,* 2006). This is one of the many constraints that have effect on milk production in the tropics; that is, the scarce

availability of natural forages during the drier seasons (Ngongoni *et al*., 2006). High temperatures during the dry season tend to reduce feed intake, and with the resultant low milk production (Ageeb and Hayes, 2000). In accordance with Epaphras *et al*. (2004) the critical period, in terms of daily average milk production was from December to February, a period of high ambient temperature and low rainfall.

Body condition score

Body condition score (BCS) is a subjective measure of available tissue reserves and can, therefore, be used to indicate energy balance of the dairy cow (Loker *et al*., 2010). It has been reported that increased BCS is genetically and favourably linked with health and fertility traits (Berry *et al*., 2003; Bastin *et al*., 2010). It has therefore been suggested that BCS is moderately heritable and may be selected for without a large negative impact on milk production (Loker *et al.,* 2010). It is imperative to note that BCS is not genetically correlated with fat percentage (Loker *et al.,* 2010). Body condition score is a moderately heritable trait across lactation and parity.

Dairy cows have physiological ability of providing nutritional substances from their body tissues by losing "body condition" for about 40 to 100 days (Gergovska *et al*., 2011). They restore the lost body reserves after calving and afterwards (Koenen *et al*., 2001; Coffey *et al*., 2004; Pryce and Harris, 2006). The interest in this mechanism is the intensive transgeneration genetic selection towards increase of the total milk yield per lactation and at the beginning of lactation (Gergovska *et al*., 2011). Body condition scores can be used on both heifers and cows, although primarily they are used on the lactating dairy herd. Body condition scores greater than 3.75 at calving can reduce dry matter intake by 1.5 to

2.0 % for every 0.25 BCS increase over 3.75 (Varga and Ishler, 2007). Therefore, monitoring feed intake and days to peak milk production to determine if cows are managed properly with adequate, but not excessive, body condition is crucial (Varga and Ishler, 2007).

Loss of BCS after calving has significant effect on milk yield for 305 days lactation and the highest is the milk yield of cows with the greatest loss of BCS after calving (Gergovska *et al*., 2011). Cows from Friesian and Brown Swiss with low BCS at calving from 2 and 2.5 points have the lowest milk yield for 305 days after calving. The milk yield and lactation period of cows with low BCS (≤ 2.5 points) is by about 1400 kg lower than that of cows with BCS ≥3.5 points (Gergovska *et al*., 2011).

Effect of udder size on milk yield

Deng *et al*. (2012) stated that, the morphological characteristics of the udder are genetic features in dairy cattle and are among the fundamental criteria for selection. Bhuiyan *et al*. (2004) reported that size and shape of udder are very important conformation traits which could play a vital role for the suitability of milking and economical milk production and they should be considered for selecting dairy cows. Ahmed and El-Barbary (2000) stated a selection based on a control table gives 50 % of the score for udder traits. Further, Seykora and Hansen (2000) found out that, the udder is 40 % of the judging score card and often becomes the deciding factor in close placing. Moreover, udder structure and frame size of milking cows are not only important to demonstrate the aesthetic characteristics, but also the high milk output and the low mastitis risk incidence (Bardakcioglu *et al*, 2011). It has been suggested that considerable emphasis be put on milking speed, because slow milking

cows are hinderance to the milking process of the herd in milking (Bardakcioglu *et al*, 2011).

On the other hand, Holmes *et al*. (1984) stated that, the udder shape, size and placement of teats are important in determining the ease with which cows can be milked. It is emphasized that good udder anatomy and milk flow rate have positively corresponded with the daily milk yield but these result do not indicate a strong genetic relationship between udder characteristics and production level. Falvey and Chantalakhana (1999) realized that, size of the udder is not a good guide to the amount of milk a cow will produced.

Effect of teat size on average milk yield

Effect of teat size on milk yield is highly essential and can, therefore, be used as guide for selection. According to Kukovics *et al*. (2006) effect of teat size is prominent for breed and its effect on milk yield seemed to be general, and can be useful in selection of the milk trait. Holmes *et al*. (1984) stated that large teat size facilitates the ease at which milking is done manually or mechanical means. However, Boettcher *et al*. (1998) noted that excessively pendulous teats reduce milking speed.

Frequency of milking

Milking frequency has been considered to positively influence milk yield. The milk yield for a single milking a day was significantly lowest when compared with twice, thrice, and four times daily milking frequency. The four times milking of dairy cows has produced the highest milk yield after which milk yield declined (Vijayakumar *et al*., 2017). Increasing milking frequency from twice to thrice a day has been shown to increase galactopoiesis by

10 to 20% in goat (Knight, 1992; Campos *et al.*, 1994) and 10% to 20% increase milk productions in cow (Vijayakumar *et al*., 2017). Milking frequency can have great effect on milk yield in goat when viewed from the producer's viewpoint (Assan, 2014). The fact remains that the mammary gland in high producing dams is highly resilient and continues lactating successfully with biologically minor decreases in milk yield even under conditions radically different from modern dairy management practices (Assan, 2014).

Regulating the quantity of milk produced involves changes in the level of activity of mammary secreting cells, and also to the cell proliferation/death balance (Boutinaud *et al.*, 2003; 2004). It has been observed that, when less frequent milking is prolonged, the decrease in milk production is sustained by chronological developmental adaptation, initially as a down regulation of cellular differentiation (Stelwagen *et al*., 1994) and later as a total loss of mammary cell number through apoptosis (Boutinaud *et al*., 2004).

2.7.2 Lactation length

Lactation length (LL), defined as the interval (days) between day of calving and drying off, and milk yield are two important traits of dairy animals which might be dictated by genotype and environmental/non-genetic factors (Afzal *et al*., 2007). Darfour-Oduro *et al*. (2010) identified that breed, individual difference and season of calving affect on-station lactation length of Sanga and Friesian-Sanga cows. In Pakistan, lactation length of Nili Ravi buffaloes has been identified to influence milk production and may range from 182 to 447 days (Afzal *et al*., 2007). In Uganda, lactation length has been found to range from 165 to 255 days on the basis of the management practices (Kugonza *et al*., 2011).

2.7.2.1 Factors affecting lactation length

Like milk yield, lactation length is also affected by factors which include genotype, feed supplementation, parity, and season of calving (Bajwa *et al*., 2004; Afzal *et al*., 2007; Darfour-Oduro *et al*., 2010).

2.7.2.1.1 Breed

It has been noted that Sokoto Gudali, together with Bunaji, Shuwa Arab and Kuri are regarded as good milk producers under traditional husbandry (Aboagye, 2002). Total milk yield per lactation is determined by the length of lactation (M'hamdi *et al*., 2012). Acoording to Bajwa *et al.* (2004), Sahiwal cattle breed of Pakistan recorded mean lactation length of 248.0 days and it is considered as one of the best cattle breed for milk production in tropical conditions. In Ghana, Aboagye (2002) reported that on-station studies on local breeds' lactation lengths of WASH, Sokoto Gudali, and N'dama ranged from 29-261, 167-252, and 182-252 days respectively. Mean lactation period of on-station Sanga and Friesian-Sanga cows has been reported to be 164.1 and 201.1 days respectively (Darfour-Oduro *et al*., 2010).

2.7.2.1.2 Parity

Number of parity may have effect on the length of lactation (Watters *et al.*, 2010). It has been noticed that lactation 1, 4 and 5 have lower milk yield as lactation length advances (Epaphras *et al.*, 2004; Darfour-Oduro *et al*. 2010; Watters *et al.*, 2010). However, Afzal *et al*. (2007) and M'hamdi *et al*. (2012) established that there were no differences in lactation length on the basis of different parities or lactation number.

2.7.2.1.3 Sex of calf

Studies conducted by Afzal *et al*. (2007) on milk yield per lactation length of buffaloes on the effect of sex of calf showed no significant effect. Habib *et al*. (2010) also reported that sex of calf has little or no influence on lactation length (days), lactation yield (kg), daily milk yield (kg), and dry period (days) in Red Chittagong Cattle. Quesnel *et al*. (1995) conversely noticed in dairy cows that milk yield over lactation period of cows with female calves had significantly lower milk yield and lactation length than those with male calves (4131 versus 4214 respectively per lactation).

2.7.2.1.4 Season of calving

Bajwa *et al*. (2004) contended that calving year and season as environmental factors affected lactation length of Sahiwal cattle in Pakistan. Warmest season calvers had lactation length of 251.0 days as compared to coldest season calvers where average lactation length was 243.8 days. Milk yield on the other hand had the opposite trend. Warmest season calvers produced 184 kg less milk (1361 versus 1545 kg) as compared to coldest season calvers (Bajwa *et al*., 2004). On the contrary, M'hamdi *et al*. (2012) noted that both lactation length and milk yield increased with decreasing dryer and warmer seasons.

2.7.2.1.5 Nutrition and management factors

Nutrition and management practices, year, and season in which lactation commences are the main environmental factors affecting lactation performance in cattle (Msanga *et al*., 2000; Epaphras *et al.*, 2004; M'hamdi *et al*., 2012). It has been stated that profitable breeding can be achieved by keeping lactation duration, dry period and service period

between optimal limits (M'hamdi *et al*., 2012). The yields of farm animals are the result of the combined effects of genotype and environmental conditions which include nutrition and all other management routines (Afzal *et al*., 2007; Darfour-Oduro *et al*., 2010; Looper, 2012). According to M'hamdi *et al*. (2012), provision of good feeding level for dairy cows boosts up lactation performance.

2.7.2.1.6 Body condition score

Body condition score (BCS) of cow at breeding season of late has been considered to affect the lactation period (Watters *et al.*, 2010). The most important areas that have been focused on are (1) the BCS of cow at the breeding time of lactation and (2) the changes in BCS from calving to time of breeding. It has been recommended that a cow should enter the dry period with a BCS between 3.25 and 3.75 (Watters *et al.*, 2010). Ensuring that the animal does not lose more than one point is important because of the increased chances of the cow being anovular—absence of ovulation (Watters *et al.*, 2010; Gergovska *et al*., 2011). Gergovska *et al*. (2011) also noted that milk yield and lactation length (LL) of cows with low body condition score (≤2.5 scores) have 1400 kg lower and shorter LL than that of cows with BCS greater than 3.5 points. A herd of cattle in good body condition (BCS ≥3) will produce more, and will be less susceptible to metabolic disorders, disease, mastitis and reproductive problems (Patton *et al*., 1988).

2.7.3 Milk composition

Milk is composed of water, carbohydrate, fat, protein, minerals and vitamins (Heinrichs *et al*., 2005; Schroeder, 2012). Milk is secreted as a complex mixture of these components. The properties and importance of milk are greater and more complex than the sum of its

components (Heinrich *et al*., 2005). Composition of milk varies from cow to cow and differs from the various breeds. Milk is, therefore, a variable biological fluid across indigenous breeds and fat content of milk shows a wide variation (Aboagye, 2002). Total solids, protein and ash contents of the milk are reported to be 10.6, 3.2 and 0.75%, respectively (Aboagye, 2002). Comparable figures from indigenous breeds (such as N'Dama, WASH and White Fulani) have documented (Coffie *et al.*, 2015c).

In addition to interspecies differences, milk composition of any particular species varies with the individuality of the animal, the breed (in the case of commercial dairying species), health (mastitis and other diseases), nutritional status, stage of lactation, age and interval between milking (O'Connor, 1995; Fox and McSweeney, 1998).

Cow's milk total protein content consists of mainly casein (80 %) and whey protein with traces of minor protein. Cow milk contains significant proportion of soluble casein (0.11g /100mL) which is about 5 % of the total casein as compared with that of buffalo's soluble casein (0.03 g/100 mL) of about 1 % of the total casein (Ahmad *et al.*, 2013). However, cow casein concentration and diameter is lesser than that found in buffalo's milk (Ahmad, 2010). The percentages of milk protein and solid-non-fat (SNF) are higher when the cows were fed rations with higher energy (Kumaresan *et al.*, 2008). Cow milk protein increased with increase in feed intake and frequency, high non-fibre carbohydrates, and low fibre of less than 26 NDF (neutral detergent fibre), however, low crude protein decreases it when it is deficient in the lactating cow's diet (Looper, 2012). It has been found that average milk protein of cows ranged from 1.57 to 4.66 %, with an average of 3.05 % (Heinrichs *et al.*, 2005).

Fat is the most sensitive milk component, and is affected by a variety of factors, such as food management or nutrition, genotype, lactation and calving phase (Simoes *et al.*, 2014). Fat component in fresh milk is also influenced by seasons. Milk fat content ranges from 1.77 to 5.98 %, with an average of 3.76 % (Heinrichs *et al.*, 2005). Fat content of 4.11 % has been reported by Okantah (1992) for Sanga cattle in smallholders' farms whilst Aboagye (2002) observed that fat content ranges from 1.3 % in WASH to 5.1 % in the Sokoto Gudali on-station. Rege *et al*. (1994), however, contended that fat component for the WASH is 4.1 %. Increase in feed intake and frequency increase milk fat whereas underfeeding energy and high non-fibre carbohydrate (NFC) decrease milk fat content (Looper, 2012).

Cholesterol is a precursor of many important steroids including bile acids, vitamin D, and steroid hormones (Strzałkowska *et al*., 2009). It has been stated that the cholesterol content in cow's milk is affected by both genetic and environmental factors (Strzałkowska *et al*., 2010) and in addition, it is related to the proportion of somatic cell counts (Strzałkowska *et al.*, 2009). Human organism assimilates 300-500 mg of cholesterol from the diet during 24 hours while 700-900 mg is created from acetyl-CoA as result of endogenous synthesis (Strzałkowska *et al*., 2009). The concentration of cholesterol in milk varies from 8.7 to 25.4 mg/dl (Strzałkowska *et al*., 2009). Studies have found a correlation between the cholesterol content of milk and the age of cows' stage of lactation (Turk *et al*., 2003), physiological condition (Polat and Cetin, 2001) and season (Paura *et al*., 2003).

Lactose is the principal carbohydrate in milk of all mammals, although it contains some trace amounts of other sugars, including glucose (50 mg/l), fructose, glucosamine,

galactosamine, neuraminic acid and neutral and acidic oligosaccharides (Fox and McSweeney, 1998). Complex oligosaccharides constitute a large portion of lactose of milk and perform biological functions that are closely related to their structural conformation (Ahmad *et al*., 2013). According to Enb *et al*. (2009), percentage composition of cow's raw, pasteurized, sterilized and cream milk lactose are 5.00, 4.80, 4.70, and 4.20 respectively.

Solid-non-fat (SNF**)** constitutes protein, lactose, minerals, vitamins, and enzymes. The SNF is more vulnerable to the environmental temperature which indirectly reflect plane of nutrition (Kumaresan *et al.*, 2008). Changes in the feeding practices occur concurrently with the change in seasonal pattern of SNF.

Total solids consist of fat and solid-non-fat and its percentage composition ranges from 10.50 to 14.50 with the average of 13.00 (O'Mahony, 1988). Enb *et al*. (2009) however stated mean total solids to be 13.40.

Percentage compositions of raw milk ash have also been reported to be relatively constant at 0.7 to 0.8 %, but the relative concentrations of the various ions vary considerably (O'Connor, 1995). Fat component of 0.72, % and 0.83 % have been documented by Fox and McSweeney (1998) and Guetouache *et al*. (2014). The ash contents of raw milk are a reflection of the mineral compositions of the milk (Ajai *et al*., 2012). O'Connor (1995) noticed that milk ash composition is influenced by a number of factors including breed, individuality of the cow, stage of lactation, feed, infection of the udder and season of the

year. In addition, certain milk salts, such as sodium and potassium chlorides are sufficiently soluble to be present almost in the dissolved phase.

Amount of water is controlled by the amount of lactose synthesized by the secretory cells of the mammary gland (Guetouache *et al*., 2014). Water activity, together with temperature and pH, is one of the most important parameters which determine the rates of chemical, biochemical and microbiological changes which occur in foods (Fox and McSweeney 1998). Abd El-Salam and El-Shibiny (2011) pointed out that, 87 % of milk is water, in which the other constituents are distributed in various forms. Several kinds of distribution are distinguished according to the type and size of particle present in the liquid milk. Fresh cow milk has a pH between 6.5 and 6.7. Values higher than 6.7 indicate mastitic milk and values below pH 6.5 indicate the presence of colostrum or bacterial deterioration. A pH lower than 6.5, therefore, indicates that considerable acid development has taken place. This is normally due to bacterial activity (O'Connor, 1995).

2.7.3.1 Factors influencing milk composition

Factors that affect milk composition include genetics, stage of lactation, level of milk production, age of cow, environment, disease (for example, mastitis) and nutrition (Looper, 2012).

Genetic/Breed factors

Heinrichs *et al*. (2005) stated that genetics and inheritance account for major difference between cows' milk protein and fat content. Fifty-five percent (55 %) of the variation in milk composition is due to heredity, while 45 % is due to environmental factors such as

feeding management (Schroeder, 2012). Heritability estimates for milk composition are fairly high at 0.50 (Looper, 2012). Heritability indicates the proportion of observed differences that are due to genetics, while the reciprocal is assumed to be due to environmental factors (Heinrichs *et al*., 2005). Jensen (1995) reported average composition of milk for some breeds of cattle as shown in Table 2.2. Level of production or yields of fat, protein, non-fat solids and total solids are highly related with milk yield (Looper, 2012).

The priority placed on each genetic trait of milk component depends upon its economic or profit impact (Looper, 2012). Milk yield per cow tends to receive the most attention by producers. However, component yields should not be overlooked. Genetic selection should be directed toward increasing fat, protein and non-fat solids yields. But, because component percentages tend to have negative genetic associations with yield traits, a change in these percentages is not likely to be achieved through genetic selection alone (Looper, 2012).

Comparison of chemical composition of buffalo's and cow's milk samples by Enb *et al*. (2009) reported that fat, total protein, ash and total solids content in buffalo's milk were (4.9, 3.6, 0.76 and 13.4 %, respectively) higher than those detected in cow's milk (3.2, 3.2, 0.65 and 12.1 %, respectively). This is an indication that variation of milk compositions is due to differences in genetic constitution with respect to different species or breed.

Non-genetic Factors

Nutrition

Environmental factors such as nutrition and feeding management can have impact on yield more than the actual percent composition of the major milk constituents (Looper, 2012). It should also be realized that yields of fat, protein, non-fat solids, and total solids are highly related to milk yield (Varga and Ishler, 2007). The effect of the dietary intake of the cow on milk composition has been reported (Slots *et al*., 2009). Nutritional strategies that optimize rumen function also maximize milk production and milk components (Varga and Ishler, 2007). As cows consume more energy than they use to be, body weight is regained, losses in body condition are minimized and cows produce milk of normal fat and protein content. It is essential to meet the cow's requirement for both crude protein and rumen undegradable protein to avoid a negative impact on dry matter intake and fibre digestibility. Studies of diets containing no supplementary fat show that each one (1) percent increase in dietary protein, within the range of 9 to 17 percent results in 0.02 percentage unit increase in milk protein (Looper, 2012). Nutrition or ration formulation changes are more strongly correlated to milk fat content than milk protein (Heinrichs *et al*., 2005). It is imperative to note that, high-producing cows eat 3.5 to 4.0 percent of their body weight daily as dry matter. If a herd is consuming less than 3.5 to 4.0 percent of body weight as dry matter, production of solids-corrected milk may be limited (Varga and Ishler, 2007).

Parity/Age

Parity or age can influence fresh milk components (Looper, 2012). Whilst milk fat content remains relatively constant, milk protein content gradually decreases with advancing parity/age. According to a survey of Holstein Dairy Herd Improvement Association

(DHIA) lactation records, milk protein content typically decreases 0.10 to 0.15 unit over a period of five or more lactations or approximately 0.02 to 0.05 unit per lactation (Looper, 2012). Milk fat falls about 0.2 % each year from the first to fifth lactation or parity (Heinrichs *et al*., 2005)

Season

Season dramatically affects milk fat and protein. The hot, humid months depress fat and protein content whereas a gradual increase of protein and fat in milk through the fall/dry period and peak levels occur in the colder months (Heinrichs *et al*., 2005). Similarly, the highest values for fat, total solids and SNF contents of Jersey milk are observed during the coldest months (Kumaresan *et al*., 2008). As temperatures increase, component levels are gradually decreased. These changes may be indicative of feed intake patterns, which are lower in warmth due to changes in weather and temperature. It has been realised, however, that seasonality influences milk composition of buffalo with fat content increase (5.27 to 5.70 %) in the dry season and similar concentrations of the other variables in both seasons (Simões *et al.*, 2014).

2.7.4 Milk quality assessment

2.7.4.1 Somatic cell count

Somatic cell count (SCC) determines the total number of cells per millilitre (mL) in milk. Somatic cells count SCC in raw milk comprises of leukocytes, or white blood cells, that are produced by the cow's immune system to fight an inflammation in the mammary gland, or mastitis (Looper, n.d.; Kelci *et al.*, 2017). Somatic cell count is higher in poorly managed cows, during mastitis problems, unhygienic milking and poor milk handling conditions

(Dehinenet *et al*., 2013). Somatic cell count increases with decreasing milk yield usually resulting from poor nutrition (Kelci *et al*., 2017).

Somatic cell count is a general indicator of the mammary gland health, as it is widely used to assess subclinical mastitis and as a standard measure for determining the quality of refrigerated or stored raw milk (Tsenkova *et al.*, 2001). According to Harmon (1994), elevated SCC and compositional changes are due to an inflammation of the mammary gland that results from the introduction and multiplication of pathogenic microorganisms in the mammary gland, which is a complex series of events leading to reduced synthetic activity. Elevated SCC above 200,000 cells/ml is a clear indicator that an animal has experienced or is developing (or recovering from) an infection. According to Schukken *et al.*, 2003 and Schwarz *et al.* (2010), the general agreement falls on a reference range of less than 100,000 cells/mL for uninfected cows and greater than 250,000 for cows infected with significant pathogens. Petzer *et al*. (2017) indicated that the optimum SCC thresholds for composite and quarter milk samples are set as 150, 000 cells/mL and 200, 000 cells/mL, respectively. The cut-off value of 200,000 cells/mL has a sensitivity and specificity of approximately 70 to 80 %; thus, around 75 % of cows with an intra-mammary infection have SCC of >200,000 cells/mL whereas around 75 % of cows without the intra-mammary infection have SCC of ≤ 200,000 cells/ml (Bradley and Green, 2005).

However, Maximum bulk-tank somatic cell count (BTSCC) levels for other countries include 400,000 cells/mL in the European Union (EU) (Hillerton and Berry, 2004), Australia, New Zealand (Smith and Hogan, 1998), and Canada (Centres for Epidemiology and Animal Health—CEAH, 2014). The maximum BTSCC level in Brazil is 1,000,000

cells/mL (Rodrigues *et al.*, 2005). In the United States, the legal maximum BTSCC for Grade A milk shipments is 750,000 cells/mL (CEAH, 2014).

Undoubtedly, the percentage contribution each cow makes to the herd SCC can be used in deciding whether to cull cows or discard a particular cow's milk from the bulk supply, hence checking limits of bulk milk somatic cell count (BMSCC). The individual cow somatic cell counts (ICSCCs) are best carefully measured monthly or at least bimonthly and can be usefully interpreted to aid herd management using the 200,000 cells/mL threshold (Bradley and Green, 2005).

Factors influencing SCC

Factors that influence SCC include sanitation, bedding for lactating cows, control of flies, correct milking procedures, state of milking equipment, season/month of the year, and good nutrition (Norman *et al.*, 2015; Kelci *et al*., 2017). Parity one heifer is noted to have low SCC (Bradley and Green, 2005). The effects of stage of lactation, age, season, and various stresses on SCC are minor if the gland is uninfected; except for normal diurnal variation, few factors other than infection status have a significant impact on milk SCC (Harmon, 1994).

Bradley and Green (2005), in summary, mentioned factors that influence SCC as including bacterial infection, number of quarter involved—more than one quarter affection increase SCC, increasing parity, stage of lactation, diurnal variation related to milking, between-cow variation thus—other than cow effect, Milking interval (SCC tends to be higher following a shortened milking interval), feed/nutritional toxins, Other systemic diseases

(e.g. leptospirosis), Bulk milk somatic cell count, Physiological stress, Storage effects, and Measurement error.

2.9.5.2 Total bacteria count in raw milked

Poor milk handling leads to high risk of illnesses from food-borne pathogens which are mainly from bacterial sources (Wanjala *et al.*, 2018). The main sources of bacterial contamination of raw milk emanate from within the udder, outside the udder, and from the surface of equipment used for milk handling and storage (Wallace, 2008). The bacteriological quality of raw milk, therefore, justifies the contamination of milk with bacterial organisms at different stages of the value chain (Reda *et al*., 2014).

According to Reda *et al*. (2014), milk is graded as very good, good, fair, and poor depending on the levels of bacterial count or colony forming units (CFU/mL). Bacterial counts not exceeding 2.0×10^5 CFU/mL is graded as very good; well if it is between 2.0×10^5 and 1.0×10^6 CFU/mL and fair if the count is between 1.0×10^6 and 5.0×10^6 CFU/mL of milk. While, samples show bacterial count above 5.0×10^6 CFU/mL are graded as poor quality (Sherikar *et al*., 2004; Reda *et al*., 2014). The bacterial limit for the EU is 100,000 cells/mL, which is also the limit for Grade A milk in the United States. However, the United States and the EU calculate compliance differently (Centres for Epidemiology and Animal Health—CEAH, 2014).

2.9.5.3 Total coliform counts in raw milk

Total coliform count is meant for bacteria that are most frequently associated with faecal or environmental contamination such as bedding, soil, water, unhygienic handling and/or

inadequate cooling of milk (Douglas, 2003). Milk samples are plated on a selective nutrient media that encourages the growth of coliform bacteria, while preventing the growth of others. Generally, counts greater than 1.0×10^2 CFU/mL would indicate poor milking hygiene or other sources (Wallace, 2008).

Total coliform counts of 3.6×10^1 – 1.7×10^6 CFU/mL are reported in milk samples in Zimbabwe (Chimuti *et al*., 2016; Wanjala *et al.*, 2018). The total coliform count is influenced by seasonal variation of weather condition in a given area. It has been observed in Eastern Europe that the average number of total coliform counts was the lowest in summer at $4.6\times10^4\pm1.0\times10^4$ CFU/mL and the highest in autumn at $2.7\times10^5\pm6.3\times10^4$ CFU/mL (Wanjala *et al*., 2018). Salman and Hamad (2011) observed differences in the prevalence of coliform counts of 76.9 % and 53.6 % in winter and summer, respectively, in Sudan.

The term faecal coliform has been used in microbiology to denote coliform organisms which grow at 44 or 44.5 ºC or 44.5-45.5 °C and ferment lactose to produce acid and gas within 48 hours (Robinson, 2005; Ugochukwu *et al*., 2015). Faecal coliforms are a subset of coliform bacteria In practice, some organisms with these characteristics may not be of faecal origin and the term "thermo-tolerant coliform" is, therefore, more correct and is becoming more commonly used (UNEP/WHO, 1996). *E. coli, K. pneumoniae, C. freundii, and some Enterobacter spp*. are considered as faecal coliforms. E. coli as well as other coliforms are often used as indicator microorganisms, so their presence in food implies poor hygiene and sanitary practices (Arafa, 2013; Bakhshi *et al*., 2017; Wanjala *et al*., 2018).

The average total coliform and faecal coliform counts in milk samples of Morocco are reported as high as 2.6×10^3 and 1.9×10^2 CFU/mL, respectively (Belbachir *et al*., 2015). The South African standard for total coliform is 20 Colony Forming Unit (CFU)/mL with no *E. coli* detection (Lues *et al*., 2010). In Zimbabwe, the dairy regulation standards for grade 'A' raw milk are <103 (1.03×10^2) CFU/mL for the total coliforms and <10 CFU/mL for E. coli (Gran *et al.*, 2003). The Kenyan acceptable level of total coliform counts in raw milk has been set as <103 CFU/mL (KEBS, 2007; 2010). National Bureau of Standards set Uganda maximum acceptable faecal coliforms count in raw milk as <100 Most Probable Number (MPN)/mL (Fuquay *et al.*, 2011; Grimaud *et al*., 2009).

The European Union (EU) limit for coliform counts in raw milk is <100 CFU/mL (Jay *et al*., 2005), which is more stringent. It is, however, imperative to note that, the main challenge to the implementation of the set limits of the standards regulatory bodies in ensuring production, distribution, and consumption of good quality and safe milk is the lack of resources in terms of personnel and equipment (Fuquay *et al*., 2011; Vairamuthu *et al*., 2010; Wanjala *et al*., 2018).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Location

This study was conducted in Ashanti, Eastern and Greater Accra Regions which lie in the transitional, deciduous and the coastal savannah zones of Ghana (Fig. 3.1). The zones differ in climatic conditions, vegetation cover, rainfall pattern and feed availability.

The transitional zone covers the middle portion of Brong Ahafo Region, Bono East, the northern part of both Ashanti and Eastern Regions and the western part of Volta Region (Figure 3.1). This zone has a bimodal rainfall with an annual rainfall of about 1200 mm. The deciduous forest zone cuts across the northern part of Western Region through southern Brong Ahafo, Ashanti and Eastern Regions. It also occupies the eastern part of Volta Region and most parts of Central Region. It has a bimodal rainfall with an annual rainfall of 1400mm. Most parts of Western Region is within the high rainfall zone. A small part of Central region also falls within this zone. Annual rainfall is over 2000 mm with a bimodal pattern. The Coastal Savannah stretches from Central Region through Greater Accra to the Volta Region. It also has a bimodal rainfall pattern that ranged from 600 to 1000 mm (Ghana District, 2006; GSS, 2013; Regions of Ghana, 2018). This study took place from July, 2015 to August, 2018.

3.1.1 Ashanti Region

Ashanti Region is located in the transitional zone of Ghana and lies between longitudes 0.15'W and 2.25'W, and latitudes 5.50'N and 7.46'N. The region shares boundaries with six of the sixteen political regions (Zurek, 2018); Bono East Region in the north, Eastern

Region in the east, Central and Western North Regions in the south and Western Region in the south-west. It occupies a total land area of 24,389 km^2 representing 10.2% of the total land area of Ghana (Ghana Districts, 2006; Regions of Ghana, 2018). Detailed description of Ashanti Region with respect to ecological zones, climatic factors and vegetation had been outlined in Coffie *et al*. (2015a).

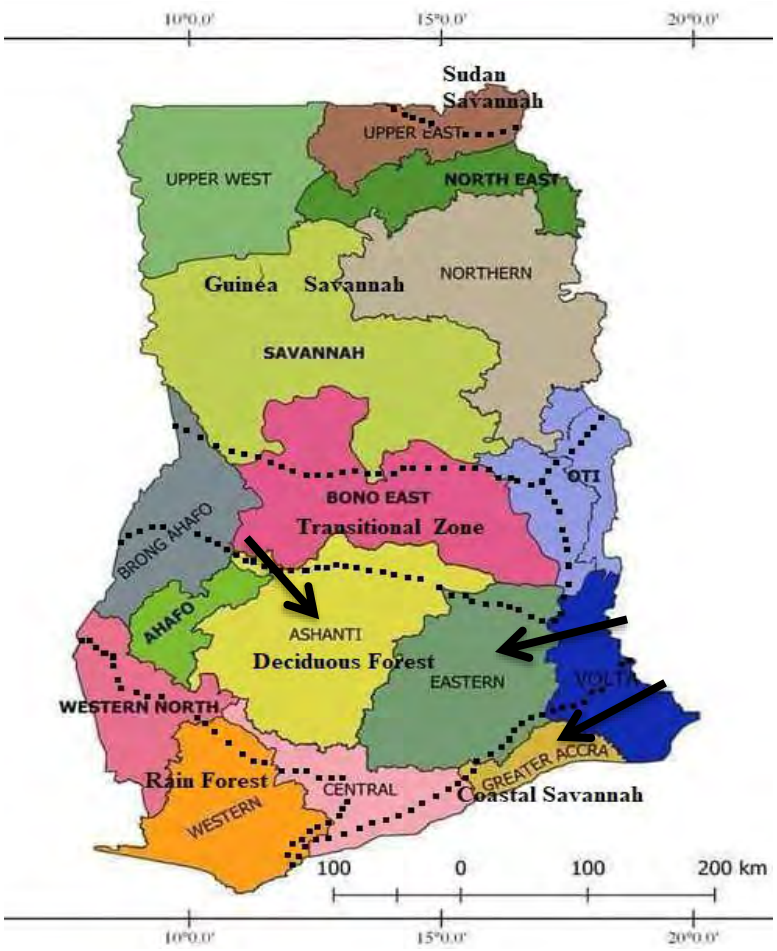

Fig.3.1. Ghana map showing the study areas (arrowed in black colour)

More than half of the Ashanti Region lies within the wet, semi-equatorial forest zone. As a result of human activities and frequent bushfires, especially during the dry season, the

forest vegetation of parts of the region, particularly the north-eastern area, has been reduced to savannah.

Ashanti Region has an average annual rainfall of 1270 mm and two rainy seasons. The major rainy season starts in March, with a major peak in May. There is a slight dip in July and a peak in August, tapering off in November. December to February is dry, hot, and dusty (Ghana Districts, 2006; Regions of Ghana, 2018).

The average daily temperature of Ashanti Region is about 27ºC. Much of the region is situated between 150 and 300 metres above sea level. The region is endowed with a spectacular geography-lakes, scarps, forest reserves, waterfalls, national parks, birds and wildlife sanctuaries (Ghana Districts, 2006). The common forage species grazed in the rangeland include *Pennisetum purpureum*, *Panicum maximum, Centrocaema pubescens, Cynodon plectostachyus*, *Andropogon gayanus* and *Axonopus sp.*

3.1.2 Eastern Region

Eastern Region occupies a land area of 19,323 km^2 and constitutes 8.1 % of the total land area of Ghana. It is the sixth largest region in terms of land area. It lies between latitudes 6 ^{O} and 7 ^{O} North and between longitudes 1^{O} 30’ West and 0^{O} 30’ East. The region shares common boundaries with the Greater Accra, Central, Ashanti, Bono East, Oti and Volta Regions (Regions of Ghana, 2018). The region has four main geographical features, viz: the Kwahu scarp with an elevation of 788.2 m above sea level; the Atiwa-Atwaredu Ranges near Kibi, reaching an elevation of 731.5 m; the Akuapem highland attaining an elevation of 466.3 m which is the southern extension of the Togo-Atakora mountain

ranges; and the isolated hills/mountains dotting the relatively low-lying plains to the south, notably the Krobo and the Yogaga mountains (Regions of Ghana, 2018). The Districts and Municipalities covered for this study included Suhum Municipal, Nsawam, Kwahuu East—Abetifi, Atiwa, Kwahu—Mpraeso, and Nkwanta.

The population of the region stands at 2,633,154 with 1,290,539 male and 1,342,615 female (GSS, 2013). The topography of the region is quite diverse with low lying areas around the valley of the Volta River and Lake and one of the highest reliefs, the Akwapim-Togo- Ranges. The region is well drained with the Volta Lake covering large stretches of the land (Ghana District, 2006; GSS, 2013).

The region lies within the wet semi-equatorial zone which is characterized by double maxima rainfall in June and October. The first rainy season is from May to June, with the heaviest rainfall occurring in June while the second season is from September to October, with little variations between the districts. Temperatures in the region are high and range between 26 ℃ in August and 30 ℃ in March. The relative humidity which is high throughout the year varies between 70 ℃ and 80 ℃ (Ghana District, 2006; Regions of Ghana, 2018).

The vegetation of the Eastern Region is tropical which supports the cultivation of several food crops and cocoa as well as rearing of animal, including cattle, sheep and goats, have been major agricultural activities in the Region. The Kwahu North is a major food crop producing area and belongs to the wide stretch of land referred to as the Afram Plains (GSS, 2013).

The main occupations of the economically active population in the region are Agriculture and related work (54.8 %), Sales (14.3 %), Production, Transport and Equipment work (14.0 %) and Professional and Technical work (6.9 %) with Services accounting for 5.0 per cent. The four principal occupations for males are agriculture and related work (56.9%), Production, Transport and Equipment work (16.6 %), Professional and Technical work (8.6 %) and Sales work (6.5 %). These occupations are similar for females, except in Sales work where females (21.8 %) feature more significantly than males (GSS, 2013).

3.1.3 Greater Accra Region

Greater Accra Region lies in the Coastal savannah zone and has a coast line of approximately 225 km^2, stretching from Kokrobite in the west to Ada in the east. The soils have low organic contents with shallow top soils which limit the capacity for crop production (GSS, 2013). Even though some trees are found in the Shai-Osudoku district, Ga South, Ga East and Ga West districts, the vegetation is mainly coastal savannah shrubs interspersed with thickets.

The region falls within the dry, coastal, equatorial climatic zone with temperatures ranging between 20° and 30° C, and annual rainfall ranging between 600 mm along the coast to 1,140 mm in the northern parts. There are two rainfall peaks, notably in June and October. The first rainfall season between April and July is associated with the major cropping season in the region. Farming is mostly done in the Shai-Osudoku and Ga districts (GSS, 2013). Farmers mainly engage in subsistence farming even though the region has the potential to provide enough food to feed the nation. The region is not well endowed with mineral resources and has only granite, clay and salt. The main rivers that flow through the

region are the Volta and the Densu. In addition, there are small seasonal streams flowing mostly from the Akwapim Ridge into the sea through numerous lagoons. There are ecologically very important but highly polluted lagoons and wetlands in the Accra Metropolitan Authority (AMA), Tema and Shai-Osudoku district. The Volta River's estuarine delta is at Ada in the Shai-Osudoku district (GSS, 2013). The national dairy farm at Amrahia is located on latitude 05º 46' N and longitude 00º 08'W in the Accra Plains.

3.2 Study Design

Horizontal survey (Lynn, 2009) involving repeated visual survey, observation, palpation, and examination of blood and/or milk for assessment of health issues, reproductive hormone levels and productive performances of the various breeds of cows, was carried out in the study areas. In study 1, five (5) breeds—Zebu, WASH/GSH, Sanga, Friesian-Sanga and Jersey cattle were used. Whereas in study 2 to 4 three (3) breeds (Sanga, Friesain-Sanga and Jersey cattle were considered.

3.3 Sample Population

The study population were dairy cattle herds, including indigenous dairy breeds, Friesian-Sanga crossbreds and Jersey cattle. A total of thousand and fifty two (1052) cattle were used for this study involving smallholder to medium scale farms, including two institutional farms. The dairy cattle breeds included Sanga (n=307), Friesian-Sanga crossbreds (n=385), Jersey (n=85), WASH (n=118) and Zebu (n=157).

3.4 Sampling Techniques (Multi-Stage Sampling)

A multi stage sampling technique involving multiple sampling methods was used for this study:

- ✓ Purposive sampling technique - used for selecting experimental animals based on the management system (e.g.level of biosecurity practices, smallholder farms), grazing management (zero-grazing or range grazing), and type of breed kept.
- ✓ Simple random sampling was used for blood, milk, tick and swab sampling.
- ✓ Snow balling sampling was used for selecting farms for the study. The details on 28 farms selected with respect to location of sampling, breed, and management characteristics are presented in Table 3.1.

3.5 Management of Experimental Animals

3.5.1 Housing and feeding

The types of housing used for this study were categorised into insect proofed barns, roofed barn and open kraal. Insect proofed barns had characteristics of a simple standard barn that had been designed to prevent entry of insects and other vectors (Plate 3.1a). Roofed barns/cattle houses were considered as a standard cattle structure for provision of shelter, usually, constructed in a wooden, metallic and/or concrete with roofs (Plate 3.1b). Open kraals are corral or fenced enclosures for holding cattle (Plate 3.1c) usually after grazing or at night.

Table 3.1: Farms selected, their location, breed and management characteristics during the time of this study

S/N	Farm (purposively chosen)	N	Location	Breed	Grazing Management	Level of Biosecurity	Level of Feed Supplementation
1	Embik	45	ASHANTI	F-S/SAN/ ZEBU	Partial ZG	Moderate	Regular
2	UEW-M	119	ASHANTI	F-S /SAN/WASH	Partial ZG	Low	Occasional/partial
3	Zare	122	ASHANTI	F-S /SAN/WASH	Partial ZG	Low	Occasional/partial
4	Husein	30	ASHANTI	SAN/ZEBU	Partial ZG	Low	Occasional/partial
5	Amadu	40	ASHANTI	SAN/ZEBU	Range grazing	Very low	No
6	Karima	30	ASHANTI	F-S/J/SAN	Partial ZG	Low	Regular
7	Bepoase/Haruna	26	ASHANTI	SAN	Range grazing	Very low	No
8	Gao Karim	26	ASHANTI	SAN/ ZEBU	Range grazing	Very low	No
9	Asmang O.K.	26	ASHANTI	SAN	Range grazing	Very low	No
10	Dromankoma	61	ASHANTI	SAN/WASH/ ZEBU	Range grazing	Very low	No
11	Imoro/Sparkx	16	ASHANTI	ZEBU	Partial ZG	Moderate	Occasional/partial
12	Adams	26	ASHANTI	SAN/ZEBU	Range grazing	Very low	No
13	Yusif / M. Ismail	26	ASHANTI	SAN/ZEBU	Partial ZG	Moderate	Occasional/partial
14	Dwomoh	21	ASHANTI	SAN/ZEBU	Range grazing	Low	No
15	Simon	26	ASHANTI	SAN/ZEBU	Range grazing	Very low	Occasional/partial
16	Suhum - Ofori	30	EASTERN	J/ ZEBU	Exclusive ZG	Moderate	Regular
17	Suhum-LAD	7	EASTERN	J	Exclusive ZG	Moderate	Regular
18	Joseph	30	EASTERN	J/SAN/ZEBU	Exclusive ZG	Low	Regular
19	Suhum-TM	8	EASTERN	F-S/J	Exclusive ZG	Moderate	Regular
20	Suhum-FE	6	EASTERN	F-S/J	Exclusive ZG	Moderate	Regular
21	Sule	34	EASTERN	SAN/ WASH	Range grazing	Very low	Occasional/partial
22	Owusu/ Chairman	26	EASTERN	J/SAN/ZEBU	Partial ZG	Low	Occasional/partial
23	AKROFONSO	26	EASTERN	SAN	Range grazing	Very low	No
24	BELK	26	EASTERN	SAN/WASH	Range grazing	Very low	No
25	REA	31	G. ACCRA	F-S/ J/ ZEBU	Exclusive ZG	Moderate	Regular
26	LARTEY	27	G. ACCRA	J/F-S	Exclusive ZG	Moderate	Regular
27	F. FRALINE	26	G. ACCRA	SAN	Range grazing	Very low	No
28	AMRAHIA	135	G. ACCRA	F-S/SAN/WASH	Range grazing	Very low	Occasional/partial
	Total	**1052**					

F-S=Friesian Sanga crossbreds; G=Greater; J=Jersey; N=Number of cattle; SAN=Sanga; WASH=West African Shorthorn; ZG=Zero grazing

All these types of housing structures were designed with different dimensions depending on the farmer's herd size.

Plate 3.1a. Insect Proof barns used for exclusive zero-grazing in the study area

Plate 3.1b. Cattle barns used for exclusive (intensive), partial zero-grazing and range grazing systems based on the production objective.

Plate 3.1c. Kraals in the study area

Cattle herds were managed under farmers' and institutional routine management practices. Water was provided *ad-libitum*. The crossbred cows of Amrahia farm and indigenous dairy breeds/cows (Plate 3.2a) were grazed on range forage including *Pennisetum purpureum*, *Panicum maximum* and *Centrocaema pubescens* whereas exotic and exotic x local crossbred (Friesian-Sanga crossbred) cows were zero grazed.

3.5.2 Feed supplementation

Feed supplementations (Plate 3.2b) were given by some farmers whilst others either gave occasional/partial feed supplements or totally relied on the available range forage (no feed supplementation). Feed supplements given included brewers' spent grain, cassava and plantain peels. Fifteen bulls were used for crossing through natural mating. Farmers provided regular feed supplementation gave plantain and cassava peels (Baawo) together with 2 kg of brewers'/malt spent grain per cow from 6:00 GMT to 9:00 GMT prior to grazing every day. Occasional feed supplementation provided Baawo and the malt spent grain to animals as and when the supplement is available. Farmers who gave no feed supplementation tatally relied on range grazing.

Plate 3.2a: Range grazing of Sanga and crossbred cow at Amrahia farm

Plate 3.2b: Feed supplementation of Jersey cows with cassava and plantain peels (baawo) and brewers' spent grain in the study area

3.5.3 Prophylactic treatment

The cows were treated against ecto-parasites mainly ticks, and mange mites using a pour-on acaricide (Flumethrin 1 % m/v and Cypermectrin 12 % m/v, Hebei New Century Pharmaceutical Co. Ltd, China) once every two months during the dry season and once a month in the minor and major seasons. Treatment against endo-parasites was done using an anti-helminthic drench, Albendazole 10 % (Hebei Yuanzheng Pharmaceutical Co. Ltd, China) once every two months during the dry season and one-and-half months in the minor and major seasons. Animals were vaccinated against Contagious Bovine Pleuropneumonia (CBPP) (T144, MmmSC) once every year. Diminazine diaceturate (Hebei Yuanzheng Pharmaceutical Co. Ltd, China) was administered to animals at every three months for the first year and six months interval for the subsequent years of the study. Cows' body condition score was taken at every crossing and calving.

3.5.4 Mating of cows and artificial insemination

Young bulls were used to open up the crossbred heifers for the third overt heat in order to avoid dystochia. Friesian-Sanga crossbred, Sanga and Jersey cows of parity one (1) or more were artificially inseminated using Friesian bull semen (United Kingdom).

3.5.5 Pregnancy diagnosis

Pregnancies of cows mated/inseminated were diagnosed through:

- Non-return to oestrus and rectal palpation (Fricke, 2010).
- Test for progesterone levels in cows' milk or serum.

3.6 Study 1: Assessment of Health Management of Ticks and Tick-borne Diseases

3.6.1 Determination of prevalence of ticks and tick load in dairy herds

3.6.1.1 Objectives

The objectives of this study were to:

- Assess incidence of tick species in the study area.
- Determine factors (breed and non-genetic factors) influencing total tick count/load (TTC) in dairy cattle herds.

3.6.1.2 Farm, breed selection and initial consideration

Nine dairy farms were purposively chosen for this study on the basis of management system/grazing regime, level of biosecurity measures observed, type of barn or kraal used and the willingness of the owner to accept interventions used for the study.

Tick species considered in this study included *Amblyomma variegatum, Rhipicephalus (Boophilus) decoloratus, Hyalomma rufipes and Rhipicephalus species.* Tick species collection and identification were done in accordance with Walker *et al.* (2003). All the tick species on each cattle were counted and recorded in their separate species.

Prior to data collection, all selected facilities (kraal, the entire premises and the cattle) were sprayed with acaricide every 5 to 6 days starting from 20th February to 10th of March, 2015, for three consecutive times in order to obtain a fair ground for build-up of ticks for total tick count (TTC). Data collected from each farm included location (regions—Ashanti, Eastern and Greater Accra), season of sampling (major rainy, minor and dry seasons), breed of cattle (Zebu, Ghana Shorthorn/WASH, Sanga, Friesian-Sanga and Jersey) (Appendix A), sex, level of feed supplementation (regular, partial/occasional and no feed supplementation), level of biosecurity practices (very low, low, and moderate/medium observation of biosecurity maesures), type of kraal/barn (insect proof barn, open kraal and roofed barn), frequencing of administering acaricide by spraying (weekly, forthnightly, monthly, and every two month).

3.6.1.3 Effect season on total tick count (TTC)

Data on seasonal effect on TTC were taken in April, May and June for rainy season; September, October and November for minor rainy season; and December, January and February for the dry season records from January 2015 to April, 2018. Tick count was done on 13th to 15th of the stated months in every given season. At the last month of each season (March, July and November), cattle were sprayed after 15th of the month with Cypermecthrin/Amitraz (Hebei New Century Pharmaceutical Co. Ltd, China) and after the

tick count exercise. This was done to prevent carrying tick load from one season to the other.

3.6.1.4 Feed supplementation

Regular feed supplementation involved provision of spent grains, cassava and unripe plantain peels almost every day whereas partial/occasional supplementation was given only when supplements are available. Farmers who did not provide feed supplement totally relied on the available range forage, as described in Coffie *et al.* (2014).

3.6.1.5 Level of biosecurity practices

Level of biosecurity practices (LBSP) were scored based on the implementation of biosecurity measures involving the three major biosecurity components: Isolation, traffic control and sanitation (Buhman, 2007; Mathis and Hagevoort, 2010) and management as well, with slight modification in scoring the farms from very low to high percentage risks of how animals were prone to predisposing and inciting factors of tick-borne diseases. Great emphasis was given to the compliance of the biosecurity management practices as indicated in Appendix B (B1 and B2). The major biosecurity principles, and management were given Key performance areas (KPA) in which an average score (S_A) in a given component was based. Each KPA was given key performance indicators (KPI) to determine how strict or weak a KPA was observed by farmers. The KPI score increased from 1 to 4 with decreasing LBSP observed.

Average score (S_A) for each biosecurity major component was estimated by summing the scores (S) obtained from KPI by farm for KPA, divided by Number of KPA (N) considered

for that major component thus, $S_A = \sum S/N$. Weighted Score (S_W) was given by percentage weight (W) assigned to each major component multiplied by S_A, i.e. $S_W = S_A \times W$; where W=24.00 % for each biosecurity component, and 28.00 % for management regimes. Overall percentage risk weighted score (S_{WRP}) was obtained by summing the four (4) S_W divided by 4 and multiplying by 100, thus, $S_{WRP} = (S_W1 + S_W2 + S_W3 + Sw4)/4 \times 100$. The overall S_{WPR} of ≤ 39 % was considered low risk farm (High biosecurity practices observed); 40 to 54 % equals moderate risk farm (moderate observation of biosecurity practices on farm); and ≥ 55 % was tagged as a high risk farm with little observation of biosecurity practices.

3.6.1.6 Types of housing

Type of housings was described as insect proof barn, roofed barns, and open kraals in 3.5.1 and Plate 1a, b and c above.

3.6.1.7 Frequency of applying acaricide

Frequency of application of acaricide was assessed by applying Amitraz (CR Industrial Co. Ltd., China) at the rate of 30 mL per 15 litre of water. Effect of weekly (at six days interval), biweekly, monthly and two months interval of acaricide application on TTC was determined.

3.6.2 Determination of prevalence of tick-borne diseases by Microscopy and Molecular (PCR) techniques

3.6.2.1 Objective of the study

The objectives of this field survey were to:

- Determine prevalence of dermatophilosis, anaplasmosis and heartwater diseases of cattle in hot-humid environment in Ghana.
- Find out factors influencing prevalence of the tick-borne diseases.

3.6.2.2 Data collection

Twenty three (23) farms were used for this study. A total of 486 cattle were randomly selected for the study. Data collected from each herd included observed cases of the TBD (dermatophilosis, anaplasmosis, and heartwater—Cowdriosis), percentage cases under farm condition, effect of grazing management, seasons, level of feed supplementation, type of housing, and level of biosecurity practices observed on TBDs as described in 3.6.1 in this study.

3.6.2.3 Determination of the TBDs by microscopy and molecular (PCR) confirmation

3.6.2.3.1 Dermatophilosis

Scabs/swabs from suspected lesions of cows showing signs of dermatophilosis were aseptically taken and stored at -20 ℃ till culturing and morphological study of the organism by microscopy. The samples were soaked with sterilized distilled water for 10 minutes and then mixed by massaging the samples in a sterile mini poly bags. A smear was made on a clean oil-free microscopical slide (Chitra *et al.*, 2017) and stained with Leishman stain, Giemsa and Gram-stain. Observation under oil immersion objective lens (1000 X) was then made.

The *aliquot* of blood samples were cultured on Luria Bertani (LB) agar (SIGMA-ALDRICH Co., Batch #:089K0004, USA) for 24 hours, since the organism is not

fastidious, (Gebreyohannes and Gebresselassie, 2013). Observations of colonies were made after 24 hour incubation at 37 ºC.

Genomic DNA extraction of Dermatophilus congolensis

Confirmation of *Dermatophilus congolensis* DNA was prepared as described by Soumet *et al.* (1994) with slight modification. *Dermatophilus congolensis* colonies were inoculated into the Luria Bertani (LB) broth and incubated at 37˚C for 24 hour in shaking incubation (100 rpm). One mL of each culture was centrifuged for 3min at 13000 xg (4 ˚C). The resulting pellets were washed twice in 50µL 1X TAE buffer (Tris, Acetic, and EDTA; pH 8.0), and further centrifuged at 13000xg for 3min (4˚C). The pellet was suspended in 200 µL Tris, EDTA (TE) buffer, boiled in water bath for 8 min, and stored at -20˚C.

Confirmation of genomic DNA templates of Dermatophilus congolensis

The concentration of extracted DNA was assessed by spectrophotometry and its purity ascertained by electrophoresis in 1.5 % agarose gels stained with 0.5 µL of ethidium bromide. Ten microlitres (10 µL) of randomly chosen DNA samples were loaded into the agarose gel and circuited for 30 minutes electrophoresis. The genomic DNA was confirmed with DNA Transilluminator (Bioimaging System) using UV light.

PCR amplification of Dermatophilus congolensis

The confirmed genomic DNAs were subjected to optimization to identify a 500 bp fragment of 16S rRNA gene of *Dermatophilus congolensis* isolates, initially using three PCR conditions to determine best PCR condition for best amplicafication to be used. The primers used and their sequences were Derma_F: Forward, 5’ ACA TGC AAG TCG AAC

GAT GA 3' and Derma_R: Reverse 3' ACG CTC GCA CCC TAC GTA TT 5' to amplify the 500 bp fragment of the *D. congolensis* DNA. A reaction mixture containing 3.75 µL Sterile, Nuclease Free water, 2 µL PCR Buffer 10x, 0.7 µL d-NTP (2 mM), 0.8 µL $MgCl_2$ (50 mM), 0.5µL Taq DNA polymerase, 0.25 µL of each of the forward (Derm F) and the reverse (Derma R) primers and 2 µL of template DNA isolated from the field samples was prepared. The PCR cycler condition was programmed with initial denaturation at 95 ℃ for 5min, 35 three-step cycles of denaturation at 94 ℃ for 30 sec, annealing at 53 ℃ for 1min, extension at 72 ℃ for 3 min, and a final extension at 72 ℃ for 7 min. The amplicons were analysed by electrophoresis in 1.5% agarose gel for 1 hour at 80V. Product size was compared to the 100 pb ladder (Fermentas SM1211) after ethidium bromide staining. The bind size of *D. congolensis* was visualized by spectrophotometry using Transilluminator (Bioimaging System).

3.6.2.3.2 Anaplasmosis

Anaplasma organism was examined in thin blood smears taken from the jugular vein. Anaplasmosis was diagnosed by confirming the presence of *Anaplasma* organism seen as marginal bodies in Giemsa´s stained blood smear (Chitra *et al*., 2017). The organisms appeared as dark blue purple bodies which range from 0.3 – 1 µm along the margin of erythrocytes (Knowles *et al*., 1996).

Genomic DNA isolation of Anaplasma marginale

Genomic DNA was isolated from whole blood in Ethylene diamine tetraacetic acid (EDTA) using QIAamp® DNA blood mini kit (QIAGEN, GmbH, Germany) in accordance with the manufacturer's instructions with slight modifications as described by Singh *et al*

(2012). Briefly, 200 µL of the blood sample were mixed with 20 µL of proteinase K and 200 µL of lysis buffer and incubated at 56 ℃ for 10 minutes. Then, 200 µL of ethanol was added to the sample, and the mixture was applied to QIAamp Mini spin column and centrifuged at 8000 rpm for 1 minute. It was then washed twice each with 500 µL wash buffer 1 and 2. Lastly, 150 µL of elution buffer were added to the column, and DNA was collected in 1.5-mL Eppendorf tubes after centrifugation and stored at −20 ◦C till use.

Genomic DNA of *Anaplasma margenale* was also extracted from infected blood with high rickettsiaemia in the standard blood smear examination protocols (Radostits *et al*., 1994) and used as positive control. Whereas genomic DNA from negative control was isolated from the blood of calf aged 4 days.

PCR Amplification of Anaplasma marginale

The MSP 5 primer, external forward (Amar msp5 eF: 5' GCA TAG CCT CCG CGT CTT TC 3' and external reverse, Amar msp5 eR: 5' TCC TCG CCT TGG CCC TCA GA 3') were used to amplify a 458 bp fragment of the conserved MSP 5 region of *Anaplasma marginale* DNA. A master solution containing 3.75 µL Sterile, Nuclease Free water, 2 µL PCR Buffer 10x, 0.7 µL d-NTP (2 mM), 0.8 µL $MgCl_2$ (50 mM), 0.5µL Taq DNA polymerase, 0.25 µL of each of the external forward (Amar msp5 eF) and external reverse (Amar msp5 eR) primers and 2 µL of template DNA isolated from the field samples was prepared. The PCR cycler condition was programmed with initial denaturation at 94 ℃ for 5min, 34 cycles of denaturation at 94 ℃ for 1 min, annealing at 58 ℃ for 1min, extension at 72 ℃ for 1 min, and a final extension at 72 ℃ for 10 min (Singh *et al*., 2012). The

amplicons were determined by electrophoresis in 1.5% agarose gel for 1 hour at 80V. Product size was compared to the 100 pb ladder after ethidium bromide staining.

3.6.2.3.3 Heartwater

Blood and Amblyomma tick samples were taken from susceptible herds in live animals. Ticks samples were crushed and soaked with sterilized distilled water for 10 minutes and then mixed to obtain aliquots. All samples were stored at -20 ℃ till use. A haematological assessment of leucocytosis in rickettsiaemia (Radostits *et al*., 1994) was also done. Post hydro-pericardium, hydrothorax, pulmonary oedema, intestinal congestion, oedema of the mediastinal and bronchial lymph nodes, petechiae on the epicardium and endocardium were assessed (Radostits *et al*., 1994). Smears were prepared from brain (Cerebrum) culture from heads cut off from dead (Plate 3.3) calves of 4 – 6 months and stained with Leishman stain to identify *Ehrlichia ruminantium* (formerly *Cowdria ruminantium*) colonies by light microscopy in the cytoplasm of endothelial of the brain **(**OIE, 2018).

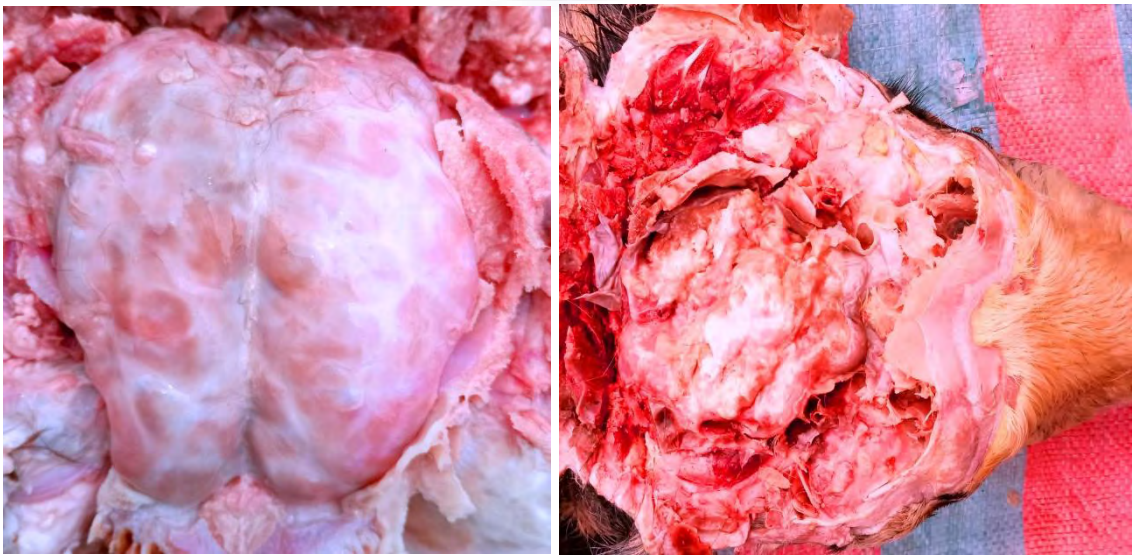

Plate 3.3. Removal of brain for diagnosis of heartwater (Cowdriosis)

DNA extraction of Ehrlichia (Cowdria) ruminantium

Blood, ticks and brain samples were collected from healthy herds whereas brain samples were taken from a clinical case confirmed by standard method (from suspected positive case). DNA was extracted from the individual tick tissue samples using the QIA-amp PCR DNA extraction tissue kits (Qiagen, Hilden, Germany) and purified as decribed by Peter *et al*. (2000).

PCR amplification of Ehrlichia ruminantium

Amplification of a 279 bp *Ehrlichia ruminantium*-specific DNA fragment of pCS20 sequence of *Ehrlichia ruminantium* was done using Oligonucleotide primers pCS20_F (5'ACT AGT AGA AAT TGC ACA ATC TAT 3') and pCS20 R (5' TGA TAA CTT GGT GCG GGA AAT CCTT 3') as previously described (Mahan *et al.,* 1992; Peter *et al.,* 2000) with slight modification. A master solution consisting of 3.75 µL Sterile, Nuclease Free water, 2 µL PCR Buffer 10x, 0.7 µL d-NTP (2 mM), 0.8 µL $MgCl_2$ (50 mM), 0.5µL Taq DNA polymerase, 0.25 µL of each of the forward (pCS20 F) and reverse (pCS20 R) primers and 2 µL of template DNA isolated from the field samples was prepared. A PCR cycler condition was set with initial denaturation of DNA done at 94 °C for 1 min. A 45 cycles of denaturation of 94 °C for 1min, annealing of primers at 55 °C for 1 min and extension at 72°C for 2 min. A final extension of 72 °C for 10 min was followed by a cooling at 4 °C for infinity. In all the PCR reactions, a reagent free control (no template DNA) and a respective positive control was included. The products from the PCR reactions were analyzed by electrophoresis on a 1.5 % agarose gel and viewed with UV light illumination and photography after staining with ethidium bromide.

3.6.3 Statistical analysis

Data on the total tick count were transformed to a scale, using the formula, Y = Log10 (X +1) + 0.5 to confer normality (Marufu, 2008) prior to analysis. The Data were then subjected to Least squares (LS) analysis using Generalized Linear Model (GLM) Type III Procedure of IBM Statistical Package for Social Sciences, version 25 (SPSS, 2017) on the following fixed models:

$$Y_{ijklmnopqrs} = \mu + B_i + C_j + F_k + G_l + H_m + L_n + M_o + P_q + T_r + X_s + BC_{ij} + BF_{ik} + BG_{il} + BH_{im} + BL_{in} + BM_{io} + BP_{iq} + BT_{ir} + BX_{is} + CF_{jk} + CG_{jl} + CH_{jm} + CL_{jn} + CM_{jo} + CP_{jq} + CT_{jr} + CX_{js} + FG_{kl} + FH_{km} + FL_{km} + FM_{ko} + FP_{kq} + FT_{kr} + FX_{ks} + GH_{lm} + GL_{ln} + GM_{lo} + GP_{lq} + GT_{lr} + GX_{ls} + HL_{mn} + HM_{mo} + HP_{mq} + HT_{mr} + HX_{ms} + LM_{no} + LP_{nq} + LT_{nr} + LX_{ns} + MP_{oq} + MT_{or} + MX_{os} + PT_{qr} + PX_{qs} + TX_{rs} + e_{ijklmnopqrs}$$

Where: $Y_{ijklmno}$ = Dependent variable (Total tick count); μ = population mean; B_i = effect i^{th} breed of cattle, i = 1, 2, 3, 4 and 5; C_j = effect of j^{th} season of tick sampling, j = 1, 2 and 3; F_k = effect of k^{th} farm, k = 1, 2, 3, …, 9; G_l = effect of l^{th} sex of cattle, l = 1and 2; H_m = effect of m^{th} type of housing, m = 1, 2, and 3; L_n = effect of n^{th} location of the study, n = 1, 2 and 3; M_o = effect of o^{th} management systems, o = 1, 2 and 3; P_q = effect of q^{th} level of biosecurity practices, q = 1, 2 and 3; T_r = effect r^{th} interval/period of acaricide spray, r = 1, 2, 3 and 4; X_s = effect of s^{th} feed supplementation, s = 1, 2 and 3; $BC_{ij} + BF_{ik} + BG_{il} + BH_{im} + BL_{in} + BM_{io} + BP_{iq} + BT_{ir} + BX_{is} + CF_{jk} + CG_{jl} + CH_{jm} + \ldots + TX_{rs}$ = corresponding first order interactions; and $e_{ijklmnopqrs}$ = the random error term. Differences between means of significant effects were separated by the pairwise comparisons of means procedure of SPSS (2017).

3.7 Study 2: Haemato-biochemical Indices of Dairy Cows

3.7.1 Objectives

The objectives of this study were to:

- Determine effect of breed on the haemato-biochemical indices.
- Find out effect of physiological state of cows on haemato-biochemical indices.
- Determine effect of feed supplementation of cows on haemato-biochemical indices.

3.7.2 Data collection

A total of one hundred and twenty (120) cows of different breeds (Sanga, n=40; Friesian-Sanga crossbreds, n=40 and Jersey cows, n=40) and of different physiological states categorized into non-cycling heifer (n=24), non-pregnant cow (n=24), early gestation (first trimester, n=24), mid gestation (second trimester, n=24) and late gestation (third/last trimester, n=24) were used for haemato-biochemical studies. Data collected on each cow included effect of breed, and physiological state on the blood indices of the cows sampled. For haematological studies, 111 blood samples of cows of the breed (Sanga, n=37; Friesian-Sanga Friesian-Sanga crossbreds, n=37 and Jersey cows, n=37) were used, Whereas 110 cows of different physiological states (non-cycling heifer,n=24; non-pregnant cow, n=24; early gestation, n=24; mid gestation, n=24) and late gestation, n=24) were taken.

3.7.3 Measurement of haematological parameters

Full blood count (FBC) was determined by a five (5) parts (differentials) fully automated BC 5800 haematology system, (Mindray, Germany) according to the manufacturer's instructions. Principles employed included impedance method for RBC and PLT counting;

Cyanide free reagent for haemoglobin test; and Flow Cytometry (FCM) + Laser light scatter + Chemical dye method for WBC differential analysis and WBC counting. Haematological indices assessed included Red blood cell (RBC) count, haemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW), platelets (PLT), mean platelet volume (MPV) and platelet distribution width (PDW). Leucocytes indices embraced level of White blood cells (WBC), percentage neutrophils, lymphocytes, monocytes, eosinophil and basophils.

3.7.4 Measurement of serum biochemical parameters

Hepatic enzymes including alanine aminotransferase (ALT), aspatate aminotransferase (AST), alkaline phosphatase (ALP) and Gamma glutamyl transferase (γGT) were determined through Liver function test (LFT) using Mindray BS 130 fully automated blood chemistry analyser (Shenzhen Mindray Bio-Medical Electronics Co., Ltd, Germany) in accordance with manufacturer's instructions.

Enzymatic, metabolic and blood electrolyte levels were assessed by performing liver function test (LFT), lipid profile, kidney function test (KFT) and electrolytes using the Mindray BS 130 fully automated blood chemistry analyser. The details of parameters assessed included ketones (beta-hyroxybutyrate), total protein (TP), globulin, albumen, NEFA, creatinine and lipids {(total cholesterol (TC), triglycerol (TG), high density lipoprotein (HDL), very low density lipoprotein (VLDL), and low density lipoprotein (LDL)}; Electrolyte: Na^+, K^+, Ca^{2+}, Cl^-, Mg, HCO_3, and P. Metabolic parameters, NEFA and βHBA were determined by using the colorimetric reaction according to the

manufacturer's instructions using Colorimetric Kits and were measured using fully-automatic biochemistry analyzer (Kovacevic *et al*., 2016). Blood glucose level was determined using portable glucometer blood glucose monitoring system, OneTouch SelectSimple, (LifeScan Inc., China) in accordance with manufacturer's manual.

3.7.5 Statistical analysis

Data on haematological and biochemistry indices were subjected to one-way-anova of IBM SPSS version 25 (SPSS, 2017), according to the following model:

$$Y_{ij} = \mu + B_i + P_j + S_k + e_{ijk}$$

Where: Y_{ij} = Dependent variables representing haemato-biological indices (RBC, WBC, HGB, PLT, BAS e.t.c.; and ALT, AST, γGT, ALP, BUN, e.t.c.); μ = overall mean of traits; B_i = effect of i^{th} breed, i = 1, 2, and 3; P_j = effect of j^{th} physiological state of cows, j = 1, 2, 3, 4 and 5; S_k = effect of k^{th} feed supplementation, k = 1, 2 and 3; e_{ijk} = error term.

3.8 Study 3: Reproductive Hormones of Dairy Cattle

3.8.1 Determination of reproductive hormonal profiles in dairy cows

Determination of serum and milk hormonal levels requires separate Enzyme Linked Immuno Sorbent Assay (ELISA) kits or Radio Immunoassay (RIA) kits specific for either plasma/sera or milk. Several commercial EIA are available for determination of hormonal profiles in human, though information on these products application and usefulness in dairy cattle in the study area is rare. This study was purposely conducted to determine assaying of gonadotrophic and reproductive steroid hormones in dairy cows using commercial EIA.

3.8.1.1 Objectives

The objectives of this study were to:

- Determine breed and feed supplementation on progesterone concentrations in heifers during prepubertal stage, onset of oestrus, first overt heat to conception using the commercial EIA.
- Determine oestradiol and progesterone concentrations in dairy breeds during oestrous cycles and gestational stages.
- Find out relationship between serum and raw milk reproductive steroid (P_4, E_2 and testosterone) and gonadotrophic (FSH and LH) hormones concentrations 45 days postpartum.
- Determine effect of age on testosterone and oestradiol concentrations in bulls.

3.8.1.2 Blood/milk Sampling

Three (3) breeds (Jersey, Crossbred and Sanga cattle) were used for this study. Twenty one (21) blood samples were taken from the cows with respect to breed, and physiological states (non cycling heifers, cycling cows, first to third trimesters and postpartum). A total of twenty four (24) cattle were used for studies on effect of age on testosterone and oestradiol levels in bulls. Blood samples were humanely taken from jugular vein using 10 ml syringe and 18 x 1.5" gauge needle (Quadat limited, United Kindom; Neomedic [PTY] Limited, Republic of South Africa) and gently dispensed into Serum tubes (Elite Medical, Nanjing; Neomedic, China) and allowed to clot. The samples were then transported on ice packs to ABC Laboratory and were centrifuged at 3000 rpm for 10 min. Sera were stored at -20 ℃ until tere were used. Milk samples were collected directly into serum tubes. Centrifugation was done, fats discarded and supernatants were frozen at -20 ℃ till

assaying. Factors considered in this study included breed (Jersey, Crossbred and Sanga cows), and physiological state (prepubertal stage, gestational stages and pospatrum period).

3.8.1.3 Principle and assaying procedure of Oestradiol

Frozen sera/milk samples obtained from cows were thawed to 22 ℃ and assayed for oestradiol concentration using Enzyme Immunoassay (EIA) (USA for SG Biotec Laboratories, Tema) for the quantitative determination of oestradiol.

Oestradiol (E_2) estimation using commercial EIA was based on the principle of competitive binding between E_2 in the test specimen and E_2 – HRP (horseradish peroxidase) conjugated for a constant amount of rabbit anti-oestradiol. In the incubation, goat anti- rabbit IgG-coated wells were incubated with 25 µL E_2 standards, controls, test samples, 100 µL oestradiol-HRP conjugate reagent and 50 µL rabbit anti-oestradiol reagent at room temperature (18-22 ℃) for 90 minutes. During the incubation, fixed number of amount of HRP labelled E_2 compete with the endogenous E_2 in the standard sample or quality control serum for a fixed number of binding sites of the specific E_2 antibody. Thus, the amount of E_2 peroxidase conjugates immunologically bound to the well progressively decreased as the concentration of E_2 increased.

Unbound E_2 peroxidase conjugate was removed and the wells washed. Solution of Tetra Methyl Benzidine (TMB) reagent was added afterward and incubated at room temperature (22 ℃) for 20 minutes, resulting in the development of blue colour. The colour development was stopped with the addition of stop solution, and the absorbance was measured spectrophotometrically at 450 nm. The intensity of the colour formed was

proportional to the amount of enzyme present and was inversely related to the amount of unlabeled E_2 in the sample. A standard curve was obtained by plotting the concentration of the standard versus the absorbance. The E_2 concentration of the specimens and control run concurrently with the standard was calculated from the standard curve (Figure 3.2). Materials used for assaying included precision pipettes 20-50 µl, 0.5-200 µl and 1.0ml; disposable pipettes tip: distilled and deionized water; vortex mixer; absorbent paper/paper towel; linear-linear graph; and microtiter well reader.

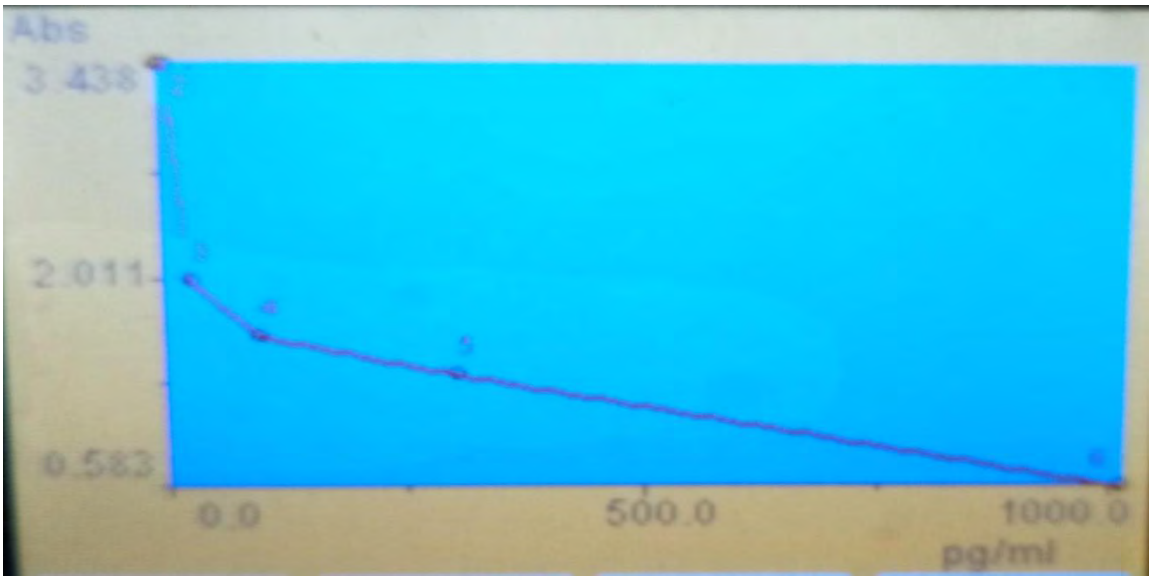

Fig. 3.2. A standard curve (E_2 ng/mL) obtained by plotting the concentration of the standard versus the absorbance at 450 nm OD.

Assay procedure or protocol was done in accordance with the manufacturer instructions without modification as briefly described as follows:

1. Ninety six (96) coated wells were secured in a micro wells holder. Different pipette tip was used for each reagent, and before pipetting each reagent, the pipette tip was

equilibrated such that reagent slowly filled the tip and gently expelled the contents, and repeated several times. Each tip was used once.

2. Twenty-five micro litre (25 µL) of standards (0.0, 10.0 30.0, 100.0, 300.0 and 1000.0 pg/mL) were dispensed into the coated wells in duplicates (for optimization calibration for standard curve) whereas samples (sera/milk) were dispensed in triplicates using 96 wells.
3. 50 µL of rabbit anti-Estradiol (E_2) reagent was dispensed into each well.
4. 100 µL of Estradiol –HRP conjugate reagent was dispensed into each well and thoroughly mixed for 30 seconds.
5. It was then incubated at room temperature (22 ℃) for 90 minute.
6. The wells were rinsed by flicking the microcells 5 times with washing buffer (1x).
7. 100 µl of TMB substrate was dispensed into each well, and gently mixed for 10 seconds and subsequently incubated at room temperature (22 ℃) for 20 minutes.
8. The reaction was stopped by adding 100 µL of stop solution to each wells.
9. The wells were gently mixed for 30 seconds. The blue colour changed to yellow colour completely.
10. Absorbance was read at 450 mm with a microtiter plate reader (‘Human’, Germany) within 15 minutes.

The concentrations of E_2 (pg/mL) were calculated from the standard curve ($r^2 = 0.98 – 1$) by evaluating the optical density (OD) at 450 nm, for each set reference standards, controls and samples.

3.8.1.4 Progesterone

Progesterone (P_4) EIA is based on the principle of competitive binding between progesterone in the test specimen and progesterone-HRP conjugate for a constant amount of rabbit anti-progesterone. The principle was the same as described in E_2. Assay procedure of sera/milk progesterone (P_4) levels were determined by EIA (Progesterone Enzyme Immunoassay test kit (SG Biotec Laboratories, USA) according to manufacturer's instructions as described below:

1. Ninety-six (96) coated wells were set in micro wells holder.
2. 25 µL of standards (0.0, 0.5, 3.0, 10.0, 25.0, 50.0 ng/mL), and samples were dispensed into wells.
3. 100 µL of Working Progesterone-HRP Conjugate Reagent was dispensed into each well.
4. 50 ml of rabbit anti-progesterone reagent was also dispensed into each well.
5. It was then thoroughly mixed for 30 seconds and incubated at room temperature (18-25°C) for 90 minutes.
6. Rinsing was done by flicking the micro wells 5 times with deionized water.
7. 100 µL of TMB Reagent was dispensed into each well, and gently mixed for 10 seconds.
8. Incubation was done at room temperature (18-25°C) for 20 minutes.
9. The reaction was stopped by adding 100 ml of stop solution to each well.
10. It was then gently mixed 30 seconds, ensuring that all the blue colour changed to yellow colour completely.

11. Absorbance was read at 450 nm with a microtiter well reader was noticed within 15 minutes. The concentrations of P_4 (ng/mL) were calculated from the standard curve (r^2 = 0.97 – 1) (Fig. 3.3) by evaluating the optical density (OD) at 450 nm.

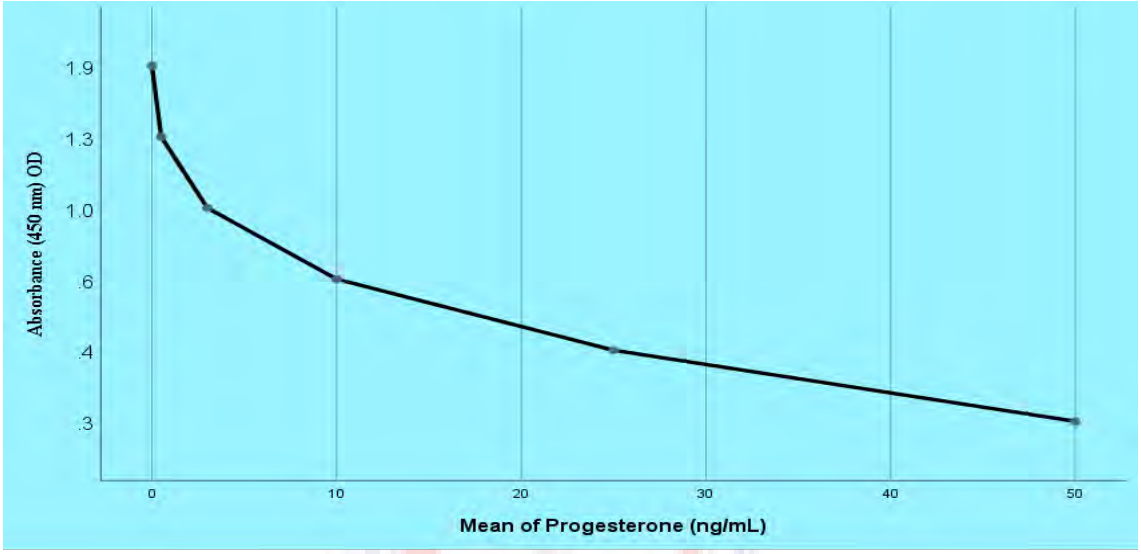

Fig.3.3. Standard curve (r^2 = 0.97 – 1) showing concentrations of P_4 (ng/mL) and absorbance at 450 nm optical density (OD).

3.8.1.5 Testosterone

Testosterone concentration in postpartum cows, and in bull was determined based on the principle of competitive binding between testosterone in the test specimen and testosterone HRP conjugate for a constant amount of the rabbit anti – testosterone. In the incubation, goat anti- rabbit IgG-coated wells were incubated with 10 µL of testosterone standard, controls sample, 100 µL testosterone-HRP conjugate reagent and 50 µL rabbit anti- testosterone reagent at 37°C for 90 minute.

During incubation a fixed amount of HRP labeled testosterone competed with the endogenous testosterone in the standard/serum/milk for a fixed number of binding sites of

the specific testosterone anti-body. Thus, the amount of testosterone peroxidase conjugate immunologically bound to the well progressively decreased as concentration conjugate with the endogenous testosterone in the specimen increased.

Reagent preparation and assay procedure were done according to the manufacturer's instructions. In brief, 96 pre-coated microwells were set in the microwells holder. Calibration were done using the standards (0.0, 0.10, 0.5, 2.0, 6.0 and 18.0 ng/mL) for the standard curve as shown in Fig. 3.4.

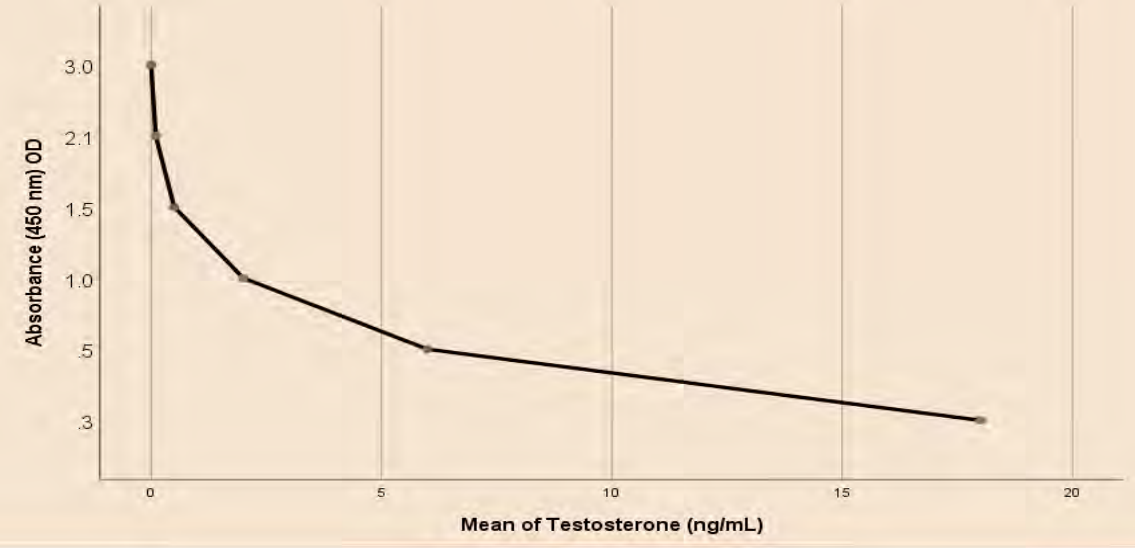

Fig.3.4. A standard curve (for testosterone ng/mL) obtained by plotting the concentration of the standard versus the absorbance at 450 nm OD

A 10 µL of standards, and sera/milk were dispensed into the coated micro wells. A 50 µL of rabbit anti-testosterone reagent was also added t to each well. Subsequently, 100 µL of testosterone –HRP conjugate reagent was dispensed into each well, and thoroughly mixed for 30 seconds. It was then incubated at 37 ℃ for 90 minutes. The microcells were rinsed

and flicked 5 times with washing buffer (1x). A 100 µL of TMB substrate was dispensed into each well, and gently mixed for 10 seconds. Incubation was done at room temperature (18-22 ℃) for 20 minutes. The reaction was stopped by adding 100 µL of stop solution to each wells and gently mixed for 30 seconds and ensuring that all blue colour changed to yellow colour completely. Absorbance was read at 450 mm with a microtiter well reader within 15 minutes.

3.8.1.6 Follicle stimulating hormone

Quantitative assessment of follicle stimulating hormone (FSH) was determined using EIA test kit (Chemux Bioscience, Inc., USA).

Principle of the test

The FSH Quantitative test kit was based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilized a polyclonal anti-FSH antibody for solid phase (microtiter wells) immobilization and a mouse monoclonal anti-FSH antibody in the antibody-enzymes (horseradish peroxidase—HRP) conjugate solution. The test sample was allowed to react simultaneously with the antibodies, resulting in the FSH molecules being sandwiched between the solid phase and enzymes–linked antibodies. After 60 minutes of incubation at room temperature, the wells were washed to remove unbound labelled antibodies. A solution of TMB was added and incubated for 20 minutes resulting in the development of a blue colour. The colour development was stopped with the addition of 2N HCL, and the colour was changed to yellow and measured spectrophotometrically at 450mm. The concentration of FSH was directly proportional to the colour intensity of the test sample.

Reagent preparation

1. All reagents were brought to room temperature (22 ºC) before use.

2. Where reference standards were lyophilized, reconstitution of each standard with 0.5ml distilled water was done. The concentrated material was allowed to stand for at least 20 minutes. Reconstituted standards were stored sealed at 2-8 ℃.

3. One (1) volume of wash buffer (50x) was used to dilute with 49 volumes (50x) into distilled water. Thus, 15 ml of distilled water was used to prepare 750ml of washing buffer.

Assay procedure

1. Ninety six (96) coated wells were set in the holders.
2. A 50µL of standards (0.0, 0.5, 20.0, 50.0,100.0 and 200.0 mIU/mL) , and sample specimens were dispensed into wells.
3. A 100 µL of enzymes conjugant reagent was put into each well.
4. The mixture was thoroughly mixed for 30 seconds. It was very important to have complete mixing in this setup.
5. Incubation was done at room temperature (22 ℃) for 60 minutes.
6. The incubation mixture was removed by flicking plate constant into waste container.
7. Rinsing and flicking the microsite wells 5 times with washing buffer were done.
8. The wells sharply stroke onto absorbent paper or paper towels to remove all residue water droplets.
9. 100µ of TMB solution was dispensed into each well and gently mixed for 5 seconds.
10. It was then incubated at room temperature for 20 minutes.

11. The reaction was stopped by adding 100 µl of stop solution to each wells, and then gently mixed for 30 seconds making sure that all the blue colour changed to yellow colour completely.

Optical density was read at 450 mm (r^2 = 0.980**)** with a microtiter well reader within 15 minutes. The concentrations of FSH (mUI/mL) were calculated from the standard curve (r^2 = 0.980) (Fig. 3.5) by evaluating the optical density (OD) at 450 nm.

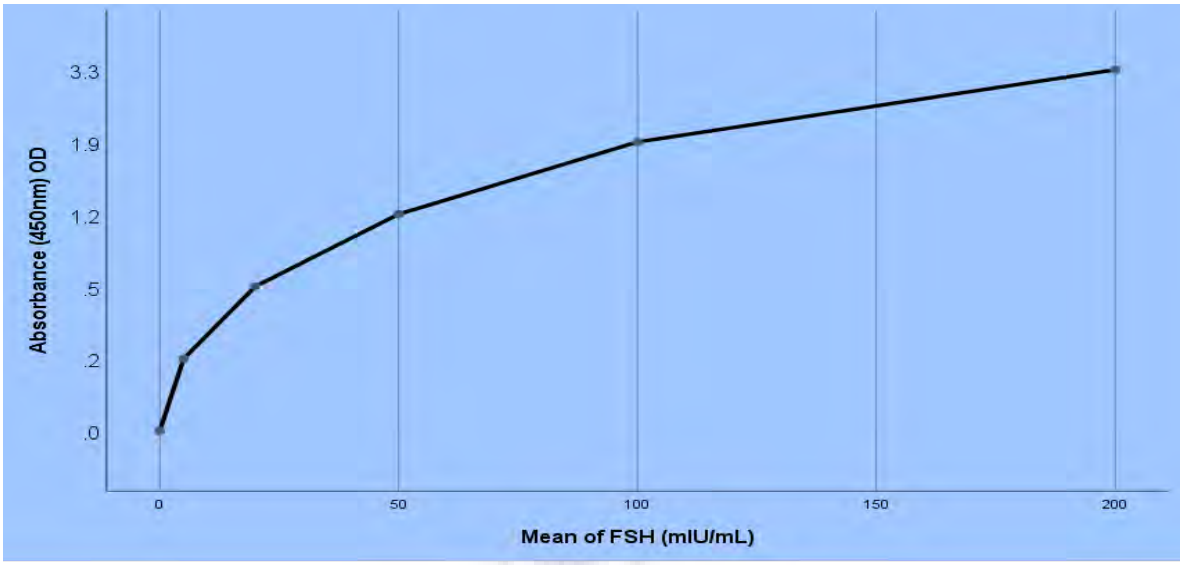

Fig.3.5. A standard curve obtained by plotting the concentration of the FSH standard versus the absorbance at 450 nm OD

3.8.1.7 Luteinizing Hormone

The quantitative determination of LH followed the same principle of FSH (Sandwich) described above. Sample collection and preparation, reagent preparation, and assay procedure were done in accordance with the manufacturer's instructions without modification. The samples were examined in triplicates, and within and between coefficients of variation were 6.38 % and 7. 44 % (mean from three wells) respectively.

Calculation of a standard curve was done by plotting the mean absorbance obtained from each reference standard against its concentration in mIM/mL on a graph paper, with absorbance value on the vertical or Y axis and concentration on the horizontal or X axis. The mean absorbance values were used for each sample to determine the corresponding concentration of LH in mlU/mL from the standard curve (Fig. 3.6) according to the manufacturer's reference manual.

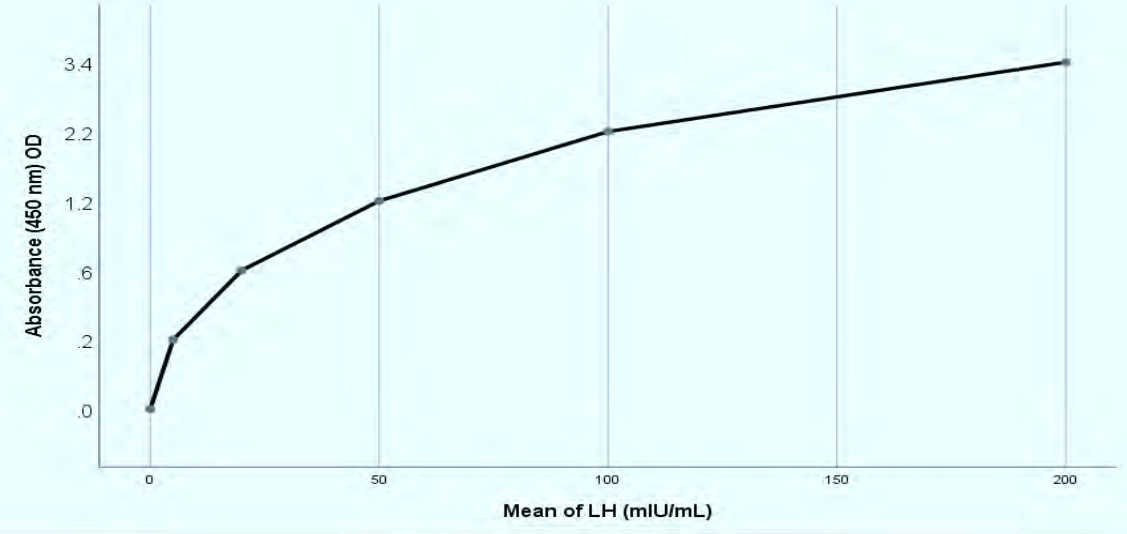

Fig. 3.6. LH standard curve obtained by plotting the concentration (mIU/mL) of the standard versus the absorbance at 450 nm OD

3.8.1.8 Statistical analysis

Data on hormonal profiles were subjected to one-way-anova of IBM SPSS version 25 (SPSS, 2017), according to the following model:

$$Y_{ij} = \mu + C_i + P_j + S_k + e_{ijk}$$

Where: Y_{ijk} = Dependent variables representing the hormonal levels (progesterone, oestradiol, testosterone, FSH and LH); μ = overall mean of traits; C_i = effect of i^{th} breed, i

= 1, 2, and 3; P_j = effect of j^{th} physiological state of cows, j = 1, 2, 3, 4, 5 and 6; S_k = effect of k^{th} physiological state of cows, k = 1, 2, and 3; e_{ij} = error term.

Correlation between traits was estimated using SPSS (2017). Correlations were classified as low (0.10 – <0.35), medium/moderate (≥0.35 – <0.50), and high (≥0.50 – 1.00).

3.9 Study 4: Production Performance of Dairy Cows

3.9.1 Determination of Milk yield, lactation length and milk composition in dairy cows

3.9.1.1 Objectives

The objectives of this study were to:

- Assess effect of breed and non-genetic factors (farm/geographical location, parity, stage of lactation, BCS, udder size, teat size, season, feed supplementation, and milking frequency) on mean milk yield and lactation length of Sanga, Friesian-Sanga crossbred and Jersey cows.
- Find out effect of breed and non-genetic factors on percentage milk composition of milk in dairy cows.

3.9.1.2 Data collection

A total of five hundred and sixty-six (566) cows were purposively sampled for this study. Data on milk yield was measured with graduated beaker (1 litre capacity), immediately after hand milking. Factors considered in this study encompassed farm (n=9), breed (Snaga, n=202; Friesian-Sanga crossbred, n=332 and Jersey, n=32), parity (1 – 6), stage of lactation (1-30 days, , body condition score (BCS—2.0, 2.5, 3.0, 3.5 and 4.0 on the 1 – 5-

scale), udder/teat size (small, medium and large), season of calving (rainy, minor rainy, dry seasons) (Coffie 2014), and frequency of milking per day (once/twice).

Udder and teat sizes were categorized into small, medium and large. The categories of udder and teat sizes used for this study were outlined in Coffie *et al*. (2015a) with slight modification in the udder sizes. Briefly, Udder base circumference [UBC] and udder length [UL] from base of udder to the base of teat were measured and categorized into small (UBC≤74cm; UL ≤13cm), medium (UBC=75-85cm; UL=14-17cm) and large (UBC ≥86cm; UL≥18cm). Teat base circumference (TBC) and its length [TL] were measured and considered as small (TBC≤5cm; TL≤ 2cm), medium (TBC=6-8cm; TL=2.5-5cm) and large (TBC≥9cm; TL>5cm).

Lactation length of dairy cows was determined as outlined in Coffie *et al.* (2015b). A total of one hundred and seventy-seven (177) cows (Sanga, n=62; Friesian-Sanga, n=83 and Jersey, n=32) were used for the study.

Milk composition (% protein, % fat, % lactose, % cholesterol, % total solids, and % solids-non-fat) of the various breeds was determined at the Biochemistry Laboratory of the Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, in accordance with A.O.A.C. (2006). Breeds of cows used for this study included Sanga (n=10). Friesian-Sanga crossbred (n=10) and Jersey cows (n=10).

3.9.1.3 Statistical analysis

Data on milk yield and lactation length of the diary cows were subjected to least squares (LS) analysis using Generalized Linear Model (GLM) Type III Procedure of IBM SPSS version 25 (SPSS, 2017) on the following fixed model:

$Y_{ijlmnopqrs} = \mu + B_i + C_j + F_l + L_m + M_n + P_o + S_p + Tq + Ur + X_s + e_{ijlmnopqrs}$;

Where: Where: $Y_{ijklmnop}$ = the dependent variable or the trait being measured (mean milk yield); μ = the population mean; B_i = the effect of i^{th} breed, i = 1, 2 and 3; C_j = the effect of j^{th} body condition score, j = 1, 2, …, 5; F_l = the effect of l^{th} farm, l = 1,2, …, 9; L_m = the effect of m^{th} stage of lactation, m = 1, 2 and 3; M_n = the effect of n^{th} frequency of milking/day, n = 1 and 2; P_o = the effect of o^{th} parity of cow/dam, o = 1, 2, …, 6; S_p = the effect if p^{th} season of lactation, p = 1, 2, 3; T_q = the effect of q^{th} teat size, q=1, 3, 3; U_r = the effect of r^{th} udder size, r = 1, 2, 3; X_s = the effect of s^{th} supplementation, s =1, 2, 3; and $e_{ijlmnopqrs}$ = residual effect.

Data on percentage milk composition of the dairycows were subjected to one-way-anova of IBM SPSS version 25 (SPSS, 2017), according to the following model:

$Y_{ijklmno} = \mu + A_i + B_j + C_k + M_l + P_m + S_n + X_o + e_{ijklmno}$;

Where: $Y_{ijklmno}$ = traits being measured (milk composition); μ= overall mean of traits; A_i = the effect of i^{th} Location, i = 1, 2, and 3; B_j = effect of j^{th} breed, j= 1, 2 and 3; C_k = effect of k^{th} body condition score, k = 1, 2 and 3; M_l = effect of l^{th} stage of lactation, l = 1, 2 and 3; P_m = effect of m^{th} parity, m = 1, 2 and 3; S_n = effect of n^{th} season of milking, n = 1, 2 and 3; X_o = effect of o^{th} feed supplementation, o = 1, 2 and 3 and $e_{ijklmno}$ = residual effect.

3.9.2 Assessment of milk quality in Ghanaian Sanga, Friesian-Sanga and Jersey cows

3.9.2.1 Objectives

This study was conducted to:

- Evaluate effect of breed and non-genetic factors on fresh/raw milk's temperature, pH and specific gravity in dairy cows.
- Determine effect of fixed factors on somatic cell count, total bacterial (viable) count and total coliform in fresh milk of dairy cows.

3.9.2.2. Measurement of specific gravity of milk by Lactometer

A lactometer (or galactometer) is a hydrometer used to test milk purity. Quevenne is an arbitrary scale used with hydrometers or lactometers in the determination of the specific gravity of milk. Degrees Quevenne = 1000 (specific gravity ≈ 1). The specific gravity of milk was determined one hour after the milk was drawn from the cows in order to avoid a lower than normal value. Hence milk samples were kept in air conditioned environment in order to achieve the temperature of 20 °C or close.

Fresh milk sample was poured into 200 mL measuring cylinder to about 8/9 full. Lactometer was then gradually lowered into the milk and allowed to float freely until equilibrium was reached. Lactometer reading was taken at the lower meniscus and recorded (as Quevenne's degree/lactometer degree—ºL). The specific gravity of milk was calculated by dividing the Quevenne's degree by 1,000 and adding 1. Thus: Specific gravity (g/mL) = $(\frac{\text{lactometer degree or } {}^{0}\text{L}}{1000}) + 1$. The lactometer reading was standardized at a temperature of 20 °C (Gachuiri *et al*., 2012), That is, lactometer reading was corrected by adding 0.2 to the lactometer reading or 0.0002 to the specific gravity for each 1 °C above

20°C and vice versa for lower temperatures (by subtracting when temperature fell below 20 °C).

3.9.2.3 Measurement of milk temperature and pH

Temperature of fresh milk sample was determined on the field of sampling using digital thermometer (Ashford Instrument Ltd, Kent). Milk pH was determined in the ABC laboratory (Commercial facility) using a digital pH meter–500X500 (Partech Scientific Instruments, Thane West, Indian) in accordance with manufacturer's instructions.

3.9.2.4 Determining somatic cell count in milk of cows

Freshly obtained milk samples were kept on ice packs and transported to the University of Education, Winneba, College of Agriculture Education laboratory (Mampong Campus) for determination of somatic cell count using haemocytometer (Superior Marienfeld, Germany) and by following guidelines for counting somatic cell count (SCC) in milk (Fitts and Murphy, 2004) with slight modification. Hundred microliters (100 µL) of milk sample was dispensed into micro centrifuge tube using micro pipette (Oxford LP Benchmate, Japan) and 100 µL of 0.4 % trypan stain (used for differentiation between live and dead cells) was added. 10 µL of the prepared sample was loaded into two well chambers of the haemocytometer with cover slide. The loaded slide was mounted on Compound microscope (GT Vision, UK) and observed (1000 X) magnification. Four (4) corner squares and one (1) centre square were counted. Cells touching top and left boundaries were counted whiles cells touching bottom and right boundaries were not counted. Calculations of counted cells were done as follows:

1. % of viable cells = $\frac{number\ of\ Viable\ somatic\ cells}{total\ number\ of somatic\ cell}$ X 100

2. Average number of cells/square = $\frac{Vaible\ somatic\ cell}{5\ squares}$

3. Dilution factor = $\frac{Final\ volume\ (milk+stain)\ =200\mu m}{Volume\ of\ milk\ =100\mu m} = 2$

4. Concentration SSC = $\frac{\text{Average number of cells}}{\text{squares}}$ X dilution factor X 10^4

Thus, only the results on the concentration of SCC were presented and expressed as SCC/mL.

3.9.2.5 Total bacterial and coliform count

Sanitary management and milking practices were considered as factors. Under sanitized management condition, milking was done in a cleaned and sanitized premises (milking yard/cubicle) together with udder cleaning, hand washing with clean running water and mopping udder and hands with a clean napkin prior to milking. Farmers who milked with partial cleaning and little or no cleaning of udders and hands were also noted.

The total bacterial and coliform counts were determined by method described by Ogot *et al.* (2015). Briefly, milk samples were serially diluted in peptone water. From the tubes with dilutions of 10^{-1} – 10^{-5}, 1 mL was pippeted and inoculated in standard plate count agar using pour plate method. The plated sample was allowed to solidify and then incubated at 37°C for 48 hours. Negative control was done using plate count agar only (Harley, 2013). For coliform count the milk samples were shaken 25 times then diluted using the same dilution procedure used for standard plate count. The samples were inoculated on MacConkey agar using the spread plate method. The plates were then incubated at 37°C

for 36 hours (Harley, 2013). The number of colonies was recorded using a colony counter. Only the plates with 30-300 colonies were considered in calculating the colony forming units (CFU) per mL of sample (Ogot *et al.*, 2015)**.** The CFU/mL of sample was determined using the formula: CFU per mL of sample = $\frac{\text{number of colonies}}{(\text{amount plated X dilution})}$ (CFU/mL).

3.9.3.6 Statistical analysis

Data on milk pH, temperature, specific gravity, somatic cells, and bacterial counts were subjected to analysis of variance (ANOVA) of SPSS (2017), version 25 under the following model:

$Y_{ijklmno} = \mu + B_i + C_j + L_k + M_l + P_m + S_n + X_o + e_{ijklmno;}$ Where:

$Y_{ijklmno}$ = Dependent variable/traits being measured; μ = population mean; B_i = the effect i^{th} breed of cattle, i = 1, 2, and 3; C_j = the effect of j^{th} body condition j = 1, 2 and 3; L_k = the effect of k^{th} location of the study, k = 1, 2 and 3; M_l = the effect of l^{th} sanitary management, l = 1, 2 and 3; P_m = the effect of m^{th} parity of dam, m = 1, 2 and 3; S_n = the effect of n^{th} stage of lactation, n = 1, 2 and 3; X_o = the effect o^{th} feed supplementation, o = 1, 2 and 3 and $e_{ijklmno}$ = residual effect.

CHAPTER FOUR

4.0 RESULTS

4.1 Health Management of Ticks and Tick-Borne Diseases

4.1.1 Prevalence of tick species and tick-borne diseases

4.1.1.1 Incidence of tick species across breeds

Mean seasonal trend in occurrence of *Amblyomma variegatum (Av.), Boophilus decoloratus (B. d.), Hyalomma rufipes (Hy.r.) and Rhipicephalus species (Rh. spp.)* from 2015 to 2017 are preseted in Fig 4.1.

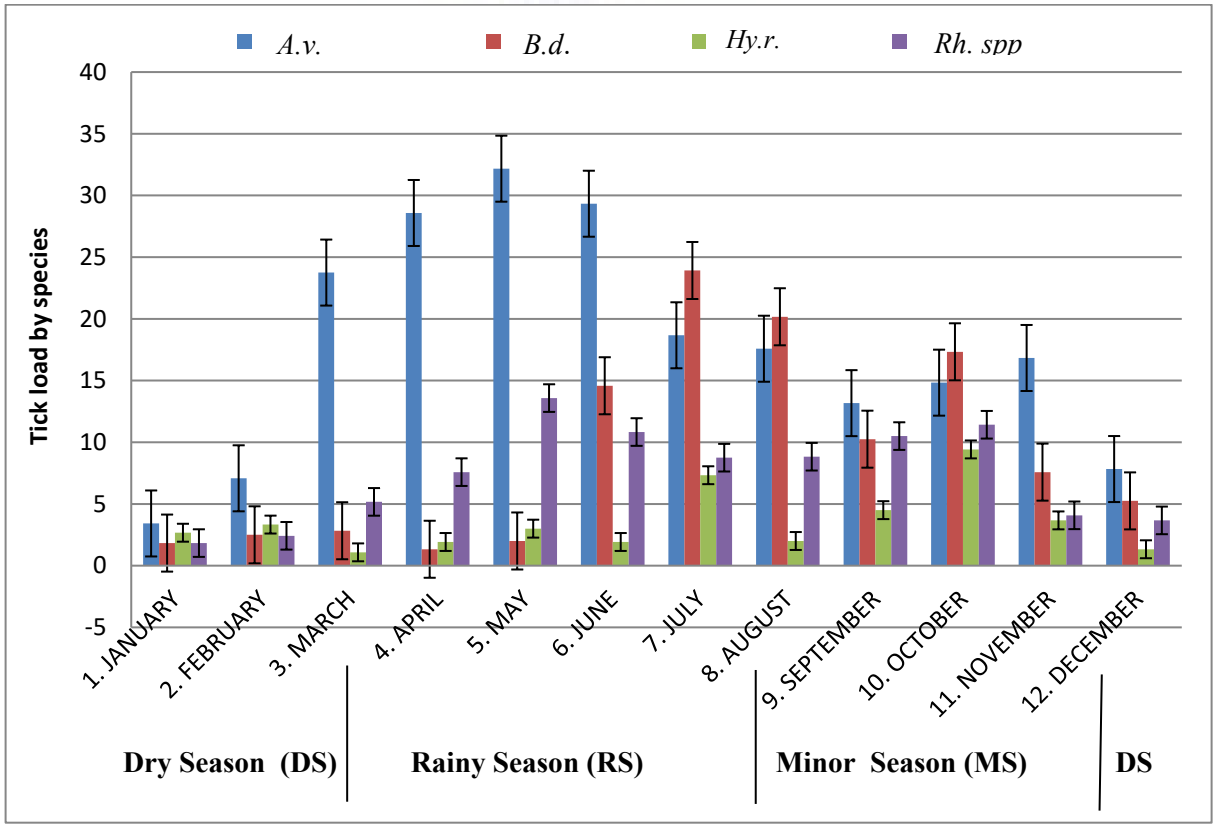

Fig. 4.1: Mean seasonal trend in tick species abundance across breeds in 2015, 2016 and 2017. Each point represents a mean from 20 cows (4 cows x 5 breeds) in each month for the three years period.

Amblyomma variegatum was the dominant tick species followed by *Boophilus spp (Rhipicephalus) decoloratus, Rhipicephalus spp. and Hyalomma rufipes* in decreasing trend in abundance. *Amblyomma variegatum* occurred throughout the year but increase in abundance with increasing rains peaking in May in the major rainy season. *A. variegatum* incidence declined in July and remained in reducing trend till September in the minor season. There was a slight increasing trend in *A. variegatum* fron October to November and sharply reduced from December to January/February.

Boophilus spp. (mainly of Rhipicephalus (Boophilus) decoloratus, Rhipicephalus (Boophilus) annulatus and *Rhipicephalus (Boophilus) geigyi*) number remained decreased during the dry season and assumed increasing abundance from May and peaked in July, where *A. variegatum* incidence had been decreasing. *Boophilus (Rh) decoloratus* incidence increased in October as compared to September and reduced in December.

Rhipicephalus spp. (*Ripicephalus senegalensis* and *Rhipicephalus evertsi evertsi*) abundance occurred with the occurrence of green vegetation, starting from March, peaked in May and slightly declined in June. *Rhipicephalus spp* numbers remained stable from June to October and progressively reduced after October and reached a minimum in December.

Hyalomma marginatum rufipes was the least abundant tick species. Its incidence was low but its highest incidence occurred in October and in the minor season followed by July in the rainy. *Hyalomma rufipes* were mostly found in the Coastal zone of the study area. The lowest incidence of the ticks occurrd in December and March.

4.1.2 Factors influencing total tick load or count

4.1.2.1 Effect of Farm and management on total tick count

Effect of fixed factors on total tick count/load is showed in Table 4.1a. Farm and farm management system had significant (P<0.01) influence on total tick count or abundance. The farm effect was determined by the management practices adopted.

Table 4.1 a: Effect of Farm and management on total tick count/load (TTC)

FIXED FACTORS	N	LEAST SQUARE MEANS (± SE) *for Y = Log10 (X +1) + 0.5)TTC*
FARM	***127***	***0.000****
1. EMBIK	13	2.1 ± 0.05^{c}
2. REA	9	2.0 ± 0.06^{d}
3. UEW-M	12	2.1 ± 0.05^{c}
4. ZARE	16	2.1 ± 0.04^{b}
5. SUHUM (A&B)	19	2.2 ± 0.04^{b}
6. KARIMA	22	2.2 ± 0.04^{b}
7. AMRAHIA	16	2.4 ± 0.05^{a}
8. HARUNA	12	2.3 ± 0.05^{a}
9. M. ISMAIL	8	2.2 ± 0.06^{b}
MANAGEMENT	***127***	***0.000****
1. RANGE GRAZING	48	2.6 ± 0.03^{a}
2. PARTIAL ZERO GRAZING	32	2.4 ± 0.04^{b}
3. EXCLUSIVE ZERO GRAZING	47	1.6 ± 0.03^{c}

**=P-value; ^{a,b,c,d}=Means bearing different superscript in the same column are significantly different.*

Farms that practised range grazing (sedentary husbandry system) had the highest (P<0.01) tick load followed by partial zero-grazing with the exclusive zero-grazing having the least total tick count.

4.1.2.2 Effect of Breed on total tick load in different management regimes

Breed also influenced (P<0.01) tick infestation load (Table, 4.1 b). Zebu (*Bos indicus*) and Friesian-Sanga crossbreds had the highest (P<0.01) tick count. Sanga and Ghana Shorthorn

(GSH) breeds had similar (P>0.05) total tick count but were higher than the count obtained in Jersey cattle. The differences in total tick count observed in this study were interfered or overshadowed by management effect. Sanga and GSH breed resisted the tick load better than the crossbreds and zebu. However, Jersey cattle recorded the least or no tick count (Table 4.1 b).

4.1.2.3 Effect of Location

Location significantly influenced (P<0.01) the total tick count in the three regions studied (Table 4.1 b). Cattle of Ashanti region had the highest total tick count, which was similar (P>0.05) to those obtained in Greater Accra. Eastern region had the least (P<0.01) count.

4.1.2.4 Effect of season on total tick count

Season was one of the determinants of total tick count. Major rainy season had the highest (P<0.01) total tick count and it was followed by the minor rainy season. Dry season recorded the least (P<0.01) count (Table 4.1b).

4.1.2.5 Effect of sex of cattle

Sex of cattle had insignificant (P>0.05) effect on total tick count in the study areas.

4.1.2.6 Effect of feed supplementation

Cattle that were regularly supplemented with feed had a fewer total tick count. Animal given partial and no feed supplementation had a similar (P>0.05) tick count and this was higher (P<0.05) than those given regular feed supplementation (Table 4.1b).

Table 4.1b: Effect of breed, and non-genetic factors on total tick count (TTC)

FIXED FACTORS	N	LEAST SQUARE MEANS (± SE) *for Y = Log10 (X + 1) + 0.5)*
BREED	***127***	***0.000****
1. SANGA	27	2.2 ± 0.03^{b}
2. WASH (GSH)	19	2.2 ± 0.04^{b}
3. FR-SANGA CB	27	2.3 ± 0.04^{a}
4. JERSEY	9	2.0 ± 0.06^{c}
5. ZEBU	45	2.3 ± 0.03^{a}
LOCATION (REGIONS)	***127***	***0.009****
1. ASHANTI	48	2.3 ± 0.06^{a}
2. EASTERN	31	2.1 ± 0.06^{b}
3. GREATER ACCRA	48	2.2 ± 0.10^{a}
SEASON	***127***	***0.000****
1. RAINY SEASON	48	2.6 ± 0.06^{a}
2. MINOR SEASON	32	2.4 ± 0.06^{b}
3. DRY SEASON	47	1.6 ± 0.10^{c}
SEX OF CATTLE	***127***	***0.847****
1. MALE	52	2.2 ± 0.03
2. FEMALE	75	2.2 ± 0.02
FEED SUPPLEMENTATION	***98***	***0.000****
1. SUPPLEMENTATION	12	2.2 ± 0.05^{a}
2. PARTIAL SUPPLEMENTATION	39	2.5 ± 0.03^{b}
3. NO SUPPLEMENTATION	47	2.5 ± 0.03^{b}
TYPE OF HOUSING	***127***	***0.000****
1. INSECT-PROOF	33	2.0 ± 0.04^{b}
2. OPENED KRAAL	28	2.3 ± 0.03^{a}
3. ROOFED BARN	66	2.3 ± 0.02^{a}
LBSP	***98***	***0.000****
2 VERY LOW BSP	48	2.6 ± 0.03^{a}
3 LOW BSP	32	2.5 ± 0.03^{b}
4 MODERATE BSP	18	2.0 ± 0.04^{c}
PERIOD OF AABS	***98***	***0.000****
1. WEEKLY (5 – 6 days interval)	20	2.2 ± 0.04^{a}
2. FORTHNIGHTLY	40	2.3 ± 0.03^{b}
3. MONTHLY	16	2.5 ± 0.04^{c}
4. TWO MONTH	22	2.5 ± 0.04^{c}

**=P-value; AABS= administering acaricide by spraying; BSP=biosecurity practicess observed; LBSP=level of biosecurity practices; ^{abcd}=Means bearing different superscript letters in the same column are significantly different.*

4.1.2.7 Types of housing

Type of housing used for keeping dairy herds significantly (P<0.01) affected tick count. Insect-proofed barn recorded little or no tick count whereas roofed and open kraal had similar (P>0.05) but more (P<0.01) total tick count than those observed in insect proof ones (Table 4.2 b).

4.1.2.8 Level of biosecurity measures employed

Level of biosecurity greatly (P<0.01) influenced total tick count (Table 4.1b). Implementation of moderate level of biosecurity practices resulted in a drastic reduction (P<0.05) in total tick count, followed by low level observation of the measures with negligence of biosecurity measures on farm registering the worst tick count.

4.1.2.9 Period of administering acaricide

The period of administering acaricide by spraying during peak tick infestation also influenced (P<0.01) total tick count. Weekly spraying of tick species had the most (P<0.01) reduced total tick count in hot-humid rainy seasons. Fortnightly or bi-weekly spraying recorded a fewer (P<0.01) tick count, with a monthly and two months spraying having similar (P>0.05) and the worst tick counts (Table 4.1b).

4.1.2.10 Interactions effect on fixed factors

Two-way interactions between breed and environmental variables are presented in Table 4.1c. Significant interactions existed between breed and season, supplementation, type of housing, level of biosecurity practices, and period or interval of administering acaricide spray. There were also significant interactions between location and type of housing,

interval of administering acaricide spray; season and type of housing, level of biosecurity practices, and interval of administering acaricide spray; feed supplementation and and type of housing, level of biosecurity practices and interval of administering acaricide spray. Interaction between breed and location and sex of cattle were not significant. There was also insignificant interaction effect between location and season, in this study. Other insignificant interactions are presented in Table 4.1c.

Table 4.1c: Two-way interactions between breed and environmental variables

Type of interaction	***Log10 (X +1) + 0.5) total tick count***
Breed *Location	Ns
Breed *Season	**
Breed *Sex of cattle	Ns
Breed*Supplementation	**
Breed *Type of housing	**
Breed *Level of biosecurity practices	**
Breed *Interval of administering acaricide spray	**
Location*Season	Ns
Location* Sex of cattle	Ns
Location* Supplementation	Ns
Location* Type of housing	**
Location* Interval of administering acaricide spray	**
Season*Sex of cattle	Ns
Season*Supplementation	Ns
Season*Type of housing	**
Season*Level of biosecurity practices	**
Season*Interval of administering acaricide spray (IAAS)	**
Sex*Supplementation	Ns
Sex*Type of housing	Ns
Sex*Level of biosecurity practices	*
Sex*Interval of administering acaricide spray	*
Supplementation*Type of housing	**
Supplementation*Level of biosecurity practices	**
Supplementation* IAAS	**
Type of housing*Level of biosecurity practices	**
Type of housing* IAAS	**
Level of biosecurity practices* IAAS	**

**=p<0.05; **=p<0.01; ns=not significant; IAAS=* Interval of administering acaricide spray.

4.1.3 Prevalence of tick-borne diseases

4.1.3.1 Assessment of prevalence of tick-borne diseases by microscopy

The prevalence of tick-borne diseases in 486 cattle surveyed for dermatophilosis, anaplasmosis and heartwater are presented in Table 4.2. Dermatophilosis had the highest overall percentage prevalence, followed by anaplasmosis, and heartwater disease recorded the least.

Table 4.2: Prevalence of tick-borne diseases in hot humid and Coastal Savanna zones

	Tick-borne Diseases	**N**	**Observed positive Cases**	**Prevalence (%)**
1.	DERMATOPHILOSIS	486	133	27.37
2.	ANAPLASMOSIS	486	104	21.40
3.	HEARTWATER	486	33	7.41

N=number of observation.

The laboratory findings on conventional determination of prevalence of dermatophilosis, anaplasmosis, and heartwater/cowdriosis are presented in Plates 4.1 a, b, and c, respectively. Plate 4.1a, shows morphological, culturing and staining characteristics of *Dermatophilus congolensis* organism used to determine positive cases in 486 heads of cattle, in which 133 heads were positive. The pleomorphic, Gram-positive non-acid fast facultative actinomycete, *Dematophilus congolensis,* with filamentous hyphae and zoospores were evident. Culture characteristics of the parasites on nutrient (III) and LB agar (IV) showing a typical fried egg features, together with staining characteristics with Leichman's (V) and Gram stain (VI) are presented in the Plate 4.1 a.

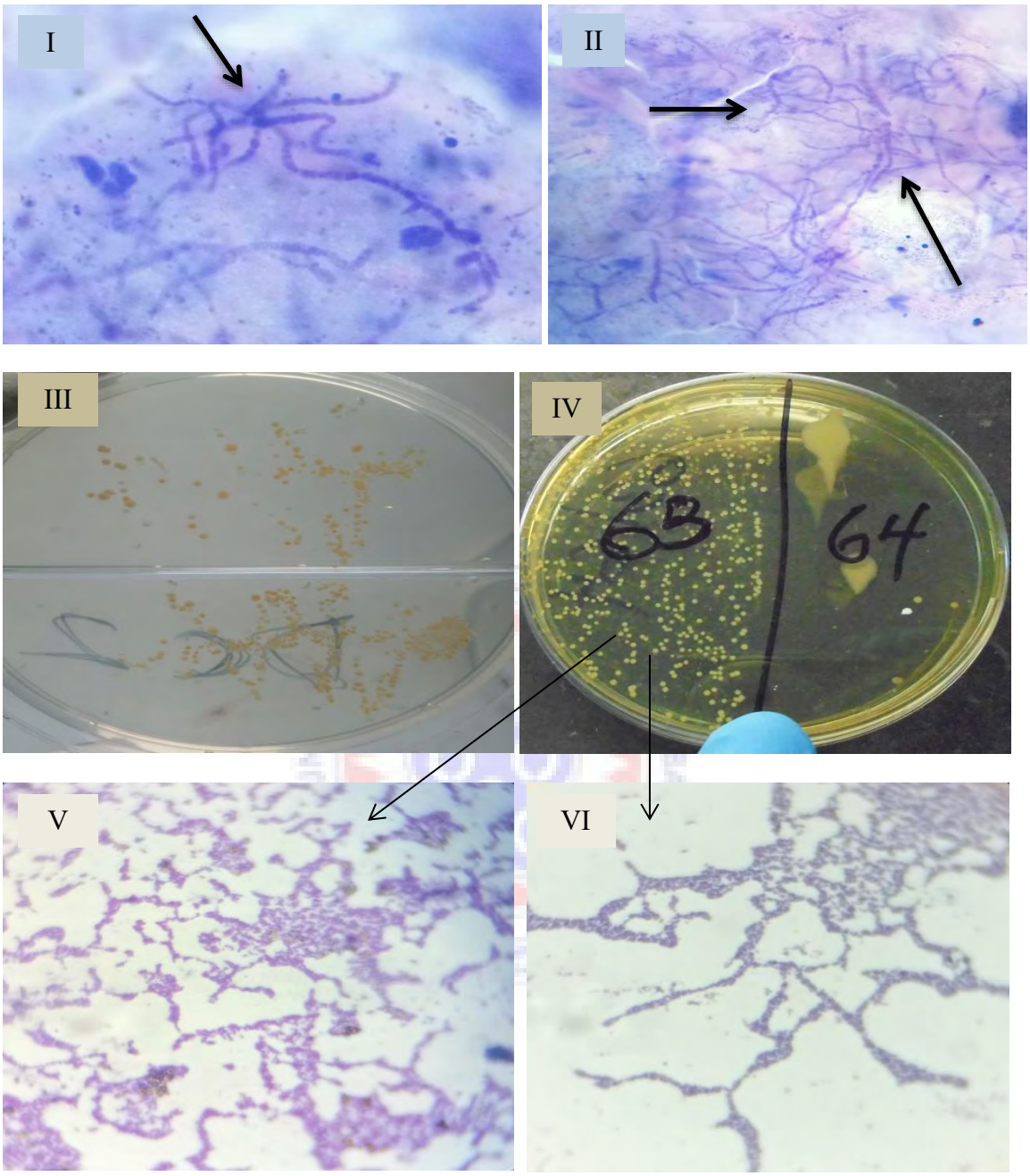

Plate 4.1a: Culturing and staining characteristics of *Dermatophilus congolensis:* I and II, morphological characteristics of filamentous hyphae and zoospores from an impression smear of the undersurface of scabs stained with Leichman stain; III and IV, Culture characteristics of the parasites on nutrient and LB agar, respectively; V and VI, staining of a colony from culture with Leichman's and Gram stain, respectively.

Plate 4.1b, indicates results on demonstrations and detection of *Anaplasma marginale* haemo-parasite (arrowed). Of 486 cattle sampled, 104 (21.40 %) were positive to anaplasmosis.

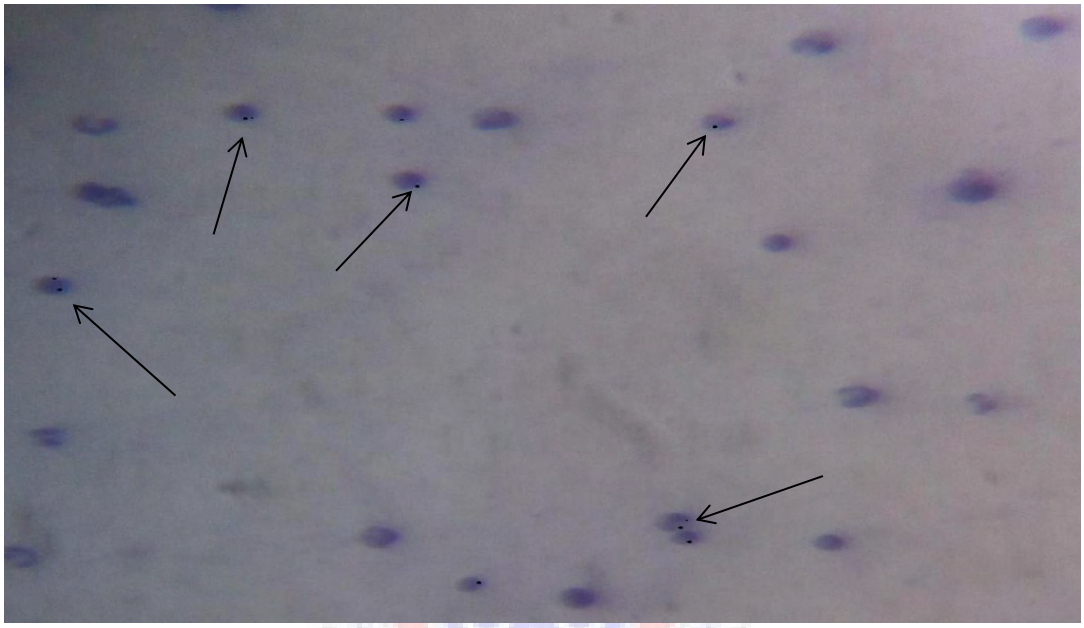

Plate 4.1b: *Anaplasma marginale* parasites appearing as dark dots (arrowed) at the margins of red blood cells; note the massive haemolysis as indicated in few RBCs' number.

Plate 4.1c shows brain stains of positive cases detected at post mortem. The prevalence of cowdriosis by conventional means was determined through PM and laboratory examinations within the survey area in 33 heads of cattle. Brain stains showed *Ehrlichia ruminantium* inclusion bodies stained with Leichman stain.

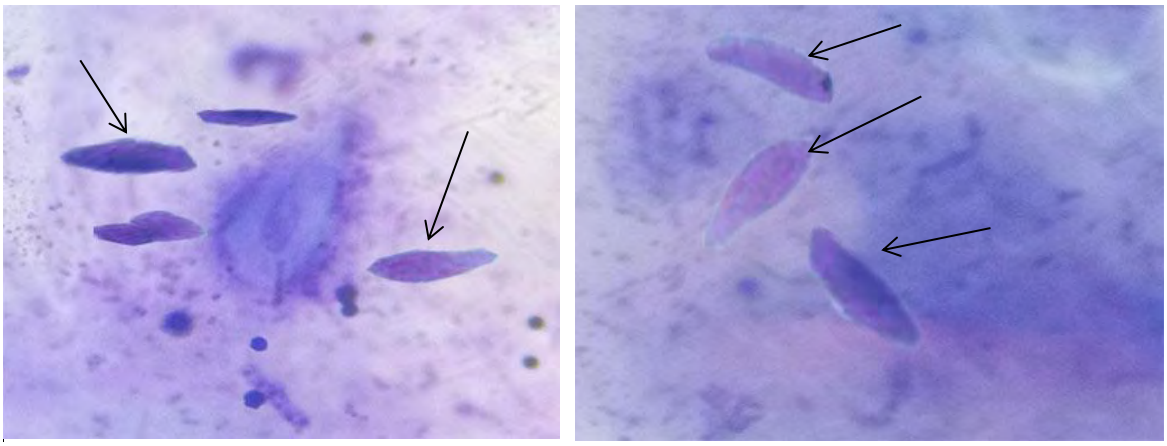

Plate 4.1c: Brain stains showing *Ehrlichia ruminantium* inclusion bodies stained with Leichman stain (arrowed)

4.1.3.2 Molecular (PCR) confirmation of the tick-borne diseases

4.1.3.2.1 Dermatophilosis

Finding on the molecular confirmation of dermatophilosis (PCR result) is presented in Plate 4.2. The isolates of *D. congolensis* (74/80 ≈ 92 %) were confirmed by amplifying a 500 bp fragment of 16s rRNA gene of *D. congolensis* from scabs and nasal swabs of carrier herds but no amplification was seen in negative control.

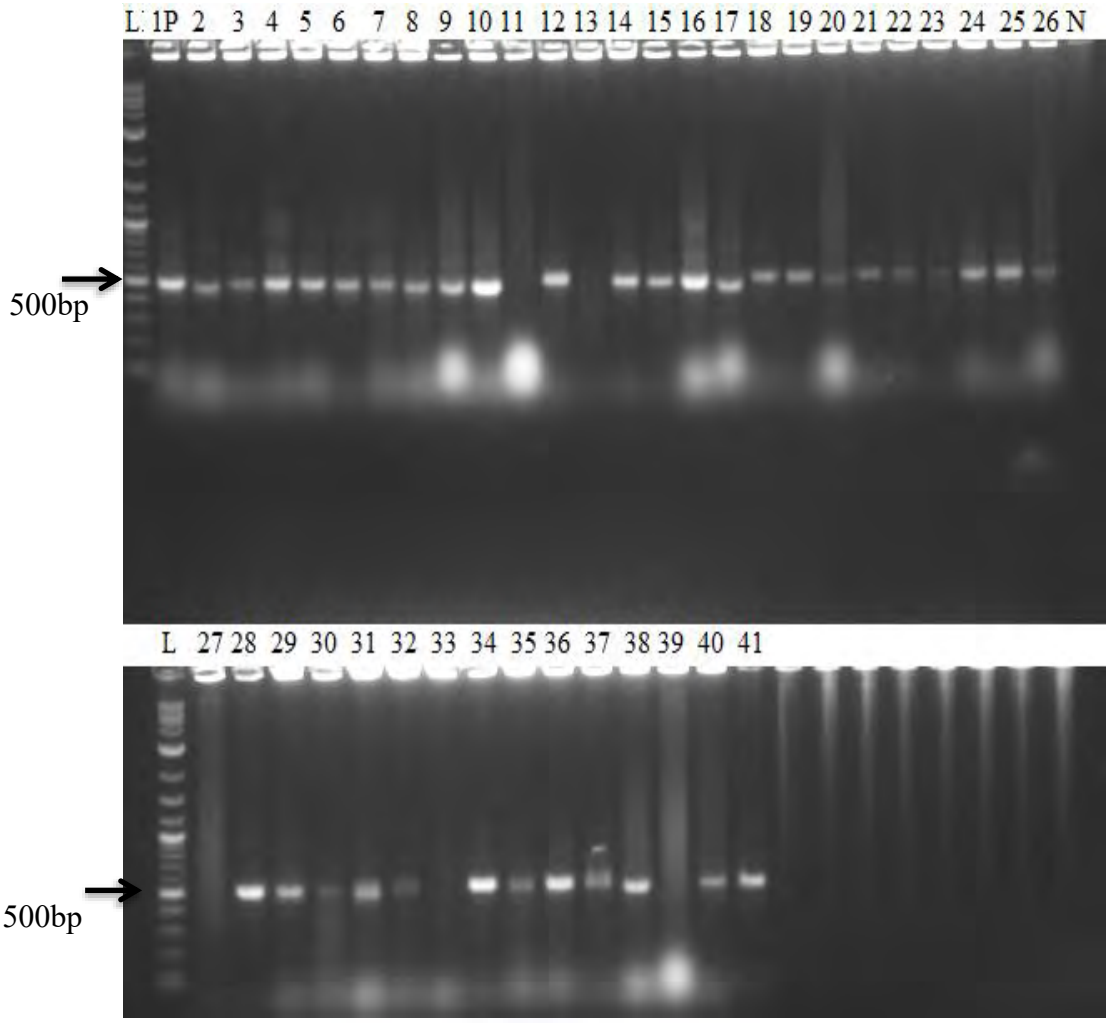

Plate 4.2: PCR amplification of a 500 bp fragment of 16S rRNA gene of *Dermatophilus congolensis* isolates from scabs (from D. congolensis infected cattle i.e. lane 2 - 10) and nasal swabs (from carrier herds i.e. lane 11 - 25).Lane L= 100 bp ladder, lane 1P= positive control from laboratory confirmed case; lane N=negative control using nuclease free water.

4.1.3.2.1 Anaplasmosis

Of the total samples subjected to primary PCR, 33.75 % (27/80) were positive for Amar MSP5 gene of *Anaplasma marginale* infection. The PCR amplification of a 458 bp fragment of msp5 gene of *A. marginale* is shown in Plate 4.3.

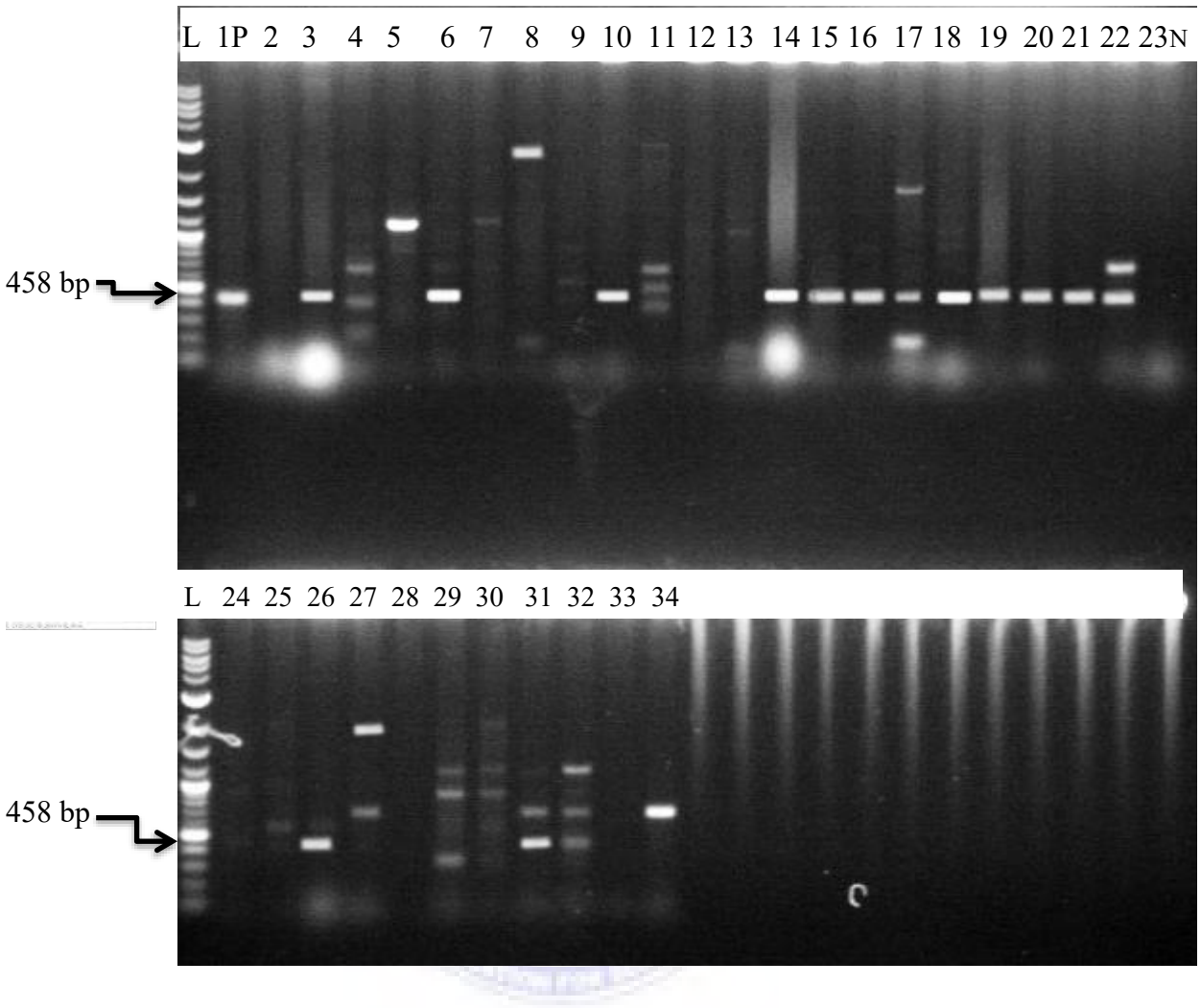

Plate 4.3: PCR amplification of a 458 bp fragment of msp5 gene of *A. marginale.*
Lane L= 100 bp DNA Ladder plus maker, Lanes 1P = positive case from field study.
Lane 23N= negative control.

4.1.3.2.3 Heartwater/Cowdriosis

Amplification of a 279-bp of a pCS20 sequence of *Ehrlichia ruminantium* were found positive in 10.0 % (8/80). PCR amplification of 279-bp of pCS20 gene sequence of *Ehrlichia ruminantium* indicated in Plate 4.4.

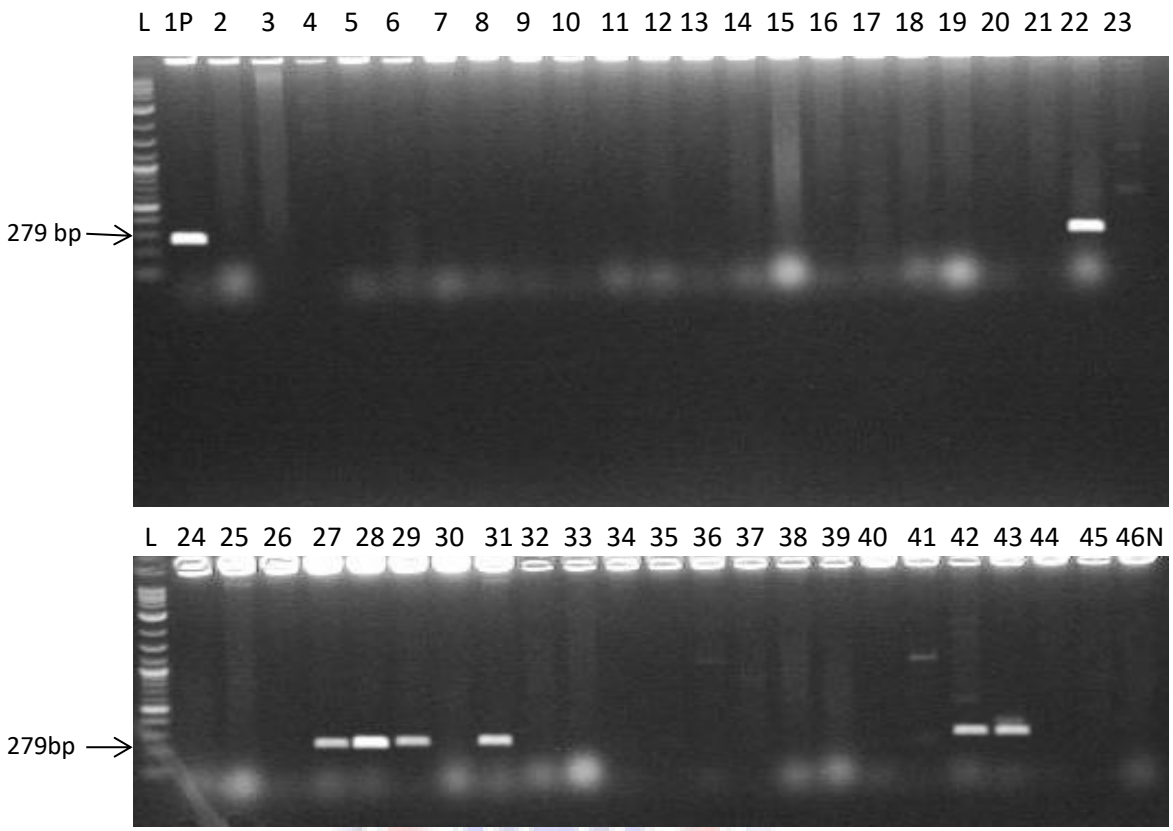

Plate 4.4: PCR amplification of 279-bp of pCS20 gene sequence of *Ehrlichia ruminantium* amplified from brain and ticks but not from blood. L=100 bp ladder plus maker; 1P= positive case from brain confirmation and isolation of *Ehrlichia ruminantium*; 46 N= negative control.

4.1.4 Factors influencing the prevalence of tick-borne diseases

4.1.4.1 Effect of farm on prevalence of dermatophilosis, anaplasmosis and heartwater

The percentage prevalence of the three tick-borne diseases differed with individual farm practices. Farms that controlled movement of biological agents and practised good sanitation had little or no outbreaks as indicated in Table 4.3a whiles farm that compromised good management and good health standards recorded higher percentage prevalence of the tick-borne diseases.

Table 4.3 a: Effect of Farm on the prevalence (%) of Dermatophilosis, Anaplaasmosis and Heartwater/Cowdrosis

FACTORS	N	DERMATOPHILOSIS		ANAPLASMOSIS		HEARTWATER	
		Observed Cases	Prevalence (%)	Observed Cases	Prevalence (%)	Observed Cases	Prevalence (%)
FARM Overall	***486***	***133***	***27.37***	104	***21.40***	36	***7.41***
1. EMBIK	19	1	5.26	0	0.00	0	0.00
2. REA	23	0	0.00	0	0.00	0	0.00
3. UEW-M	15	5	33.33	5	33.33	2	13.33
4. ZARE	21	1	4.76	1	4.76	1	4.76
5. SUHUM	22	0	0 .00	0	0.00	0	0.00
6. HUSEIN	18	2	9.09	2	9.09	2	11.11
7. AMADU	20	10	50.00	4	20.00	2	10.00
8. SULE	17	10	58.82	6	35.29	3	17.65
9. KARIMA	21	3	14.29	2	9.52	2	9.52
10. BEPOASE	21	7	33.33	4	19.05	3	14.29
11. F. FRALINE	21	9	42.86	7	33.33	1	4.76
12. GAO KARIM	21	4	19.05	3	14.29	3	14.29
13. OWUSU	21	9	42.86	7	33.33	2	9.52
14. AKROFONSO	21	8	38.10	6	28.57	3	14.29
15. ASMANG OK	21	11	52.38	9	42.86	1	4.76
16. DROMANKOMA	56	17	30.36	14	25.00	2	3.57
17. IMORO	11	0	0.00	0	0.00	0	0.00
18. BELKO	21	7	33.33	7	33.33	2	9.52
19. ADAMS	21	6	28.57	4	19.05	0	0.00
20. YUSIF (DR)	21	0	0.00	0	0.00	0	0.00
21. DWOMOH	16	10	62.50	10	62.25	2	12.50
22. SIMON	15	6	40.00	6	40.00	1	60.67
23. AMRAHIA	23	6	27.27	7	30.43	4	17.39

N=Number of observation; %=Percentage

4.1.4.2 Effect of other non-genetic factors on prevalence of the tick-borne diseases

Cattle that were managed under range grazing had the highest percentage prevalence of dermatophilosis, followed by partial zero-grazing (Table 4.3b). Similar trend in the percentage prevalence was recorded in anaplamosis and heartwater/cowdriosis. Exclusive zero grazing management regime had no prevalence of the three tick borne diseases studied.

Feed supplementation influenced percentage prevalence of dermatophilosis, anaplasmosis and heartwater in that the observed cases decreased with increasing feed supplementation (Table 4.3b). Cattle supplied with regular feed supplement had the least (percentage) outbreaks of the tick-borne diseases whereas cattle that had no feed supplementation recorded the highest percentage prevalence in all the three tick-borne diseases surveyed.

Season also affected the prevalence of dermatophilosis, anaplasmosis and cowdriosis/heartwater. Percentage prevalence of dermatophilosis increased with the increasing intensity of rains resulting in the highest clinical conditions in the major rainy season, minor rainy and dry seasons in descending order (Table 4.3 b). Anaplasmosis and heartwater had a similar seasonal trend such that minor rainy season recorded the highest percentage occurrence, followed by the major rains and dry season had the least percentage outbreaks.

Table 4.3 b: Non-genetic factors influencing the prevalence of dermatophilosis, anaplaasmosis and heartwater

FIXED FACTORS	N	DERMATOPHILOSIS		ANAPLASMOSIS		HEARTWATER	
		Observed Cases	PREVALENCE (%)	Observed Cases	PREVALENCE (%)	Observed Cases	PREVALENCE (%)
MANAGEMENT	**486**	***133***	***27.37***	***104***	***21.40***	***36***	***7.41***
1. RANGE ZG	404	128	31.68	102	25.25	34	8.42
2. PARTIAL ZG	21	5	23.81	2	9.52	2	9.52
3. EXCLUSIVE ZG	61	0	0.00	0	0.00	0	0.00
SUPPLEMENTATION	***486***	***133***	***27.37***	***104***	***21.40***	***36***	***7.41***
4. SUPPL.	128	5	3.91	4	3.13	3	2.34
5. PARTIAL SUPPL.	216	60	27.78	47	21.76	13	6.02
6. NO SUPPL.	142	68	47.89	53	37.32	20	14.08
SEASON	***441***	***133***	***30.16***	***104***	***21.40***	***36***	***7.41***
4. RAINY SEASON	126	68	53.97	37	29.37	12	9.52
5. MINOR SEASON	168	44	26.19	51	30.36	20	11.90
6. DRY SEASON	147	21	14.48	16	10.88	4	2.72
TYPES OF HOUSING	***486***	***133***	***27.37***	***104***	***21.40***	***36***	***7.41***
4. INSECT- PROOF	60	0	0.00	0	0.00	0.00	0.00
5. OPEN KRAAL	287	115	86.47	83	28.91	20	6.97
6. ROOFED BARN	139	18	13.53	21	15.11	16	11.51
LEVEL OF BSM	***486***	***133***	***27.37***	***104***	***21.40***	***36***	***7.41***
1. LITTLE/NO LBSM	370	129	34.86	101	27.30	35	9.46
2. LOW LBSM	63	4	6.35	3	4.76	1	1.59
3. MODERATE LBSP	53	0	0 .00	0	0.00	0	0.00
OVERALL	**486**	***133***	***27.37***	***104***	***21.40***	***36***	***7.41***

N=Number of cattle; ZG=Zero grazing;SUPPL=Feed supplementation; LBSP=Level of biosecurity practices

Types of housing influenced percentage prevalence of the tick-borne diseases (Table 4.3 b). Dairy herds kept in insect proof barns had no outbreak of dermatophilosis, anaplasmosis and heartwater diseases. Nevertheless, open kraals with range grazing regime had the greater percentage outbreaks of dermatephilosis and anaplasmosis as compared with those raised under roofed barns. Heartwater disease was, on the other hand, prevalent in roofed barns than in open kraals

Level of biosecurity measures observed by farm categories greatly impacted on the percentage prevalence rate of the tick-borne diseases. Dairy herds reared under little to no level of biosecurity measures had the highest prevalence of dermatophilosis, anaplasmosis and cowdrosis. Dairy herds maintained in low to moderate level of biosecurity practices (LBSP) had fewer percentage outbreaks whiles those kept under moderate LBSP had no outbreaks of the tick-borne diseases (Table 4.3 b).

4.2 Haemato-biochemical Indices of Dairy Cows

4.2.1 Haematological indices of Ghanaian dairy cows

4.2.1.1 Effect of breed on haematological indices of cows

Effect of breed on haematology of Ghanaian Sanga, Friesian-Sanga crossbred and Jersey cows are presented in Table 4.4.

Table 4.4: Effect of breed on erythrocytes and leucocytes of dairy cows

Factors	Sanga	Crossbred	Jersey	*P-value*	*Reference*
Erythrocyte indices	***N=37***	***N=37***	***N=37***		*Range***
RBC ($X10^6/\mu L$)	8.6 ± 0.70^a	6.5 ± 0.13^b	6.2 ± 0.33^b	*0.000*	*5.0-10.0*
HGB (g/dL)	12.3 ± 0.80^a	10.0 ± 0.22^b	9.9 ± 0.22^b	*0.001*	*8.0-15.0*
HCT (%)	38.3 ± 3.91^a	28.4 ± 2.19^b	26.0 ± 3.81^b	*0.006*	*24.0-46.0*
MCV (fL)	$43.\pm0.52^c$	45.4 ± 0.52^b	50.0 ± 1.03^a	*0.000*	*40.0-60.0*
MCH (pg)	14.0 ± 0.17^c	15.2 ± 0.16^b	16.3 ± 0.4^a	*0.000*	*11.0-17.0*
MCHC (gdL)	32.3±0.13	33.6±0.37	32.7±0.46	*0.086*	*30.0-36.0*
RDWSD (%)	31.4±1.04	31.6±0.71	33.6±0.64	*0.346*	*16.7-23.3*
PLT ($x10^3/\mu L$)	299.5±51.9	301.3±52.0	82.6±9.00	*0.135*	*233-690*
MPV (fL)	6.6±0.29	7.3±0.35	6.8±0.11	*0.377*	*4.0-4.8*
PDW (%)	14.9±0.10	15.8±0.38	15.5±0.12	*0.314*	*56.0-80.0*
PCT (%)	10.1±±8.65	1.1±0.24	0.4±0.10	*0.172*	*0.15-0.40*
Leucocytes	***N=22***	***N=22***	***N=22***	*P-value*	
WBC ($x10^9/L$)	11.2±0.61	12.3±1.03	12.0±0.94	*0.784*	*4.6 -12.0*
GRAN %	28.3 ± 1.19^b	43.8 ± 1.38^a	34.1 ± 2.08^b	*0.000*	
NEU %	29.2±2.66	29.7±2.45	27.2±1.61	*0.916*	
LYM %	64.7±2.38	59.0±2.44	57.8±3.99	*0.365*	*40.0 – 70.0*
MON %	4.1±1.10	7.2±1.31	10.6±3.32	*0.151*	*1.0 – 6.0*
EOS %	0.7±0.13	0.6±0.10	0.3±0.10	*0.384*	*2.0 – 5.0*
BAS %	0.6 ± 0.10^b	1.1 ± 0.11^a	0.7 ± 0.11^b	*0.016*	*<1.0*

RBC=Red blood cell, HGB=Haemoglobin, HCT=Haematocrit, MCV=Mean corpuscular volume; MCH= mean corpuscular haemoglobin; MCHC=mean corpuscular haemoglobin concentration, RDW=red cell distribution width; PLT=platelet; Mean platelet volume; Platelet distribution width; PCT=Plateletcrit; WBC=White blood cell; GRAN=Granulocytes; NEU=Neutrophils; LYM=Lymphocytes; MON=Monoccytes; EOS=Eosinophils; BAS=Basophils; ^{a,b,c,d}*=Means bearing different superscript in the same row are significantly different.*

***=Normal Reference Range* (Radostits *et al*., 2007; Tornquist and Rigas, 2010; Reece, 2015a).

4.2.1.1.1 Effect of breed on erythrocytes

Effect of breed on erythrocytes and leucocytes of indigenous dairy cows are presented Table 4.4. Breed had significant (P<0.01) influence on erythrocyte indices. Red blood cells, HGB and HCT were highest (P<0.01) in Sanga, but Friesian-Sanga and Jersey cows

had similar values. Values of MCV and MCH were highest (P<0.01) in Jersey, followed by crossbreds and Sanga in descending order. Breed had little (>0.05) effect on mean values of MCHC, RDW, PLT, MPV, PDW and PCT.

4.2.1.1.2 Effect of breed on leucocytes

Breed had significant influence on mean percentage granulocytes (P<0.01) and basophil (P<0.05). Granulocytes (GRAN) and basophil (BAS) levels were highest (P<0.01) in Friesian-Sanga crossbred, followed by Jersey cows. Sanga and Jersey cows had similar (P>0.05) granulocytes and basophil values. Breed had little (P>0.05) effect on percentage count of white blood cell (WBC), neutrophils (NEU), lymphocytes (LYM), monoccytes (MON) and eosinophils (EOS).

4.2.1.2 Effect of physiological state on haematological indices of cows

4.2.1.2.1 Erythrocyte indices

Effect of various physiological states on haemotological parameters are shown in Table 4.5 and 4.6. The physiological states [non-cycling heifer, cycling cow (CC), early gestation (G_E), mid gestation (G_M), and late gestation (G_L)] had great (P<0.01) effect on haematocrit (HTC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red blood cell distribution width (RDW %) and platelet (PLT) of cows (Table 4.5).

Table 4.5: Effect of physiological state on erythrocytes of dairy cows

PHYST	RBC	HGB	HCT	MCV	MCH	MCHC	RDW	PLT	MPV	PDW	PCT
Unit	$X10^6/\mu L$	g/dL	%	fL	pg	g/dL	%	$10^3/\mu L$	fL	%	
P-value	*0.105*	*0.583*	*0.002*	*0.000*	*0.000*	*0.004*	*0.000*	*0.000*	*0.701*	*0.271*	*0.451*
Heifer yet to cycle (22)	6.9 ±0.21	10.3 ±0.28	27.7 ±2.50^{a}	43.1 ±0.58^{e}	14.4 ±0.17^{c}	32.3 ±0.28^{b}	28.1 ±0.9^{c}	104.6 ±9.12^{b}	7.2 ±0.51	15.5 ±0.51	6.5 ±5.20
Cycling Cow (22)	5.7 ±0.22	9.3 ±0.31	24.7 ±3.14^{a}	45.33 ±0.41^{d}	15.1 ±0.28^{b}	32.1 ±0.15^{b}	31.8 ±0.61^{b}	158.6 ±13.90^{b}	7.0 ±0.12	15.7 ±0.11	0.3 ±0.10
Early Gestation (22)	7.1 ±021	10.6 ±0.32	21.3 ±3.21^{b}	51.1 ±1.00^{a}	16.5 ±0.37^{a}	33.4 ±0.33^{a}	33.1 ±1.13^{ab}	441.7 ±75.87^{a}	6.8 ±0.06	15.3 ±0.17	0.64 ±0.08
Mid Gestation (22)	6.9 ±0.42	10.4 ±0.60	19.4 ±2.93^{b}	47.1 ±0.60^{cd}	15.1 ±0.30^{b}	33.6 ±0.48^{a}	32.3 ±0.67^{b}	215.2 ±49.95^{b}	6.6 ±0.22	14.4 ±0.51	1.0 ±0.33
Late Gestation (22)	6.5 ±0.28	10.5 ±0.41	20.5 ±3.20^{b}	49.0 ±0.51^{b}	16.2 ±0.16^{a}	33.0 ±0.28^{a}	33.2 ±0.64^{ab}	115.7 ±15.70^{b}	6.7 ±0.08	15.23 ±0.17	0.5 ±0.10
Overall (110)	6.8 ±0.13	10.3 ±0.18	24.9 ±1.23	46.4 ±0.30	15.2 ±0.11	32.7 ±0.24	31.9 ±0.37	201.4 ±22.11	6.9 ±0.13	15.3 ±0.16	1.9 ±1.09

HYTC=Heifer yet to cycle, CC=Cow cycling, G_E=Early gestation, G_M=Mid Gestation, G_L=Late gestation; PHYST=Physiological state, RBC=Red blood cell, HGB=Haemoglobin, HCT=Haematocrit, MCV=Mean corpuscular volume; MCH= mean corpuscular haemoglobin; MCHC=mean corpuscular haemoglobin concentration, RDW=red cell distribution width; PLT=platelet; Mean platelet volume; Platelet distribution width; PCT=Plateletcrit; Values in parenthesis ( ) are the number of observations. ^{a,b,c,d}=Means bearing different superscript in the same column are significantly different.

Table 4.6: Effect of physiological state on leucocytes of dairy cows

PHYST	N	WBC($x10^9$/L)	GRAN%	NEU%	LYM%	MON%	EOS%	BAS%
P-value		*0.014*	*0.000*	*0.000*	*0.023*	*0.262*	*0.021*	*0.002*
Heifer yet to cycle	22	11.7±1.34^{b}	32.1±1.41^{b}	30.1±3.58^{c}	58.5±2.66^{b}	8.2±1.22	0.3±0.05^{c}	1.0±0.16^{b}
Cycling Cow	22	11.7±0.24^{b}	33.58±1.85^{b}	27.2±1.75^{d}	50.2±2.21^{c}	12.9±3.53	0.5±0.10^{b}	0.9±0.04^{b}
Early Gestation	22	18.4±2.80^{a}	35.9±1.84^{b}	40.7±2.27^{a}	62.0±3.25^{ab}	6.5±2.00	0.9±0.17^{a}	1.3±0.12^{a}
Mid Gestation	22	13.2±0.43^{b}	40.7±0.72^{a}	33.4±2.74^{b}	55.4±2.72^{bc}	7.5±1.62	0.8±0.13^{a}	1.3±0.20^{a}
Late Gestation	22	12.6±0.82^{b}	42.5±1.74^{a}	16.1±2.07^{e}	66.6±3.75^{ab}	8.6±1.85	0.8±0.16^{a}	0.5±0.09^{c}
Overall	110	13.3±0.65	37.7±0.78	29.7±1.27	59.2±1.29	7.9±0.75	0.7±0.06	1.1±0.07

WBC=White blood cell; GRAN=Granulocytes; NEU=Neutrophils; LYM=Lymphocytes; MON=Monoccytes; EOS=Eosinophils; BAS=Basophils; PHYST=Physiological state; ^{a,b,c,d}=Means bearing different superscript in the same column are significantly different.

However, physiological state had little effect on red blood cells (RBC), haemoglobin (HGB), mean platelet volume (MPV), platelet distribution width (PDW), and plateletcrit (PCT).

Haematocrit (%) was similar ($P>0.05$) in heifer and non-pregnant cycling cows (Table 4.5) The HCT value decreased ($P<0.01$) in early gestation (G_E), the value of which was similar ($P>0.05$) to that of mid and late gestational periods.

Mean corpuscular volume (MCV) was relatively low ($P<0.01$) in heifer, and increased ($P<0.01$) significantly in cycling cows. The MCV was also similar in early gestation (G_E) and mid gestation (G_M) but higher ($P<0.01$) in late gestation (G_L). The MCV values were relatively higher in gestational period than those observed in heifer and cycling cows (CC) (Table 4.5).

Mean corpuscular haemoglobin (MCH) was lowest ($P<0.01$) in heifer and increased in cycling cows (Table 4.5). The highest ($P<0.01$) values of MCH were observed in G_E and G_L. Cows of G_M had similar ($P>0.05$) value of MCH as obtained in CC. Physiological state of cows had significant influence on mean corpuscular haemoglobin concentration (MCHC) such that heifer and CC had similar ($P>0.05$) values but significantly lower ($P<0.01$) than the values observed in G_E, G_M and G_L. The MCHC values were similar ($P>0.05$) in the three semesters of gestation. Values of RDW (%) were lowest in heifer followed by CC. Highest ($P<0.01$) values of RDW were obtained in G_E and G_L. Cycling cows, G_E, G_M, and G_L had similar ($P>0.05$) value of RDW. Platelet values were similar

(P>0.05) in heifer and CC. The highest (P<0.01) platelet count was observed in GE. Cows in GM and GL had similar platelet count as realized in CC.

4.2.1.2.2 Leucocyte indices

White blood cells values were similar (P<0.05) in the heifer, CC, G_E, and G_L (Table 4.6). The highest WBC was recorded in G_E. Heifer, CC and GE had little effect on Granulocyte (%) values whereas the G_L had the highest. Neutrophil was highest in G_E, followed by GM, heifer, CC, and G_L in descending order. Lymphocytes were highest (P<0.01) in cows of G_L and lowest (P<0.01) in CC. Lymphocytes (%) value did not significantly differ (P>0.05) in heifer, G_E, G_M and G_L. Eosinophil level (%) was lowest (P<0.01) in heifer followed by CC. Cows in G_E recorded the highest (P<0.01) eosinophil level and it was similar in all the trimesters. Basophil was highest (P<0.01) in G_E and G_M while G_L had the least (P<0.01). Heifer and CC had similar (P>0.05) basophil records. Monocyte values, however, did not differ significantly across the physiological states (Table 4.6).

4.2.1.3 Effect of feed supplementation on haematology of dairy cows

4.2.1.3.1 Erythrocyte indices

Provision of feed supplementation to dairy cows had significant (P<0.01) effect on erythrocyte indices. HCT values increased (P<0.01) with increasing levels of feed supplementation (Table 4.7). Levels of MCV, MCHC and RCD CV were higher (P<0.01) in supplemented cows than those that were not given feed supplementation. Platelet counts increased (or improved) with increasing level of feed supplementation. Feed

supplementation had insignificant (P>0.05) effect on RBC, HGB, MCH, PWD and PCT levels.

Table 4.7: Effect of Supplementation on haematology of dairy cows

Haematological indices	RFS *(24)*	OFS *(24)*	NFS *(24)*	*P-value*	*Reference Range***
ERYTHROCYTE INDICES					
RBC ($X10^6$/µL)	6.8 ± 1.96	7.3 ± 0.36	6.4 ± 0.17	*0.090*	*5.0-10.0*
HGB (g/dL)	10.4 ± 0.28	10.5 ± 0.43	10.0 ± 0.25	*0.413*	*8.0-15.0*
HCT (%)	25.8 ±1.76^{ab}	23.7 ± 1.91^{b}	14.2 ± 3.08^{c}	*0.012*	*24.0-46.0*
MCV (fL)	46.6 ± 0.38^{a}	47.4 ± 0.34^{a}	44.0 ± 1.09^{b}	*0.000*	*40.0-60.0*
MCH (pg)	15.3 ± 0.14	14.8 ± 0.34	15.3 ± 0.17	*0.126*	*11.0-17.0*
MCHC (gdL)	32.9 ± 0.15^{a}	33.8 ± 0.64^{a}	31.8 ± 0.56^{b}	*0.008*	*30.0-36.0*
RDW CV (%)	18.6 ± 0.28^{a}	18.1 ± 023^{a}	16.7 ± 0.14^{b}	*0.000*	*16.7-23.3*
PLT ($x10^3$/µL)	484.8 ±106.37^{a}	178.5 ±16.83^{b}	100.2 ± 11.01^{b}	*0.000*	*233-690*
MPV (fL)	6.5 ± 0.07^{b}	8.2 ± 0.62^{a}	6.7 ± 0.12^{b}	*0.000*	*4.0-4.8*
PDW (%)	15.1 ± 0.12	15.7 ± 0.23	15.3 ± 0.41	*0.493*	*56.0-80.0*
PCT (%)	0.4 ± 0.08	2.0 ± 0.48	2.9 ± 2.17	*0.599*	*0.15-0.40*
LEUCOCYTES					
WBC ($x10^9$/L)	12.9 ± 1.15	13.8 ± 1.60	13.5 ± 0.56	*0.849*	*4.6 -12.0*
GRAN %	38.1 ± 1.22^{a}	39.6 ± 0.7^{a}	29.3 ± 1.9^{b}	*0.000*	
NEU %	31.5 ± 1.71^{a}	31.5 ± 2.45^{a}	23.8 ± 2.57^{b}	*0.036*	
LYM %	59.5 ± 1.62^{b}	54.2 ± 2.47^{b}	67.9 ± 2.68^{a}	*0.001*	*40.0 – 70.0*
MON %	5.9 ± 0.84^{b}	8.5 ± 1.84^{ab}	10.4 ± 1.46^{a}	*0.0.2*	*1.0 – 6.0*
EOS %	0.8 ± 0.10^{a}	0.4 ± 0.09^{b}	0.5 ± 0.08^{b}	*0.010*	*2.0 – 5.0*
BAS %	0.7 ±0.10^{b}	1.1 ± 0.09^{a}	1.2 ± 0.12^{a}	*0.014*	*<1.0*

*RBC=Red blood cell, HGB=Haemoglobin, HCT=Haematocrit, MCV=Mean corpuscular volume; MCH= mean corpuscular haemoglobin; MCHC=mean corpuscular haemoglobin concentration, RDW=red cell distribution width; PLT=platelet; Mean platelet volume; Platelet distribution width; PCT=Plateletcrit; WBC=White blood cell; GRAN=Granulocytes; NEU=Neutrophils; LYM=Lymphocytes; MON=Monoccytes; EOS=Eosinophils; BAS=Basophils; RFS=Regular feed supplementation; OFS= Occasional/Partial supplementation; NFS= No feed Supplementation; ^{a,b,c,d}=Means bearing different superscript in the same row are significantly different.**=Normal Reference Range* (Radostits *et al*., 2007; Torngquist and Rigas, 2010; Reece, 2015a).

4.2.1.3.2 Leucocyte indices

Feed supplementation markedly (P<0.05) influenced leucocyte indices, such that, percentage levels of GRAN and NEU were higher (P<0.05) in cows regularly/occasionally supplemented with feed than cows without feed supplementation (Table 4.7). Percentage levels of LYM, MON and BAS were lower (P<0.05) in cows that were given feed supplementation than those that had no supplementation. Eosinophil levels were higher (P<0.01) in cows given feed supplementation than those which had little or no feed supplementation. Feed supplementation had little effect (P>0.05) on WBCs.

4.2.2 Serum biochemical indices of dairy cows

4.2.2.1 Effect of breed on serum biochemistry

Breed had little or no effect on mean values of hepatic enzymes including ALT, AST and γGT (Table 4.8). Breed, however, had significant influence on ALP concentration such that Friesian-Sanga had the mean highest (P<0.05) serum concentration of ALP but the value was similar (P>0.05) to that observed in Sanga cows. Jersey cows had the lowest (P<0.05) value of ALP. Breed also influenced (P<0.01) serum mean total protein (TP) and globulin levels, in that, Sanga had the highest (P<0.01) concentrations. Crossbred and Jersey cows had similar mean TP and globulin levels. Breed had insignificant (P>0.05) effect on mean serum albumin concentration.

Mean total bilirubin and indirect bilirubin concentration were not significantly (P>0.05) influenced by breed. Direct bilirubin, however, was highest (P<0.01) in Sanga followed by the crossbred and Jersey having the lowest (P<0.01) concentration (Table 4.8).

Table 4.8: Effect of breed on blood biochemistry of indigenous dairy cows

Factors	Sanga (40)	Crossbred (40)	Jersey (40)	*P-value*	*R R*
ENZYMES					
ALT (u/L)	19.7±1.34	25.7±±1.80	25.0±1.32	*0.135*	*14.0-38.0***
AST (u/L)	45.7±5.21	54.6±2.16	55.8±2.38	*0.112*	*60.0-118.0**
γGT (u/L)	15.7±0.7	16.9±0.90	20.0±1.92	*0.126*	*0.0 – 31.0**
ALP (u/L)	165.4±22.89^{a}	200.3±20.49^{a}	101.5±5.14^{b}	*0.044*	*35.0-126.0**
METABOLITES					
Total protein (g/L)	84.0±1.89^{a}	69.6±2.35^{b}	63.3±3.15^{b}	*0.000*	*51.2-88.6*$^{++}$*
Albumin (g/L)	32.5±1.44	32.9±0.53	30.5±0.73	*0.221*	*21.0-36.0**
Globulin (g/L)	51.5±1.69^{a}	33.4±1.07^{b}	32.8±3.23^{b}	*0.000*	*30.0-35.0**/ 22.5-51.8*$^{++}$*
Total bilirubin (µmol/L)	4.7±0.44	3.9±0.28	4.1±0.40	*0.332*	*1.7-8.6**
Direct bilirubin(µmol/L)	2.1±0.06^{a}	1.7±0.13^{a}	1.4±0.08^{b}	*0.046*	*0.7-2.4**
Indirect bilirubin (µmol/L)	2.1±0.41	2.3±0.21	2.8±0.35	*0.440*	*0.0-5.1**
Urea (mmol/L)	3.4±0.36^{b}	2.6±0.19^{b}	9.9±0.97^{a}	*0.000*	*2.0-7.5**
BUN (mmol/L)	9.3±1.05^{b}	7.2±0.53^{b}	15.6±2.73^{a}	*0.000*	*3.6-8.9**$^{+}$*
Creatinine (µmol/L)	127.9±5.60	118.5±5.70	98.8±6.27	*0.078*	*44.0-194.0**$^{+}$*
Triglyceride-TG (mg/L)	0.5±0.14	0.4±0.05	0.50.06	*0.494*	*1.2 -3.1**
Total cholesterol (mmol/L)	2.4±0.14^{b}	2.7±0.13^{b}	4.2±0.33^{ae}	*0.000*	*2.1-4.7*$^{+}$ /1.0-4.6**
HDL (mmol/L)	1.5±0.04^{c}	1.9±0.06^{b}	2.3±0.10^{a}	*0.000*	*2.6-3.5**
LDL (mmol/L)	1.7±0.13	1.5±0.13	2.0±0.40	*0.155*	
VLDL (mmol/L)	0.2±0.06	0.2±0.02	0.02±0.03	*0.712*	
Ketone (mmol/L)	1.0±0.16^{a}	0.8±0.07^{ab}	0.5±0.06^{b}	*0.040*	*0.3-6.4**
Glucose (mmol/L)	3.2±0.18	3.0±±0.12	2.7±0.08	*0.300*	*2.2-5.6**$^{+}$*
NEFA (mmol/L)	0.4±0.07	0.6±0.11	0.2±0.01	*0.302*	*0.05-0.25*$^{¤}$/ <0.4*^{oo}*
ELECTROLYTES					
Sodium (Na^{+}) (mmol/L)	137.8±2.01	139.3±1.03	140.8±0.88	*0.526*	*132-152*$^{+}$*
Chloride (mmol/L)	99.4±1.06^{c}	102.4±0.65^{b}	106.4±0.17^{a}	*0.000*	*99.0-107.0**$^{+}$*
Potassiun (K^{+}) (mmol/L)	6.0±0.36^{a}	5.2±0.08^{b}	4.9±0.11^{b}	*0.000*	*3.9-5.8*;*$^{+}$*
Calcium (Ca^{2+}) (mmol/L)	2.1±0.06	2.3±0.09	2.2±0.07	*0.429*	*2.1-2.8**
HCO_3 (mmol/L)	22.6±0.86	21.9±0.39	21.6±0.66	*0.568*	*20.0-30.0**
Magnesium (mmol/L)	1.1±0.01	1.1±0.03	1.0±0.00	*0.796*	*0.6-1.2**$^{+}$*
Phosphorus (mmol/L)	2.2±0.10^{a}	1.1±0.06^{b}	1.8±0.06^{b}	*0.001*	*1.1-2.8**

*NEFA= Nonesterified fatty acids; HCO_3=Bicarbonate; NEFA=Non esterified fatty acid; HDL=High density lipoprotein; LDL=Low density lipoprotein; VLDL= Very low density lipoprotein; Values in parenthesis ( )=Number of observation; ^{a,b,c,d}=Means bearing different superscript in the same column are significantly different; RR=Reference range [**= Meyer et al., 1992; *=Radostits et al., 1994; ¤= Fonseca et al., 2004; ^{oo}=Oetzel, 2004; *$^{+}$=Merck Manual, 2012; $^{+}$=Reece et al., 2015; $^{++}$=Garcia et al., 2017].*

Mean urea and BUN were significantly highest (P<0.01) in Jersey cows (Table 4.8). Sanga and Friesian-Sanga crossbred cow had similar (P>0.05) mean serum urea and BUN concentrations. Breed had little (P>0.05) effect on creatinine and triglyceride components. Mean serum total cholesterol level was highest (P<0.01) in Jersey cows but Sanga and crossbred had similar (P>0.05) concentrations. Jersey cows also recorded the highest concentration of HDL, followed by crossbred and Sanga cows in descending order. Breed had little effect (P>0.05) on mean serum VLDL levels.

Sanga had the highest (P<0.01) mean serum ketone level, followed the crossbreds, and Jersey having the lowest concntration. Crossbred and Jersey cows had similar (P>0.05) mean serum ketones. Breed did not significantly influence mean serum glucose and NEFA concentrations (Table 4.8).

Breed had slight (P>0.05) influence on serum sodium, calcium, bicarbonate, and magnesium (Table 4.8). Jersey recorded the highest (P<0.01) concentration of chloride, followed by Friesian-Sanga crossbred and Sanga cows in descending order. The highest (P<0.01) concentration of potassium was recorded value in Sanga but values observed in crossbred and Jersey cows were similar (P>0.05). Phosphorus concentration was highest in Sanga. Crossbred and Jersey had a slight (P>.0.05) difference in P concentration.

4.2.2.2 Effect of physiological state on serum biochemistry

Physiological state had significant (P<0.05) effect on alanine transaminase (ALT), aspartate aminotransferase (AST), and gamma glutamyl tansferases (γGT), but had little

(P>0.05) effect on Alkaline phosphatase (ALP) (Table 4.9). Non-cycling heifer had the highest (P<0.05) serum ALT concentration, which was not insignificantly different from the value observed in G_E. Cows of G_E and G_L had similar (P<0.05) serum ALT but significantly higher than the value recorded in G_M. Cycling cows (CC) had the least (P<0.05) serum ALT. Serum AST was higher in G_M and G_L but did not differ significantly (P>0.05) in heifer, CC, GE. Cows in G_M had the highest γGT level. Heifer, CC, G_E and G_L had similar γGT.

Physiological state also influenced (P<0.01) serum total protein concentration. Serum total protein was lowest (P<0.01) in G_L whiles levels in Heifer, CC, G_E and G_M were similar (P>0.05). Serum albumin recorded the highest value in heifer and CC. The least albumin concentration was obtained in G_L but the value did not differ significantly from those observed in G_E and G_M. Globulin level was similar (P>0.05) in heifer, CC, G_E and G_M but G_L had the lowest (P<0.01) level.

Total bilirubin (TBIL) concentration was highest (P<0.01) in CC but heifer and cows in gestational trimesters had similar (P>0.05) serum TBIL levels. Direct bilirubin (DBIL) was also highest in G_E and the value was similar to those noticed in CC and G_L. Cycling cow had the highest serum indirect bilirubin, followed by G_M. Non-cycling heifer, GE, GM and GL had similar IBIL levels.

Table 4.9: Effect of physiological state on serum biochemistry of dairy cows

Physiological State	Heifer (*N=24*)	Cycling Cow (CC; *N=24*)	Early Gestation (G_E; *N=24*)	Mid Gestation (G_M; *N=24*)	Late Gestation (G_L; *N=24*)	*P-Value*
ALT (u/L)	30.1 ± 1.91^{a}	20.8 ± 1.31^{d}	28.2 ± 2.85^{ab}	24.0 ± 1.31^{c}	26.8 ± 1.95^{bc}	*0.019*
AST (u/L)	48.6 ± 5.14^{b}	52.2 ± 3.01^{b}	46.0 ± 2.63^{b}	62.8 ± 5.18^{a}	62.0 ± 5.34^{a}	*0.016*
γGT/GGT (u/L)	19.3 ± 1.31^{b}	20.6 ± 1.88^{b}	14.1 ± 0.95^{b}	58.0 ± 19.79^{a}	16.0 ± 1.13^{b}	*0.017*
ALP (u/L)	219.7±27.32	102.6±24.08	170.1±15.05	193.1±35.14	172.6±29.42	*0.223*
Total protein (g/L)	76.6 ± 2.58^{a}	75.8 ± 2.49^{a}	75.6 ± 2.66^{a}	73.2 ± 2.64^{a}	66.2 ± 1.78^{b}	*0.001*
Albumin (g/L)	34.3 ± 0.79^{a}	32.6 ± 0.57^{a}	31.2 ± 1.56^{b}	31.2 ± 0.44^{b}	30.6 ± 0.82^{b}	*0.031*
Globulin (g/L)	44.6 ± 1.62^{a}	42.6 ± 2.63^{ab}	41.3 ± 2.23^{ab}	45.5 ± 2.39^{a}	33.7 ± 1.93^{b}	*0.001*
Total bilirubin (µmol/L)	2.9 ± 0.24^{b}	5.4 ± 0.60^{a}	3.9 ± 0.27^{b}	3.9 ± 0.25^{b}	3.6 ± 0.10^{b}	*0.000*
Direct bilirubin (µmol/L	1.3 ± 0.14^{b}	1.8 ± 0.22^{ab}	2.0 ± 0.17^{a}	1.5 ± 0.08^{b}	1.9 ± 0.17^{ab}	*0.004*
Indirect bilirubin (µmol/L)	1.9 ± 0.15^{b}	3.4 ± 0.45^{a}	2.0 ± 0.23^{b}	2.5 ± 0.22^{ab}	1.9 ± 0.1^{b}	*0.001*
Urea (mmol/L)	2.5 ± 0.33^{c}	7.8 ± 1.13^{a}	3.7 ± 0.35^{b}	5.5 ± 0.60^{b}	4.7 ± 0.45^{b}	*0.000*
BUN (mmol/L)	6.8 ± 0.93^{b}	5.0 ± 16.44^{b}	9.3 ± 1.00^{a}	10.7 ± 1.64^{a}	6.3 ± 1.26^{b}	*0.000*
Creatinine (µmol/L)	120.3 ± 5.87^{a}	123.6 ± 8.48^{a}	118.0 ± 3.15^{a}	103.8 ± 3.7^{b}	108.9 ± 6.0^{b}	*0.003*
Triglyceride (mg/L)	0.3±0.03	0.4±0.05	0.4±0.05	0.4±0.80	0.6±0.06	*0.093*
Total cholesterol (mmol/L)	2.7 ± 0.11^{b}	3.3 ± 0.39^{a}	2.9 ± 0.16^{b}	3.2 ± 0.22^{a}	3.4 ± 0.21^{a}	*0.002*
HDL (µmol/L)	0.8±0.13	1.0±0.11	1.0±0.09	0.9±0.07	1.0±0.14	*0.422*
LDL (µmol/L)	1.9 ± 0.2^{ab}	2.1 ± 0.34^{a}	1.3 ± 0.09^{b}	1.7 ± 0.13^{ab}	2.0 ± 0.23^{ab}	*0.001*
VLDL (µmol/L)	0.1±0.02	0.2±0.02	0.2±0.03)	0.2±0.04	0.2±0.03	*0.317*
Ketone (mmol/L)	0.8±0.14	0.9±0.08	1.5±0.63	1.1±0.12	0.7±0.16	*0.312*
Glucose (mmol/L)	3.2 ± 0.21^{a}	2.2 ± 0.14^{b}	2.8 ± 0.19^{a}	2.8 ± 0.19^{a}	2.8 ± 0.08^{a}	*0.030*
NEFA (mmol/L)	0.3 ± 0.01^{b}	0.3 ± 0.02^{b}	0.3 ± 0.03^{b}	0.6 ± 0.12^{b}	1.2 ± 0.32^{a}	*0.000*
Sodium (Na^{+}) (mmol/L)	140.8±1.71	140.2±1.21	139.0±1.19	138.2±1.63	145.2±1.19	*0.669*
Chloride (mmol/L)	104.3±1.15	105.6±0.77	103.6±0.78	102.4±1.06	105.0±0.92	*0.070*
Potassiun (K^{+}) (mmol/L)	5.8 ± 0.31^{a}	4.9 ± 0.12^{b}	5.7 ± 0.19^{a}	5.3 ± 0.11^{b}	5.9 ± 0.41^{a}	*0.015*
Calcium (Ca^{2+}) (mmol/L)	2.3±0.04	2.2±0.11	2.3±0.10	2.1±0.05	2.0±0.12	*0.115*
HCO_3 (mmol/L)	23.4 ± 0.52^{a}	20.7 ± 0.52^{b}	22.0 ± 0.58^{a}	22.5 ± 0.57^{a}	22.4 ± 0.80^{a}	*0.015*
Magnesium (mmol/L)	1.1±0.05	1.0±0.03	1.1±0.04	1.4±0.29	1.0±0.03	*0.320*
Phosphorus (mmol/L)	2.0 ± 0.04^{a}	1.9 ± 0.14^{a}	2.0 ± 0.13^{a}	2.0 ± 0.07^{a}	1.6 ± 0.03^{b}	*0.005*

N=Number of observation; NEFA= Nonesterified fatty acids; HCO_3=Bicarbonate; ^{a,b,c,d}=Means bearing different superscript in the same row are significantly different.

The highest (P<0.01) serum urea concentration was observed in CC while the lowest (P<0.01) level was noticed in heifer. Cows of G_E, G_M and G_L had similar serum urea concentrations but were significantly lower than that observed in CC. Blood urea nitrogen (BUN) was highest in cows of G_M and the value was similar to the value observed in G_E (Table 4.9). Non-cycling heifer, CC, and GL had similar (P>0.05) BUN concentration.

Creatinine concentration was highest in CC and the value was similar to those in heifer, cows in G_E. Cows in G_M and G_L had creatinine levels that did not differ from each other significantly. Triglyceride was not significantly (P>0.05) influenced by the physiological state considered in this study.

Physiological state also had significant (P<0.01) influence on serum total cholesterol concentration. The highest (P<0.01) value of cholesterol concentration was recorded in cows in G_M, which was similar to the values obtained in CC and cows in G_L. Heifer and cows in G_E had similar (P>0.05) values of cholesterol but were lower (P<0.01) in concentrations (Table 4.9). The highest low density lipoprotein (LDL) concentration was recorded in CC but the value was similar to those observed in heifer, cows in G_M and G_L. Cows in G_L had the lowest value of LDL. High density lipoprotein (HDL) and VLDL levels across physiological states considered were insignificant (P>0.05).

Serum ketone (BHBA) concentration was similar across physiological state (Table 4.9). Blood glucose levels varied significantly (P<0.05). The CC had the lowest glucose concentration whereas heifer had the highest (P<0.05) but the value was insignificant

(P>0.05) to those observed in G_E, G_M and G_L. Non-esterified fatty acids was highest (P<0.01) in G_L, followed by G_M. Level of NEFA was similar (P>0.05) in Heifer, CC, G_E.

Physiological state had little (P>0.05) effect on sodium, chloride, calcium, and magnesium cation/ions concentrations. Potassium levels were similar in heifer, G_E, and G_L but higher than the values observed in CC and G_M. Serum bicarbonate concentration was lowest (P<0.05) in CC. The levels of HCO_3^- were similar (P>0.05) in heifer, G_E, G_M, and G_L but significantly (P<0.05) higher than the value observed in CC. Cows in GL had the lowest serum phosphorus (P) concentration whiles P levels did not significantly vary in heifer, CC, G_E, and G_M (Table 4.9).

4.2.2.3 Effect of feed supplementation on serum biochemistry

Hepatic enzymes

Serum hepatic enzymes including AST, γGT, and ALP were significantly (P<0.01) influenced by feed supplementation (Table 4.10). The enzyme, AST, increased (P<0.01) with increasing feed supplementation whereas γGT and ALP levels were similar in cows regularly and occasionally supplemented but were higher than the values observed in cows that were not given feed supplementation. Feed supplementation had little (P>0.05) effect on ALT level.

Serum metabolites

Serum total protein decreased (P<0.05) with decreasing intensity of feed supplementation. Albumin and globulin were not significantly affected by feed supplementation. Total and

Table 4:10 Effect of feed supplementation on serum biochemistry of dairy cows

Haematological indices	RFS	OFS	NFS	*P-value*	*Reference Range*
ENZYMES	***(24)***	***(24)***	***(24)***		
ALT (u/L)	23.3 ± 0.95	30.8 ± 6.29	22..7 ± 1.58	*0.080*	*14.0-38.0***
AST (u/L)	58.9 ± 1.85^{a}	50.0 ± 3.67^{b}	43.3 ± 1.58^{b}	*0.007*	*60.0-118.0**
γGT (u/L)	19.1 ± 1.58^{a}	18.3 ± 1.98^{a}	14.7 ± 0.73^{b}	*0.006*	*0.0 – 31.0**
ALP (u/L)	177.5 ± 14.44^{a}	162.3 ± 27.02^{a}	138.0 ± 10.06^{b}	*0.031*	*35.0-500.0**
METABOLITES					
Total protein (g/L)	77.2 ± 3.58^{a}	70.3 ± 2.86^{ab}	67.3 ± 1.48^{b}	*0.029*	*51.2-88.6*$^{++}$
Albumin (g/L)	33.6 ± 0.90	31.3 ± 0.85	31.7 ± 0.59	*0.122*	*21.0-36.0**
Globulin (g/L)	38.8 ± 2.36	39.0 ± 3.10	35.5 ± 1.41	*0.430*	*30.0-35.0**/ 22.5-51.8*$^{++}$
TBIL (µmol/L)	4.5 ± 0.37^{a}	4.2 ± 0.51^{ab}	3.3 ± 0.21^{b}	*0.001*	*1.7-8.6**
DBIL (µmol/L)	1.9 ± 0.16	1.9 ± 0.12	1.5 ± 0.093	*0.211*	*0.7-2.4**
IBIL (µmol/L)	3.1 ± 0.28^{a}	2.3 ± 0.53^{ab}	1.9 ± 0.14^{b}	*0.002*	*0.0-5.1**
Urea (mmol/L)	3.3 ± 0.28	5.4 ± 1.59	3.9 ± 0.53	*0.138*	*2.0-7.5**
BUN (mmol/L)	9.3 ± 0.38^{a}	10.6 ± 1.21^{a}	6.1 ± 0.40^{b}	*0.000*	*3.6-8.9**$^{+}$
Creatinine (µmol/L)	121.9 ± 6.01	112.4 ± 5.35	95.3 ± 3.92	*0.053*	*44.0-194.0**$^{+}$
TG (mg/L)	0.3 ± 0.04^{b}	0.6 ± 0.20^{a}	0.6 ± 0.05^{a}	*0.010*	*1.2 -3.1**
T. Chol. (mmol/L)	2.5 ± 0.13^{b}	3.2 ± 0.34^{a}	2.9 ± 0.12^{a}	*0.014*	*1.0/2.1-4.7*$^{+*}$
HDL (mmol/L)	0.7 ± 0.04^{b}	0.8 ± 0.10^{b}	1.1 ± 0.09^{a}	*0.001*	*2.6-3.5**
LDL (mmol/L)	1.6 ± 0.10	2.1 ± 0.37	1.4 ± 0.18	*0.088*	
VLDL (mmol/L)	0.1 ± 0.02^{b}	0.3 ± 0.09^{a}	0.2 ± 0.03^{a}	*0.005*	
Ketone (mmol/L)	1.0. ± 0.11	0.7 ± .015	0.7 ± 0.07	*0.070*	*0.3-6.4**
Glucose (mmol/L)	3.3 ± 0.13^{a}	2.9 ± 0.25^{a}	2.8 ± 0.11^{b}	*0.024*	*2.2-5.6**$^{+}$
NEFA (mmol/L)	0.3 ± 0.04^{b}	0.3 ± 0.05^{b}	0.7 ± 0.17^{a}	*0.036*	*0.05-0.25*$^{¤}$/ <0.4*^{oo}*
ELECTROLYTES					
Sodium (mmol/L)	139.6 ± 1.52	140.2 ± 1.32	138.5 ± 1.01	*0.734*	*132-152*$^{+}$
Chloride (mmol/L)	101.6 ± 0.84	103.3 ± 1.41	102.8 ± 0.77	*0.431*	*99.0-107.0**$^{+}$
Potassium (mmol/L)	6.1 ± 0.25^{a}	5.1 ± 0.31^{b}	5.3 ± 0.09^{b}	*0.005*	*3.9-5.8**$^{+}$
Calcium (mmol/L)	2.3 ± 0.08	2.0 ± 0.08	2.2 ± 0.12	*0.264*	*2.1-2.8**
HCO_3 (mmol/L)	21.4 ± 0.57	22.6 ± 0.97	22.4 ± 0.33	*0.310*	*20.0-30.0**
Mg (mmol/L)	1.1 ± 0.01^{a}	1.1 ± 0.02^{a}	1.0 ± 0.05^{b}	*0.013*	*0.6-1.2**$^{+}$
P (mmol/L)	1.8 ± 0.13	2.9 ± 0.25	3.3 ± 0.13	*0.066*	*1.1-2.8**

*ALT=Alanine aminotransferase; AST=Aspatate aminotransferase; ALP=Alkaline Phosphatase; BUN=Blood urea nitrogen; γGT= γ-glutamyl transferase (Gamma glutamyl transferase); HCO_3=Bicarbonate; TBIL= Total bilirubin; DBIL= Total bilirubin; IBIL= Indirect bilirubin; NEFA=Non esterified fatty acid; HDL=High density lipoprotein; LDL=Low density lipoprotein; TG=Triglyceride; T.Chol.=Total cholesterol; VLDL= Very low density lipoprotein; Values in parenthesis ( )=Number of observation; RFS=Regular feed supplementation; OFS= Occasional/Partial supplementation; NFS= No feed Supplementation; ^{a,b,c,d}=Means bearing different superscript in the same column are significantly different; RR=Reference range [**= Meyer et al., 1992; *=Radostits et al., 1994; ¤= Fonseca et al., 2004; ^{oo}=Oetzel, 2004; *$^{+}$=Merck Manual, 2012; $^{+}$=Reece et al., 2015; $^{++}$=Garcia et al., 2017].*

indirect bilirubin levels increased with increasing intensity of feed supplementation whiles direct bilirubin was similar across the different levels of feed supplementation. Feed supplementation had little (P>0.05) influence on urea concentration but it had significant (P<0.01) effect on BUN levels such that cows supplemented with feed had higher (P<0.01) BUN concentration than those that did not have feed supplementation (Table 4.10).

Feed supplementation had little (P>0.05) influence on creatinine concentration in dairy cows. Triglyceride, total cholesterol, and VLDL levels were lower in cows regularly supplemented than those that received occasional or no feed supplementation. High density lipoprotein (HDL) concentration was higher (P<0.01) in cows that were given feed supplementation than cows that had no feed supplementation (Table 4.10). Low density lipoprotein (LDL) was not significantly (P>0.05) influenced by feed supplementation.

4.3 Reproductive Hormones of Dairy Cattle

4.3.1 Progesterone profile of dairy heifers using commercial EIA

4.3.1.1 Effect of breed on progesterone concentration during prepubertal stage to Conception

Trends in progesterone concentrations (ng/mL) in blood of crossbred and Sanga cows from prepubertal stage (non-cycling) till conception are presented in Fig.4.2a. Serum progesterone concentrations from 45 to 290 days were < 0.95 ng/mL and showed very low pulsatiles (Fig. 4.2a).

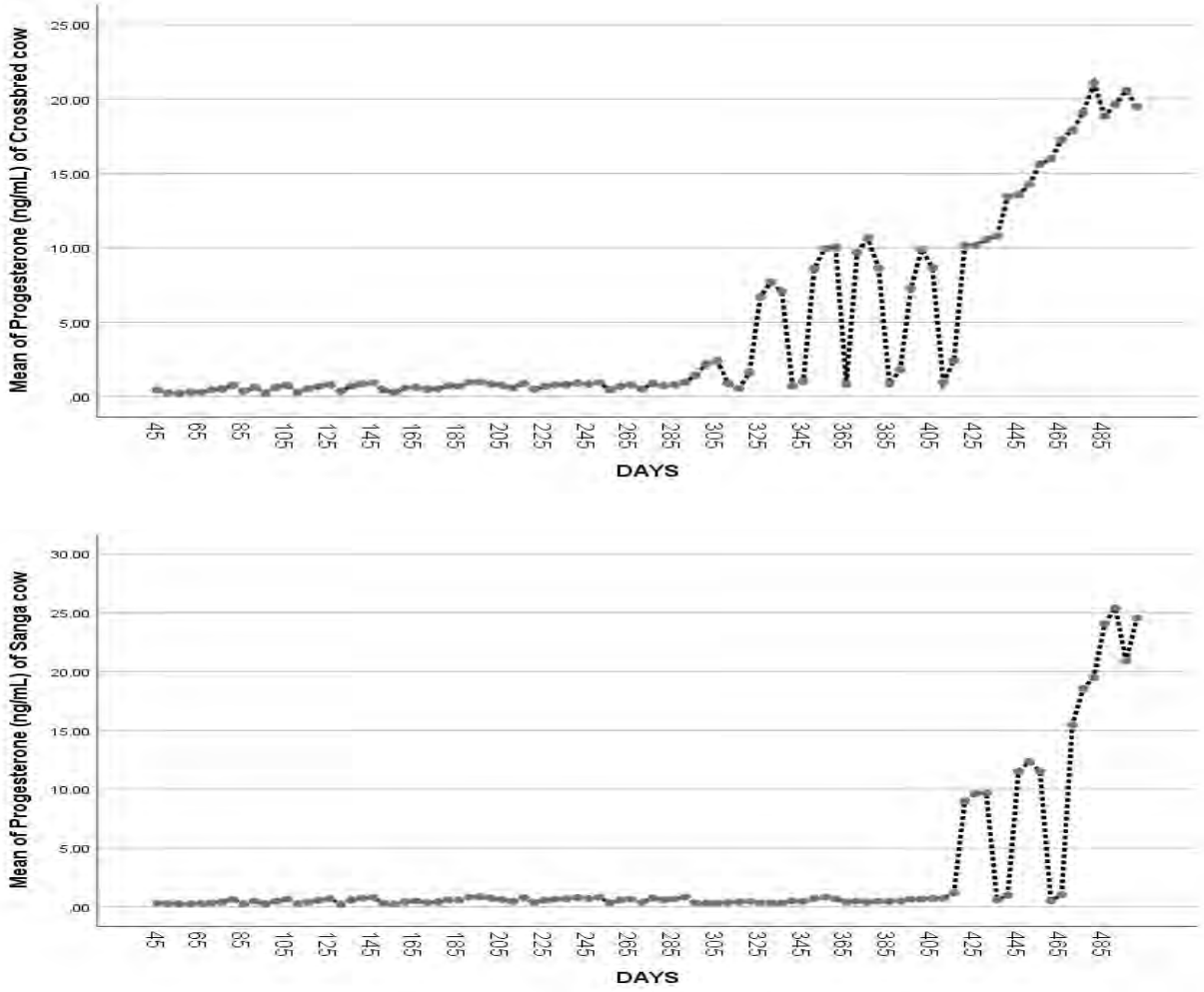

Fig. 4.2 a: Progesterone concentrations of prepubertal stage (from day 45) to conception in crossbred and Sanga cows

The first rise in serum P_4 concentration ≥ 1 ng/mL in crossbred cows occurred at the 295th day from birth while first overt oestrus was seen on the 340th day. Rise in P_4 concentration of 10.15 ng/mL occurred in the 420th day. The P_4 concentration sharply increased and showed high pulsatiles from 465 to 500 days when crossbred cows conceived (Fig. 4.2 a).

Sanga cow showed a similar trend in prepubertal serum P_4 levels as indicated in the crossbreds. However, the first rise in P_4 ≥ 1 ng/mL occurred on 415th day and exhibited overt oestrus 20 days afterward (435th day). Second overt oestrus occurred 25 days later from the

first oestrus/heat, and was crossed and never returned to oestrus (maintained P_4 concentration of 15.46 – 25.39 from day 470 to 500).

Jersey cows experienced first P_4 rise of ≥1 ng/mL earliest (285th day) followed by the crossbred and the Sanga cows in ascending order. The interval between two successive oestrus ranged from 20 to 24 days. The Jersey cow had a persistent P_4 concentration of 14.50 to 31.50 ng/mL during 120 days conception in pulsatile pattern, and on day 500, the mean P_4 concentration was 28.72 ng/mL (Fig. 4.2 b).

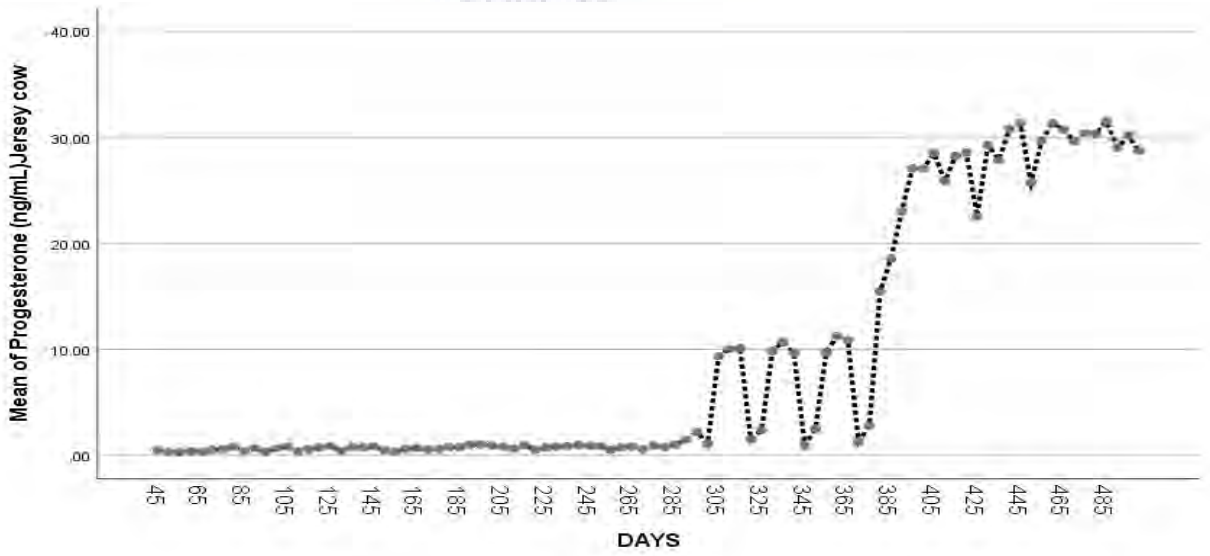

Fig. 4.2 b: Progesterone concentrations of prepubertal period till conception in Jersey cows

Effect of feed supplementation

4.3.1.2 Effect of feed supplementation on P_4 concentration in heifers during Pre-pubertal stage to conception

The effect of regular, occasional/partial and no feed supplementation on P_4 concentrations in indigenous dairy heifers from prepubertal stage to conception are presented in Fig. 4.3.

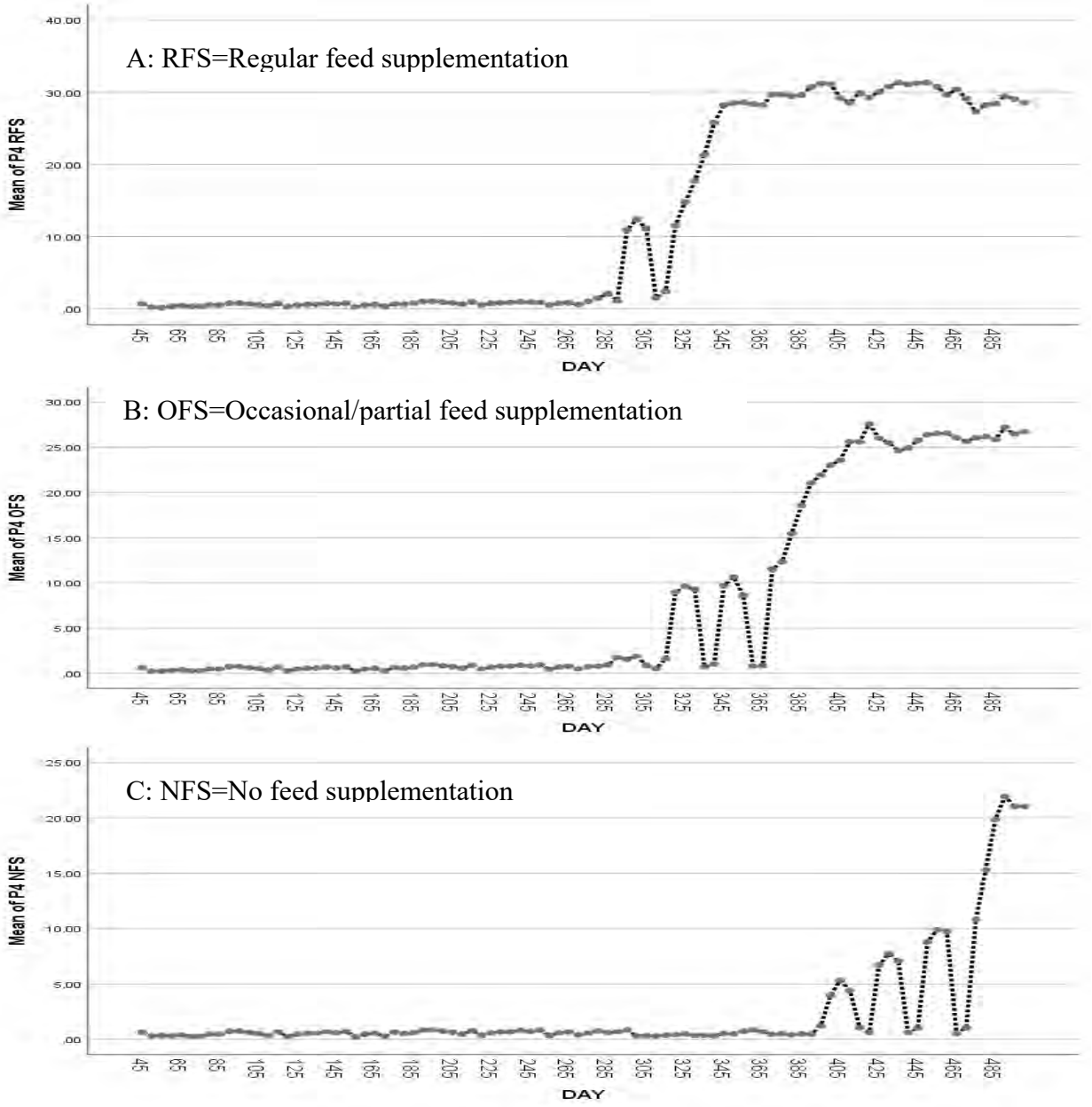

Fig. 4.3: Mean plots for effect of feed supplementation on P_4 concentrations in dairy heifers from pre-pubertal stage to conception.

Heifers that had regular feed supplementation were the first to show high P_4 pulsatile followed by occasional and no feed supplementation in descending order. The first rise in P_4

concentrations of ≥ 1.00 ng/mL was observed in 275, 290 and 395 days in heifers that had regular, occasional/partial and no feed supplementation respectively.

The progesterone concentrations in heifers during oestrus increased (P<0.01) with increasing level/intensity feed supplementation. The mean peak in P_4 concentrations that led to first overt oestrus for heifers regularly, occasionally given feed supplementation and those that had no supplementation was 12.4 ± 0.26, 9.6 ± 0.06 and 7.7 ± 0.12 ng/mL respectively. Heifers having regular, occasional feed supplementation and those that received no supplement conceived on the day 335, 390 and 490 respectively.

The highest (P<0.01) mean concentration of P4 was recorded in heifers that had regular feed supplemented, followed by occasional and no feed supplementation heifers in descending order (Fig. 4.3). The ranges of P_4 levels during conception were 21.4 ± 1.08 – 31.3 ± 0.7, 21.0 ± 0.02 – 27.12 ± 0.90 and 19.8 0.60 – 21.92 ± 0.18 ng/mL.

4.3.2 Oestradiol and progesterone levels in dairy breeds during oestrous cycles

Oestradiol and progesterone in sera of Sanga, crossbred, and Jersey cows during oestrous cycle in hot humid environment of Ghana are presented in Fig. 4.4a, b and c. Oestradiol threshold concentrations in Sanga cow ranged from 15.12±4.10 to 125.28±8.43 pg/mL (grand mean = 33.5±3.57 pg/mL). The Sanga cows experienced oestradiol peak on day 20 of oestrous cycle and standing to be mounted (arrowed) occurred on day 22 (Fig. 4.4 a, I). The second oestrus occurred in day 43 which was 21 days after first heat of the previous cycle.

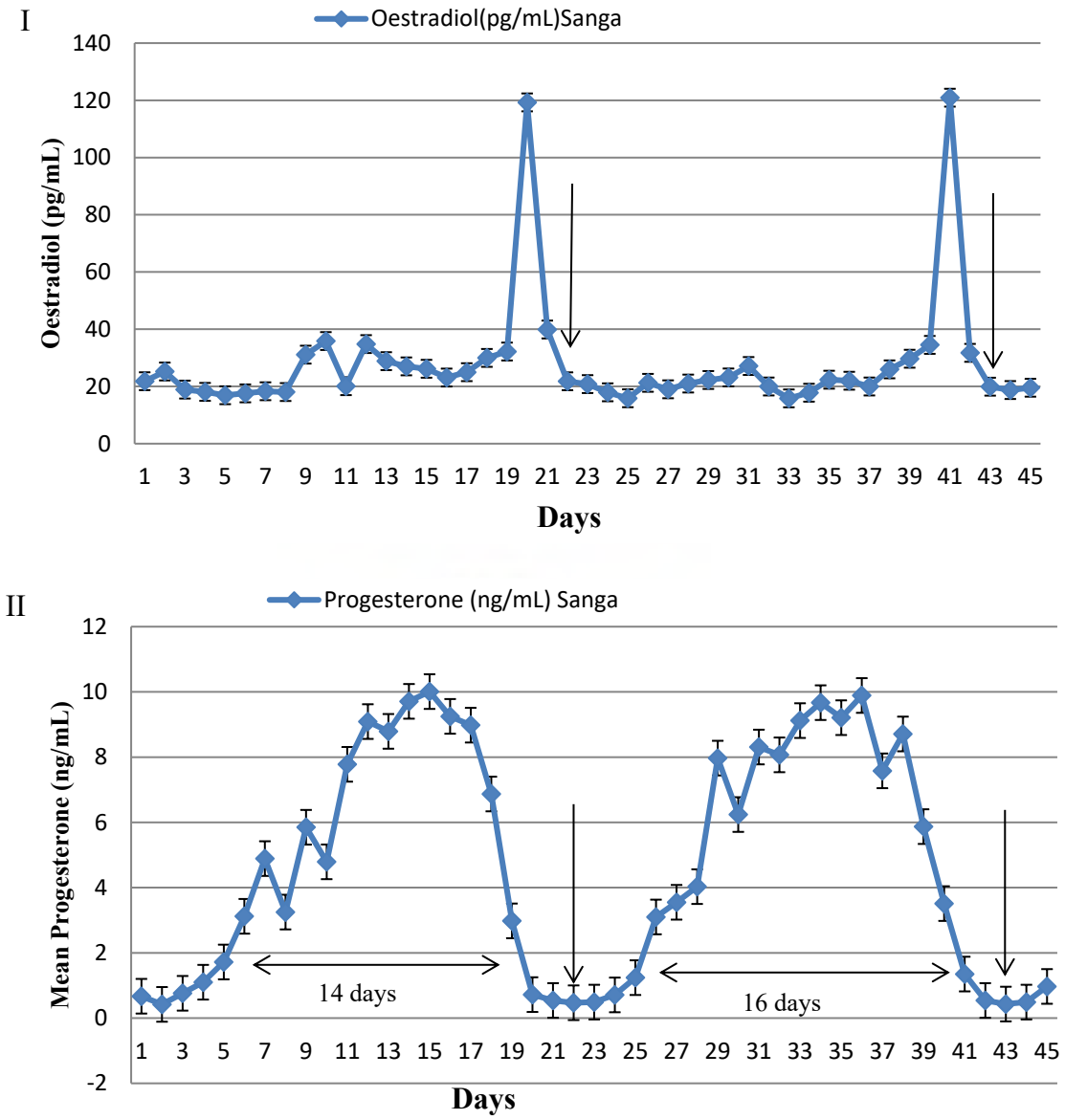

Fig. 4.4a: Mean oestradiol (I) and progesterone (II) concentrations in serum of Sanga cows during Oestrous cycle. Each dot was the mean of 3 observations.

Mean serum progesterone level in Sanga ranged from 0.42±0.19 to 10.0±1.98 ng/mL (grand mean = 4.7±0.53 ng/mL). Progesterone levels were high for 14 days (from day 5 to 19) while in the second cycle, P_4 was high for 16 days. Progesterone levels in the two cycles were not

regular, but in both overt oestrus occurred at minimum threshold P_4 concentration (Fig. 4.4 a, II).

The result on mean (from 3 cows) E_2 and P_4 concentrations in the crossbred cow is shown in Fig. 4.4b. The mean minimum and maximum (peak) threshold levels of oestradiol concentrations in serum of crossbred cows during oestrous cycle were 18.98±2.12 and 187.30±9.30 pg/mL respectively, with the overall mean of 48.56±4.86 (n=45). The first and second peaks of oestradiol concentrations in the two cycles occurred in day 17 and day 39, with associated standing heat observed at day 20 and day 41 for the two oestrous cycles, respectively (Fig. 4.4 b, I).

The minimum and maximum threshold concentrations of P_4 in crossbred cows were 0.41±0.01 and 9.81±1.85 ng/mL. Progesterone level increased above 1 ng/mL from day 4 and plateaued in pulsatility in day 12 to 17. The P_4 concentrations were high for 15 days and 17 day for the first and second cycles, respectively (Fig.4.4b, II). The interval between two oestrus was 20 days.

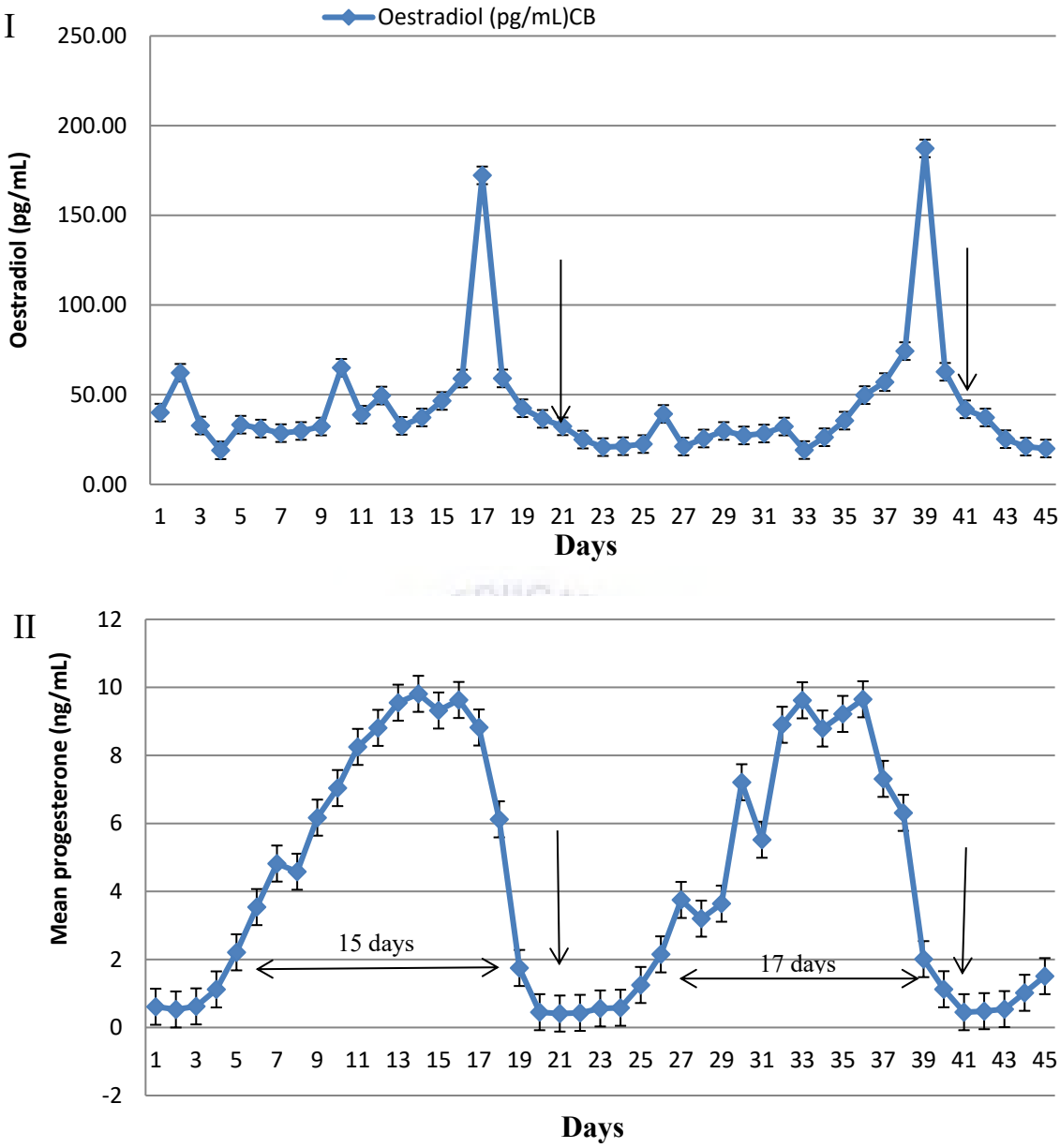

Fig. 4.4 b: Mean oestradiol and progesterone concentrations in sera of crossbred cows (CB) during Oestrous cycle. Each dot was a mean of 3 observations.

Oestradiol and progesterone concentrations (means) in sera of Jersey cows are presented in Fig.4.4c. Minimum and maximum serum oestradiol levels during oestrous cycle were 15.12±4.10 and 138.44±9.24 pg/mL, respectively. The two peaks in oestradiol concentrations of the two oestrous cycles were observed in day 20 and day 41 with the accompanying heats

occurring in day 23 and day 44 downs (arrowed), respectively. The progesterone rise in the first cycle was maintained for 15 days whereas the 17 days was observed in the second cycle.

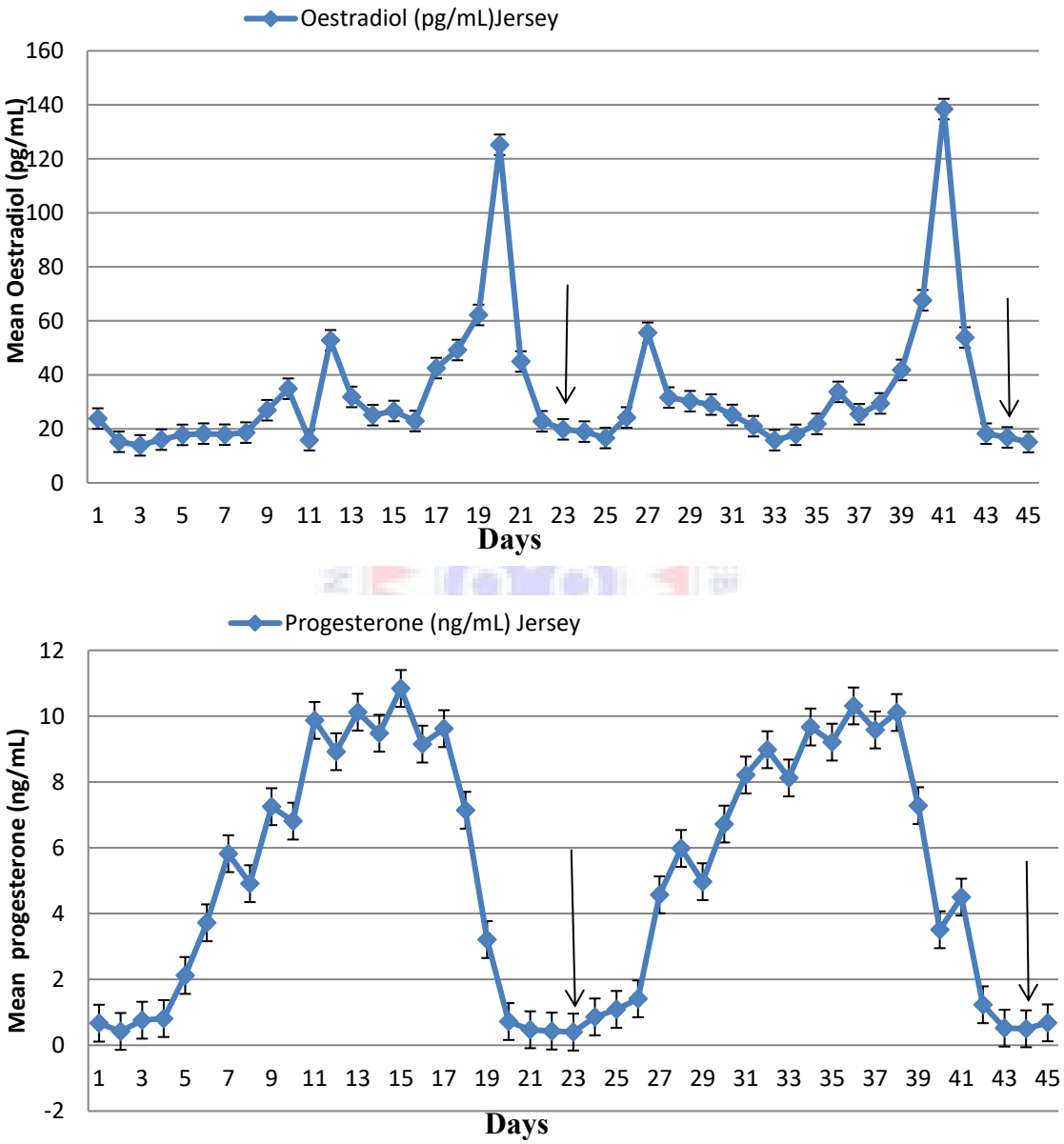

Fig. 4.4 c: Mean oestradiol and progesterone concentrations in sera of Jersey cows during Oestrous cycle. Each dot is the mean of 3 observations

4.3.3 Progesterone and oestradiol levels in dairy breeds during gestational stages

Progesterone and Oestradiol concentrations at different stages of gestation period and first 10 days after parturition are shown in Fig. 4.5. Progesterone significantly ($P<0.01$) increased from the first trimester and plateaued ($P>0.05$) in the second and third trimesters of gestation (Fig. 4.5, I). Nevertheless, P_4 concentrations superseded E_2 during the three trimesters until the last 10 day to parturition where E_2 levels over took P_4.

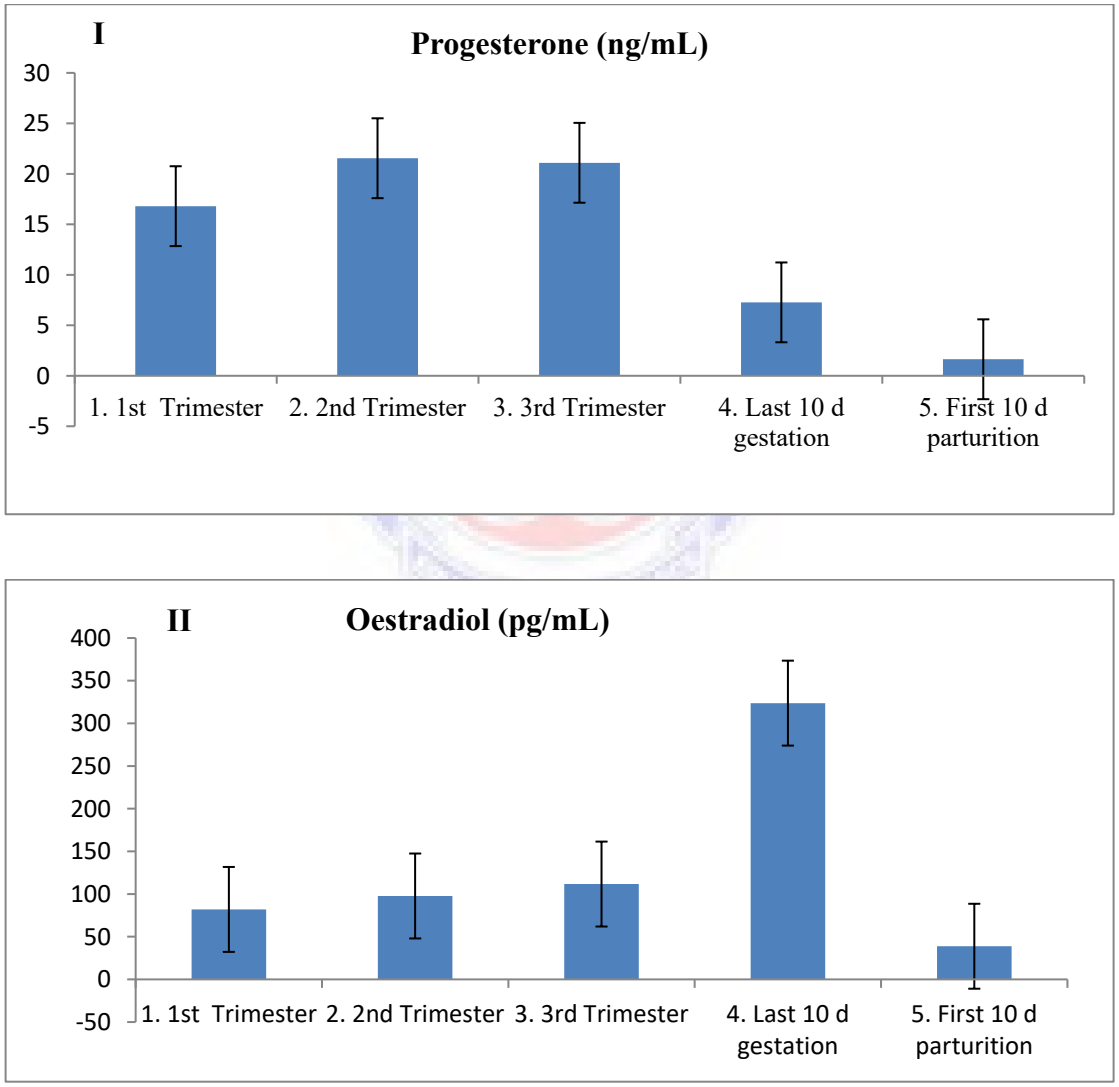

Fig. 4.5: Mean progesterone (I) and oestradiol (II) concentrations in serum of crossbred cows at different stages of gestation.

Oestradiol concentrations in the first three semesters were similar up to about 260 days (Fig 4.5, II). There was a significant ($P<0.01$) decrease in P_4 concentration in the last 10 days before parturition while oestradiol levels massively ($P<0.01$) increased (Fig. 4.5; mean=323.72±23.03 pg/mL). In the first 10 days after parturition, progesterone concentration remarkably ($P<0.01$) reduced to a level averaging 1.63 ±0.24 ng/mL whereas oestradiol level was 38.81±4.91 pg/mL.

4.3.4 Reproductive steroids, FSH, and LH, concentrations in serum/raw milk postpartum

Concentrations of reproductive steroid hormones (oestradiol and progesterone) in blood and milk of crossbred cows during the first 45 days postpartum are presented in Fig. 4.6. The EIA assaying of oestradiol concentration produced a significantly ($P<0.01$) high positive correlation (0.92) between serum and milk oestradiol levels (4.6). The oestradiol concentration ranged from 3.42 – 76.76 pg/mL and 0.56 – 39.86 pg/mL in serum and milk respectively. In addition, concentrations of E_2 level was significantly ($P<0.01$) higher in serum than in raw milk.

There was also positive correlation of 0.90 progesterone concentration in serum and milk during 45 days postpartum period (Fig. 4.7). Progesterone levels ranged from 0.41 – 5.19 ng/mL and 0.22 – 2.72 ng/mL in serum and raw milk respectively. There was also a significantly ($P<0.01$) higher concentration of P_4 in serum than in raw milk.

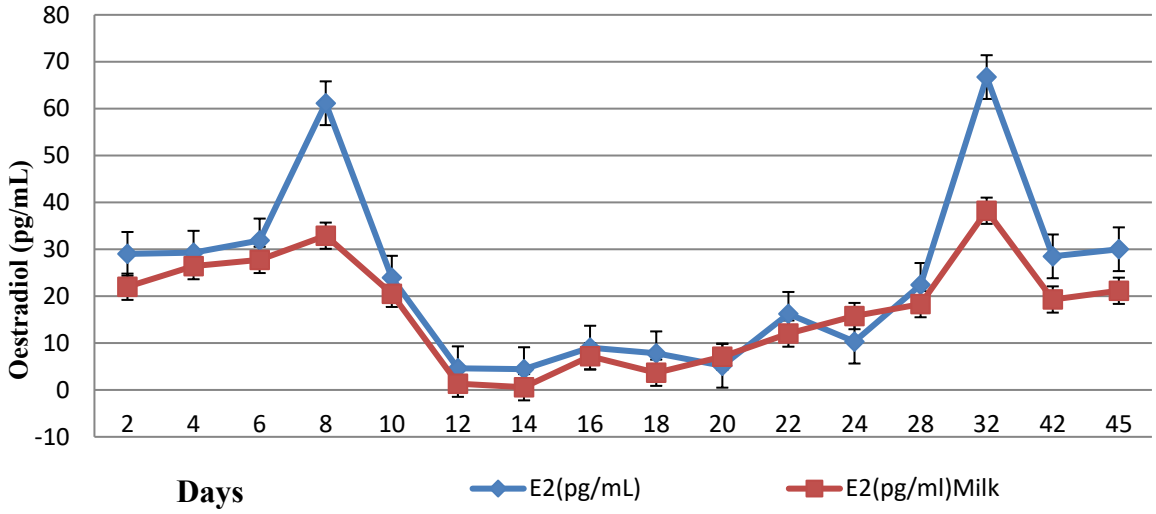

Fig. 4.6: Relationship between mean oestradiol (E_2) of concentrations in serum (in blue) and milk (in red) in three (3) crossbred cows 45 days postpartum using commercial EIA

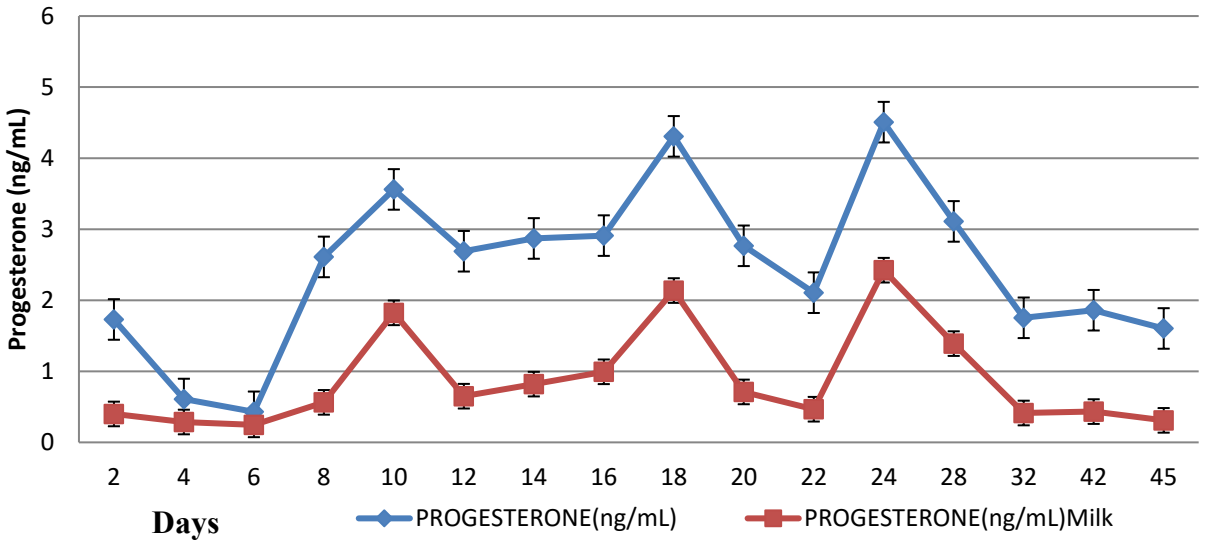

Fig. 4.7: Relationship between mean concentrations of serum (in blue) and milk (in red) progesterone (P_4) levels. Each dot represents the mean of three (3) crossbred cows 45 days postpartum using commercial EIA

Serum and milk testosterone concentrations of crossbred cows during the first 45 days postpartum are presented in Fig. 4.8. Testosterone levels were significantly ($P<0.01$) higher in

serum (range: 0.04 – 0.76 ng/mL; mean: 0.15±0.02 ng/mL; n=48) than that observed in raw milk (range: 0.01 – 0.28 ng/mL, mean: 0.10±0.01 ng/mL; n=48). Testosterone concentrations in serum and raw milk were highly (P<0.01) and positively correlated (0.71), an indication of a good association (Fig. 4.8).

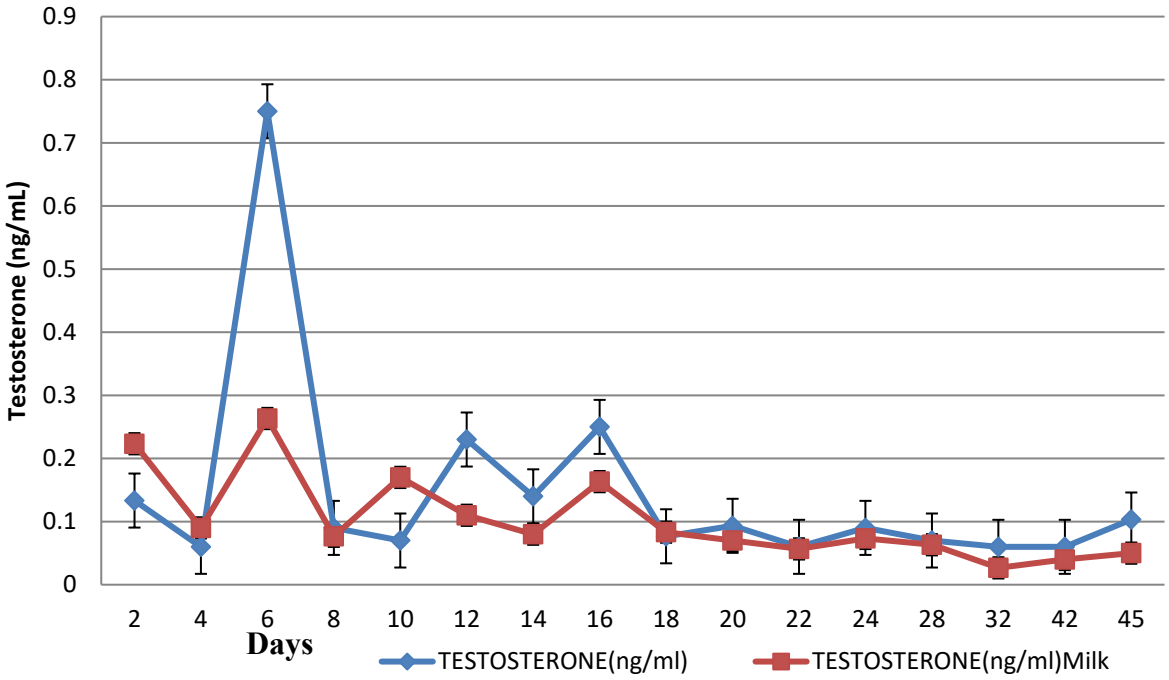

Fig. 4.8: Relationship between mean serum (in blue) and milk (in red) testosterone levels in three (3) crossbred cows 45 days postpartum using commercial EIA.

Results obtained on the relationship between FSH levels in serum and raw milk with EIA assaying are presented in Fig. 4.9. Mean concentrations of FSH were similar in serum and raw milk, and ranged from 0.01 – 22.65 mIU/mL (mean: 3.3±0.99 mIU/mL) and 0.00 – 21.76 mIU/mL (mean: 3.4±1.02 mIU/mL) in serum and milk respectively. There was significant (P<0.01), and high positive correlation (0.93) between serum and raw milk concentration of FSH (Fig. 4. 9). There were low concentrations in FSH from day 1 to day 20 postpartum. A slight pulsatile in serum was observed in day 22 and significant surge in FSH was noticed in day 42 and dropped at day 45 postpartum.

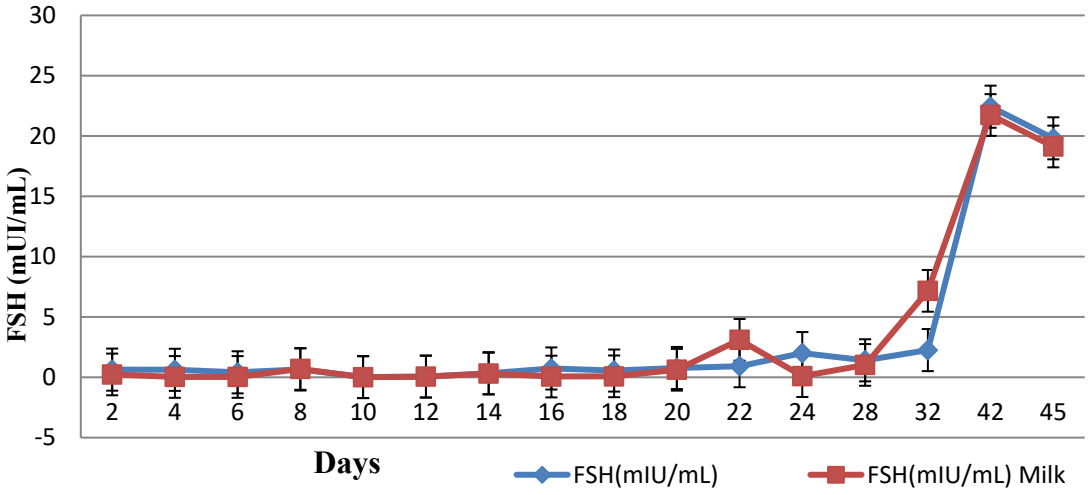

Figure 4. 9: Relationship between mean concentrations of serum and milk FSH in three crossbred cows 45 days postpartum with commercial EIA

Results obtained on the relationship between serum and raw milk LH concentrations using EIA assaying are shown in Fig., 4.10.

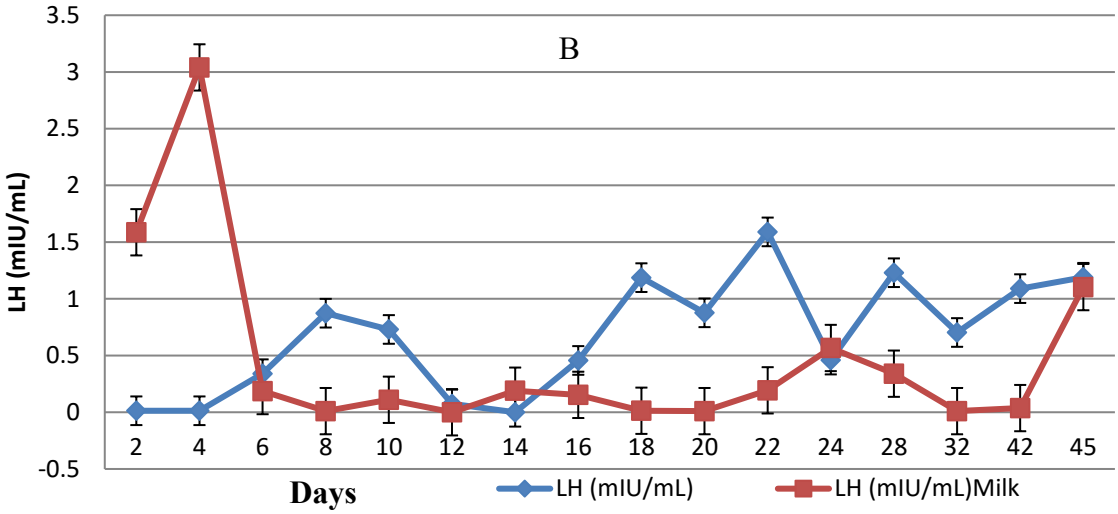

Figure 4. 10: Relationship between mean concentrations of serum and milk LH in three crossbred cows 45 days postpartum

Concentration of LH in serum and milk ranged from 0.00 – 1.63 mIU/L and 0.00 – 3.06 mIU/mL with the overall means of 0.68±0.07 mIU/L and 0.47±0.12 mIU/L (n=48),

respectively. Serum and raw milk concentrations of LH were significantly (P<0.01), moderately and negatively correlated (- 0.38). An increase in serum LH concentration moderately led to a decrease in raw milk LH concentration and vice versa (Fig. 4.10).

4.3.5 Effect of age on testosterone and oestradiol concentrations in bulls

Testosterone and oestradiol concentrations in bulls of different ages are presented in Table 4.11. Young bulls of up to six (6) months had least (P<0.05) testosterone concentration. Testosterone concentration increased (P<0.05) with increasing age from one (1) year and peaked at age four (4). Testosterone concentrations in young bulls, yearlings and those of twelve (12) years and above were similar (P>0.05).

Oestradiol concentrations were significantly (P<0.05) influenced by bulls of different ages. The highest level of oestradiol was observed in 1 year old bulls, whereas bulls of up to 6 months old, and 2, 3, 4 and 12 years old had similar (P>0.05) oestradiol concentrations.

Table 4.11: Testosterone and oestradiol concentration in sera of bull

Age goup of bull	**N**	**♂ Testosterone (ng/mL)**	**♂ Oestradiol (pg/mL)**
		0.012*	***0.042****
6 months	4	0.04 ± 0.014^{b}	26.58 ± 17.18^{b}
1 year	4	1.11 ± 0.30^{b}	93.48 ± 19.18^{a}
2 years	4	2.24 ± 0.85^{a}	24.73 ± 6.28^{ab}
3 years	4	2.37 ± 0.91^{a}	49.06 ± 18.68^{b}
4 years	4	3.27 ± 1.43^{a}	38.19 ± 15.85^{b}
≥12 years	4	0.82 ± 0.37^{b}	$10.30\pm0.83B^{b}$
Overall Mean	*24*	*1.64 ±0.28*	*40.39* ±9.73

**=P-values;* ^{a,b,c,d}*=Means bearing different superscript in the same column are significantly different.*

4.4 Productive Performance of Dairy cows

4.4.1 Factors influencing milk yield, lactation length and milk composition of dairy cows

4.4.1.1 Milk yield of dairy cows

Least square (LS) means for effect of farm, breed, parity, body condition score (BCS), stage of lactation, udder and teat sizes, season, feed supplementation and number of milking a day on milk yield of dairy cows is presented in Table 4.12. The overall LS mean milk yield was 5.4 ± 0.20 litres/day across the breeds studied.

Effect of farm on mean daily milk yield

Different farms had significant ($P<0.01$) effect on LS mean milk yield. Ofori and Rea Farms recorded a similar ($P>0.05$) milk yield but had the highest mean milk yield (Table 4.12). Nartey Farm was the next ($P<0.01$) highest, followed by Joseph, Amrahia, Karima, Zare, UEW_MF, and Embik Farms in descending order. Karima, Zare, UEW Mampong and Embik Farms had similar ($P>0.05$) and the lowest ($P<0.01$) mean milk yield.

Effect of breed on mean daily milk yield

Breed had a prominent influence ($P<0.01$) on mean milk yield. Jersey cows had the highest mean milk production, with the Sanga recording the least ($P<0.01$). Jersey and Friesian-Sanga crossbred cows had similar ($P>0.05$) mean milk yield.

Table 4.12: Least square means (± SE) of effect breed and non-genetic factors influencing milk yield of dairy cows in Ghana

Factors	N	Milk Yield (litres/cow/d)
Farm		***0.000****
Amrahia	135	4.3 ± 0.19^{d}
Embik	43	2.4 ± 0.39^{e}
Joseph	30	6.1 ± 0.53^{c}
Karima	34	3.4 ± 0.62^{e}
Nartey	27	8.7 ± 0.62^{b}
Ofori	30	10.3 ± 0.57^{a}
Rea	26	10.4 ± 0.30^{a}
UEW Mampong	119	3.2 ± 0.25^{e}
Zare	122	3.4 ± 0.23^{e}
Breed		***0.000****
Sanga	202	5.1 ± 0.32^{c}
FS Cross	332	6.6 ± 0.29^{ab}
Jersey	32	7.4 ± 0.37^{a}
Parity		***0.000****
1	178	5.4 ± 0.23^{b}
2	97	5.7 ± 0.25^{b}
3	88	7.2 ± 0.25^{a}
4	114	7.2 ± 0.24^{a}
5	63	7.4 ± 0.32^{a}
6	26	5.7 ± 0.34^{b}
SOL		***0.000****
1 – 30 days	239	6.6 ± 0.21^{a}
31 – 149 day	152	6.1 ± 0.24^{b}
Above 150 days	175	5.7 ± 0.23^{c}
BCS		***0.000****
2.0	32	5.2 ± 0.35^{c}
2.5	110	5.6 ± 0.30^{c}
3.0	207	6.7 ± 0.20^{b}
3.5	190	6.8 ± 0.21^{b}
4.0	27	7.6 ± 0.34^{a}
Udder Size		***0.000****
1. Small	187	2.5 ± 0.24^{b}
2. Medium	180	3.0 ± 0.43^{b}
3. Large	199	5.4 ± 0.27^{a}
Teat Size		***0.000****
4. Small	189	1.7 ± 0.41^{c}
5. Medium	156	3.6 ± 0.27^{b}
6. Large	221	5.5 ± 0.26^{a}
Season		***0.013****
1. Rainy	209	6.4 ± 0.22^{a}
2. Minor	208	6.1 ± 0.23^{ab}
3. Dry	149	5.8 ± 0.23^{b}
Supplementation		***0.000****
1. Suppl.	169	6.3 ± 0.18^{a}
2. Partial Suppl.	206	2.9 ± 0.14^{b}
3. No Suppl.	191	2.4 ± 0.19^{b}
Milking freq./d		***0.000****
1. Once/day	263	2.5 ± 0.16^{b}
2. Twice/day	303	5.1 ± 0.14^{a}

**=P-values; BCS=Body condition score; d=days; freq,=frequency; FS=Friesian-Sanga; SOL=Stage of lactation; Suppl=Supplementation; No.= Number; N= number of observation; UEW_MF=University of Education, Winneba, Mampong farm. ^{abcd}=Means bearing different superscript in the same column are significantly different.*

Effect of parity of dam on daily milk yield

Parity of birth had a great effect (P<0.01) on mean milk yield. Milk yield at the first parity was relatively low (Table 4.12). Thereafter, average milk yield assumed a sustained increase from second to fifth parities and then declined at the sixth parity.

Effect of Stage of lactation on mean daily milk yield

Stage of lactation influenced (P<0.01) mean daily milk production such that, mean daily milk yield decreased (P<0.01) with increasing months of the stage of lactation recorded. The highest (P<0.01) mean milk production per stage of lactation was observed in the first month of lactation (1 – 30 days). This was followed by 31 – 149 days and 150 and above days in descending order.

Effect of body condition score on mean daily milk yield

Body condition score (BCS) had great influence (P<0.01) on average milk yield. The least average milk yield was recorded by BCS 2 which was not different (P>0.05) from that of BCS 2.5. Better (P<0.01) mean milk productive performances were recorded by BCS 3 and 3.5 with the BCS 4 having the best record (P<0.01) of the LS mean milk production (Table 4.12).

Effect of udder size daily milk production

Large udder size pulled the highest (P<0.01) mean daily milk yield performance among the three categories of the udder sizes (Small. Medium and large) considered. Cows with

medium sized udder had slightly higher milk yield potential than small udder counterparts, although the difference was insignificant (P>0.05) mean milk yield.

Effect of teat size on daily milk yield performance

Least square means of milk yield performance was significantly influenced (P<0.01) by small, medium and large teat sizes. Mean daily milk yield increased with increasing size of teat size. Dairy cows with large teat size had the best (P<0.01) mean milk yield potential. This was followed by cows with medium and small teat sizes.

Effect of season on daily milk yield performance

Season of the year had significant (P<0.05) effect on the mean milk yield. Mean daily milk yield significantly increased (P<0.01) in major rainy season with the dry season recording the least (P<0.01) mean daily milk production. Minor and dry seasons did not differ (P>0.05) in the mean daily milk yield (Table 4.12)

Effect of feed supplementation on daily milk yield performance

Feed supplementation had a significant (P<0.01) impact on the average milk yield. Regular feed supplementation gave the best (P<0.01) milk yield followed by occasional, and no feed supplementations in that order.

Effect of number of daily milking on milk yield performance

Number of daily milking of dairy cows had great effect on LS mean milk yield such that, cows that were milked twice (morning and evening) a day had higher mean milk yield than cows that were milked once a day (Table 4.12).

4.4.1.2 Lactation length of dairy cows

Grand LS mean lactation length was 271.4 ± 2.96 days (n=177) across the breeds. Least square means for effect of effect of farm, breed, parity, body condition score (BCS), season of lactation, udder and teat sizes, feed supplementation and number of milking a day on lactation length (LL) of dairy cows are presented in Table 4.13.

Effect of farm on lactation length of dairy cows

It was observed that Ofori farm had the longest ($P<0.01$) LL but the value did not differ ($P>0.05$) from LL obtained by REA, Nartey and Joseph Farms. Zare, Amrahia, and UEW_MF farms had intermediate LL, followed by Embik farm while Karimal Farm had the least LL.

Effect of breed of cows on lactation length

Friesian-Sanga crossbred cows recorded slightly higher LL, followed by Jersey and Sanga cow (Table 4.13), although there was no significant ($P>0.05$) difference among the breeds with respect to LL.

Effect of parity of cows on lactation length

At parity one (1), LL was relatively low (P<0.01). Lactation length then assumed an increase (P<0.01) in parity 2, peaking at parities 3 to 5 and declined at parity 6 (Table 4.13). Parity 1 and 6 had similar LL and fewest in days among the parities considered in this study.

Table 4.13: Least square means (± SE) of effect breed and non-genetic factors on lactation length (in days) of dairy cows

Factors	N	Lactation Length
Farm		***0.000****
Amrahia	30	256.5 ± 6.81^{bc}
Embik	40	222.0 ± 10.64^{c}
Joseph	13	276.3 ± 11.05^{a}
Karima	8	193.0 ± 24.80^{d}
Nartey	11	280.8 ± 12.54^{a}
Ofori	13	304.7 ± 12.64^{a}
REA	20	303.9 ± 7.37^{a}
UEW_MF	30	242.5 ± 8.06^{c}
Zare	17	259.1 ± 9.51^{bc}
Breed		***0.873****
Sanga	62	259.6 ± 11.13
FS cross	83	264.7 ± 6.69
Jersey	32	261.9 ± 8.40
Parity		***0.000****
1	26	228.5 ± 7.00^{c}
2	41	267.4 ± 7.49^{b}
3	46	283.3 ± 7.10^{a}
4	31	269.6 ± 7.88^{b}
5	26	273.6 ± 9.05^{a}
6	7	250.1 ± 15.26^{bc}
Season		***0.091****
Rainy	88	282.1 ± 4.43
Minor	67	279.5 ± 5.50
Dry	22	261.3 ± 8.78

Factors	N	Lactation Length
BCS		***0.000****
BCS 2.0	2	198.2 ± 17.53^{b}
BCS 2.5	16	225.1 ± 11.25^{b}
BCS 3.0	84	290.0 ± 6.47^{a}
BCS 3.5	59	296.1 ± 6.33^{a}
BCS 4.0	16	300.9 ± 10.28^{a}
Udder Size		***0.000****
Small	33	235.1 ± 8.74^{b}
Medium	78	283.8 ± 4.05^{a}
Large	65	295.2 ± 5.44^{a}
Teat Size		***0.020****
Small	57	259.7 ± 5.97^{b}
Medium	43	284.0 ± 6.21^{a}
Large	76	270.4 ± 5.65^{b}
Supplementation		***0.000****
Suppl.	67	293.1 ± 6.20^{a}
Partial	71	282.0 ± 6.84^{a}
No. Suppl.	39	247. 9 ± 7.65^{b}
Milkingfreq./d		***0.004****
1. Once/day	103	258.3 ± 4.97^{b}
2. Twice/day	74	290.3 ± 8.12^{a}
Overall	**177**	**271.4 ± 2.96**

**=P-values; BCS=Body condition score; d=days; FS=Friesian-Sanga; Suppl=Supplementation; No.=Number; N= number of observation; UEW_MF=University of Education, Winneba, Mampong farm.* ^{a,b,c,d}*=Means bearing different superscript in the same column are significantly different.*

Effect of season of calving on lactation length

Lactation length of cows slightly increased with increasing rains though it was insignificant (P>0.05) (Table 4.13).

Effect of body condition score on lactation length

Body condition score (BCS) at the time of calving had significant (P<0.01) effect on LL of dairy cows (Table 4.13). Lactation length increased (P<0.01) with increasing BCS.

Effect Udder and teat sizes on lactation length

Udder size had remarkable effect (P<0.01) on LL (Table 4.13). Lactation length increased with increasing size of udder size. Cows with large udder size had the longest (P<0.01) LL. This was followed by medium and small sized udders. Teat size also influenced (P<0.05) LL such that medium udder had the highest, followed by large and small teat sizes in descending order.

Effect of feed supplementation on lactation length

Lactation length significantly (P<0.01) increased with increasing feed supplementation. The differences observed in regular and occasional feed supplementation of lactating cows were insignificant (P>0.05). Cows without feed supplementation had the shortest LL.

Effect of number of milking on lactation length

Number of milking per day had significant (P<0.01) effect of LL. Cows that were milked twice a day had longer (P<0.01) LL than cows milked once a day (Table 4.13).

4.4.1.3 Milk composition of the dairy cows

Effect of breed, parity, stage of lactation and season on fresh milk protein, fat, lactose, cholesterol, solid-non-fat (SNF), and total solids (TS) of milk composition of the Sanga, Friesian-Sang and Jersey cows are presented in Table 4.14.

Effect of location on percentage milk composition of dairy cows

Location influenced percentage protein ($P<0.05$), total solids ($P<0.01$) and solid-non-fat (SNF) ($P<0.05$). Milk from Ashanti Region recorded the highest ($P>0.01$) protein and total solids whereas milk from Eastern and Greater Accra Regions had similar ($P>0.5$) values. It was observed that the SNF component was highest in milk of cows from Ashanti Region but the value was similar to those observed in the Greater Accra Region. Milk from the Eastern Region had the lowest value of percentage SNF.

Location, however, had little ($P>0.05$) effect on percentage fat, lactose, cholesterol, water and ash component of raw milk.

Breed of dairy cows had significant ($P<0.01$) effect on percentage milk composition (Table 4.14). Breed greatly influenced percentage milk protein ($P<0.05$), fat ($P<0.01$), total solids ($P<0.01$), and ash ($P<0.05$) components. Friesian-Sanga crossbred cows had the highest ($P<0.01$) total solids (TS) which was also similar to the value observed in Sanga cows. Jersey cows had the least ($P<0.01$) protein component. Percentage fat was significantly highest in Jersey cow. Sanga cows had slightly higher percentage fat than those observed in Friesian-Sanga crossbred cows though it was not significant ($P>0.05$). Total solids were higher in

Table 4.13: Effect of breed and non-genetic factors on percentage milk composition of dairy cows in Ghana

FIXED FACTORS	N	%Protein	%Fat	%Lactose	%Chol.	%TS	%SNF	%Water	%Ash
Overall	30	4.63±0.23	3.8±0.18	4.7±0.09	0.64±0.07	14.1±0.19	8.6±0.11	86.3±0.32	0.77±0.01
Location		***0.047****	***0.476****	***0.886****	***0.114****	***0.001****	***0.049****	***0.374****	***0.083****
Ashanti Region	10	5.4±0.44^{a}	3.6±0.24	4.8±0.16	0.66±0.03	14.8±0.22^{a}	9.0±0.19^{a}	86.8±0.67	0.81±0.02
Eastern Region	10	4.2±0.38^{b}	3.8±0.39	4.7±0.12	0.59±0.02	13.6±0.23^{b}	8.3±0.23^{b}	86.4±0.38	0.75±0.02
Greater Accra	10	4.2±0.27^{b}	4.1±0.31	4.7±0.20	0.66±0.03	14.0±0.13^{b}	8.5±0.11^{a}	85.7±0.55	0.74±0.02
Breed		***0.021****	***0.000****	***0.732****	***0.193****	***0.001****	***0.061****	***0.691****	***0.024****
Sanga	10	4.9±.025^{a}	3.3±0.04^{b}	4.8±0.15	0.62±0.02	14.7±0.24^{a}	8.9±0.20	86.7±0.54	0.81±0.02^{a}
Friesian-Sanga Cross	10	5.2±0.56^{a}	3.1±0.09^{b}	4.7±0.12	0.61±0.02	13.5±0.20^{b}	8.2±0.23	86.2±0.47	0.73±0.02^{b}
Jersey	10	3.8±0.11^{b}	5.1±0.20^{a}	4.6±0.20	0.68±0.04	14.1±0.16^{b}	8.7±0.10	86.1±0.65	0.76±0.02^{ab}
Parity		***0.853****	***0.051****	***0.118****	***0.486****	***0.718****	***0.248****	***0.955****	***0.929****
1	10	4.5±0.33	3.2±0.08^{b}	4.8±0.15	0.62±0.02	13.9±0.28	8.4±0.27	86.3±0.44	0.77±0.02
2	10	4.6±0.39	4.2±0.39a	4.5±0.19	0.66±0.03	14.2±0.22	8.6±0.13	86.5±0.63	0.76±0.03
3	10	4.8±0.50	4.1±0.32^{a}	4.9±0.11	0.63±0.03	14.2±0.27	8.8±0.15	86.2±0.60	0.76±0.02
Body Condition Score		***0.041****	***0.695****	***0.717****	***0.443****	***0.000****	***0.553****	***0.002****	***0.007****
BCS 2.5	10	3.8±0.30^{b}	4.03±0.34	4.7±0.19	0.64±0.04	13.5±0.18^{c}	8.5±0.11	85.0±0.26^{b}	0.71±0.01^{b}
BCS 3.0	10	5.4±0.48^{a}	3.64±0.29	4.6±0.14	0.61±0.03	14.1±0.19^{b}	8.5±0.13	86.3±0.62^{b}	0.78±0.02^{a}
BCS 3.5	10	4.7±0.24^{ab}	3.83±0.34	4.8±0.15	0.66±0.02	14.8±0.21^{a}	8.8±0.30	87.6±0.38^{a}	0.81±0.02^{a}
Stage of Lactation		***0.021****	***0.000****	***0.732****	***0.193****	***0.001****	***0.061****	***0.691****	***0.024****
1 – 30 days	10	4.9±0.25^{a}	5.1±0.11^{a}	4.6±0.20	0.68±0.04	14.1±0.16^{b}	8.7±0.10	86.1±0.65	0.75±0.02^{ab}
31 – 149 day	10	3.8±0.11^{b}	3.3±0.56^{b}	4.7±0.13	0.61±0.02	13.5±0.20^{b}	8.2±0.23	86.2±0.47	0.73±0.02^{b}
Above 150 days	10	5.2±0.56^{a}	3.3±0.25^{b}	4.8±0.15	0.64±0.02	14.7±0.24^{a}	8.7±0.11	86.7±0.54	0.81±0.02^{a}
Supplementation		***0.035****	***0.700****	***0.747****	***0.963****	***0.000****	***0.003****	***0.000****	***0.000****
Regular	10	5.2±0.43^{a}	3.9±0.32	4.8±0.15	0.64±0.2	14.8±0.21^{a}	9.1±0.14^{a}	88.2±0.30^{a}	0.85±0.01^{a}
Occasional	10	4.8±0.35^{ab}	3.6±0.30	4.8±0.16	0.63±0.3	14.0±0.15^{b}	8.3±0.22^{b}	85.9±0.39^{b}	0.73±0.02^{b}
No supplementation	10	3.8±0.30^{b}	4.0±0.35	4.6±0.17	0.63±0.3	13.5±0.18^{c}	8.4±0.12^{b}	84.9±0.27^{c}	0.71±0.01^{b}
Season of calving		***0.048****	***0.732****	***0.645****	***0.791****	***0.000****	***0.004****	***0.000****	***0.000****
Rainy	10	5.0±0.31^{a}	3.9±0.32	4.7±0.15	0.65±0.02	14.9±0.20^{a}	9.1±0.14^{a}	87.9±0.37^{a}	0.85±0.01^{a}
Minor	10	5.1±0.47^{a}	3.6±0.30	4.8±0.16	0.62±0.03	14.0±0.13^{b}	8.3±0.22^{b}	86.2±0.47^{b}	0.74±0.02^{b}
Dry	10	3.8±0.31^{b}	4.0±0.35	4.6±0.17	0.63±0.03	13.5±0.18^{c}	8.4±0.12^{b}	84.9±0.28^{c}	0.71±0.01^{b}

**=P-values; BCS=Body condition score; Chol=Cholesterol; N=Number of observation; SNF=solid-non-fat; ST=Total solids*

^{a,b,c,d}=Means bearing different superscript in the same column are significantly different.

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Sanga's milk than those observed in the crossbred and Jersey cows. The crossbred and Jersey cows had insignificant (P>0.05) values of percentage TS. Ash content of milk was highest (P<0.05) in Sanga, followed by Jersey and Friesian-Sanga crossbred cows. Sanga and Jersey cows had similar (P>0.05) ash level, likewise did Crossbred and Jersey cows. Breed, however, had little effect (P>0.05) on percentage lactose, cholesterol, SNF, and water components.

Parity of dam had insignificant (P>0.05) effect on all the percentage milk components of dairy cows considered in this study.

Body condition score influenced (P<0.01) percentage milk protein (Table 4.14), TS, water and ash composition. Body condition score 3 had the highest protein component followed by BCS 4 and BCS 2.5 recorded the least (P<0.05). The values of protein component for BCS 3 and BCS 4 were similar (P>0.05). Percentage composition of TS, water and ash increased significantly (P<0.01) with increasing BCS points. Body condition score had little (P>0.05) influence on fat, lactose, cholesterol, and SNF in this study.

Stage of lactation (1 – 30, 31 – 149 and above 150 days) had effect on percentage protein (P<0.05), fat (P<0.01), TS (P<0.01), and ash (P<0.05). Lactation stage of above 150 days had the highest percentage protein value but it was similar (P>0.05) to the value observed in 1 – 30 days (Table 4.14). Lactating cows of the stage of 31 – 149 days had the lowest (P<0.05) percentage protein component. Stage of lactation category 1 – 30 days had significantly higher (P<0.01) percentage fat whereas 31 – 149 and above 150 days had similar (P>0.05)

values but lower in fat component. The TS and ash components were also highest in above 150 day, followed by 1 – 30 days with 31 – 149 days recording the lowest percentage fat. The ash component was similar (P>0.05) in 1 – 30 days and above 150 days. The three categories of stages of lactation had insignificant (P>0.05) effect on percentage lactose, cholesterol, SNF, and water compositions.

Feed supplementation influenced percentage protein (P<0.05), TS (P<0.01), SNF (P<0.01), water (P<0.01), and ash (P<0.01), in that, these components increased significantly (P<0.01) with increasing intensity of feed supplementation. Feed supplementation, however, had little (P>0.05) effect on percentage fat, lactose, and cholesterol composition in milk.

Season of calving/milking also had effect on percentage protein (P<0.05), TS (P<0.01), SNF (P<0.01), water (P<0.01), and ash (P<0.01) compositions. Milk sampled from cows during the rainy and minor rainy seasons had similar (P>0.05) protein composition but higher (P<0.05) than those observed in dry season. Percentage TS, SNF, water and ash components also increased greatly (P<0.01) with decreasing intensity of dryness in the study area (Table 4.14).

4.4.2 Milk quality assessment

4.4.2.1 Effect of breed and non-gentic factors on milk temperature, pH and specific gravity

Effect of fixed factors on milk temperature, pH and specific gravity is presented in Table 4.15. The overall means for raw milk temperature, pH and specific gravity among the dairy cows were 2.6 ± 0.43, 6.5 ± 0.03 and 1.026 ± 0.00, respectively.

Table 4.15: Effect of fixed factors on milk temperature, pH and specific gravity of dairy in Ghana

FIXED FACTORS	N	Temperature	pH	Specific gravity**
Overall	*30*	*25.6±0.43*	*6.5±0.03*	*1.029±0.00*
Location		***0.871****	***0.423****	***0.681****
Ashanti Region	10	25.9±1.12	6.4±0.08	1.03±0.00
Eastern Region	10	25.4±0.48	6.5±0.04	1.03±0.00
Greater Accra	10	25.4±0.56	6.5±0.04	1.03±0.00
Breed		***0.302****	***0.018****	***0.180****
Sanga	10	24.9±2.33	6.4±0.06[b]	1.028±0.00
Friesian-Sanga Cross	10	26.5±0.90	6.5±0.05[a]	1.030±0.01
Jersey	10	25.3± 0.56	6.6±0.04[a]	1.029±0.00
Parity		***0.083****	***0.261****	***0.082****
1	10	25.1±0.59	6.4±0.03	1.028±0.00
2	10	2437±1.00	6.5±0.08	1.029±0.00
3	10	26.9±0.41	6.6±0.05	1.028±0.00
Body Condition Score		***0.688****	***0.261****	***0.052****
BCS 2.5	10	25.4±0.56	6.6±0.03	1.030±0.00
BCS 3.0	10	26.1±1.02	6.4±0.07	1.029±0.00
BCS 3.5	10	25.2±0.65	6.5±0.05	1.028±0.00
Stage of Lactation		***0.302****	***0.018****	***0.180****
1 – 30 days	10	25.3±0.56	6.6±0.04[a]	1.029±0.00
31 - 60	10	26.5±0.90	6.5±0.05[a]	1.029±0.00
Above 61 days	10	24.9±0.73	6.4±0.06[b]	1.028±0.00
Feed Supplementation		***0.842****	***0.214****	***0.088****
Regular	10	25.2±0.77	6.4±0.07	1.028±0.00
Occasional	10	25.8±0.98	6.5±0.05	1.028±0.00
No supplementation	10	25.7±0.50	6.6±0.03	1.030±0.00
Season of calving		***0.532****	***0.214****	***0.088****
Rainy	10	24.9±0.71	6.4±0.07	1.028±0.00
Minor	10	26.1±0.99	6.5±0.05	1.028±0.00
Dry	10	25.7±0.50	6.6±0.03	1.030±0.00

**=P-values; **=Specific gravity of milk values were read to 3 decimal places due to technicality in reading and recording the values using lactometer; BCS=Body condition score (1-5 scale); N=number of observation. [a,b,c,d]=Means bearing different superscript in the same column are significantly different.*

All the fixed factors had little effect on raw milk temperature and specific gravity. However, raw milk pH was influenced (P<0.05) by breed and stage of lactation. Jersey cow had

significantly higher pH value which was similar to the value observed in Sanga's raw milk. Raw milk of Friesian-Sanga cows had lower (P<0.05) pH as indicated in Table 4.15. Raw milk pH reduced in the third stages of lactation.

4.4.2.2 Effect of fixed factors on milk somatic cell count

Location had little effect on raw milk somatic cell count (MSCC). Management with distinct sanitary practices had significant (P<0.05) influence on MSCC such that cows under range grazing with poor sanitary practice had the highest count with those maintained under exclusive zero grazing/good sanitary environment recording the least (P<0.05) MSCC (Table 4.16). Cows kept in partial and exclusive zero grazing management had similar (P>0.05) MSCC.

Breed had significant influence on milk somatic cell count (MSCC). Jersey cows had the highest (P<0.01) MSCC but the value was similar (P>0.05) to the MSCC in Friesian-Sanga crossbred cows. Sanga recorded the lowest (p<0.01) MSCC. The MSCC increased with increasing proportion of high milking potential among the breeds considered.

Parity of cows also had significant (P<0.01) effect on MSCC. Parity one had the lowest (P<0.01) somatic cell count. Parity two and three had similar (p>0.05) MSCC but were significantly higher than the value of count observed in parity one. The MSCC significantly decreased with increasing stage of lactation (Table 4.16).

Table 4.16: Mean ± SE for effect of fixed factors on somatic cell count, total bacterial and total coliform count

Factors	**N**	**Somatic cell count**	**TBC/mL**	**TCC/mL**
Unit		***cells/mL***	***cfu/mL***	***cfu/mL***
Overall	30	134000.0 ± 7498.7	$3.0\ x10^6 \pm 7.4\ x10^5$	$2.9x10^4 \pm 1.4x10^4$
Location		***0.136****	***0.008****	***0.001****
Ashanti Region	10	154000.0 ±14621.4	$5.6\ x\ 10^6 \pm 2.0\ x10^{6a}$	$1.8x10^4 \pm 9.4x10^{3a}$
Eastern Region	10	118000.0 ± 12454.3	$1.2\ x\ 10^6 \pm 2.3x10^{5b}$	$9.7x10^2 \pm 5.6x10^{2b}$
Greater Accra	10	130000.0 ± 10000.0	$2.1\ x10^6 \pm 3.3\ x10^{5b}$	$6.7x10^4 \pm 4.0x10^{4c}$
Sanitary Management		***0.042****	***0.016****	***0.002****
Poor sanitation (RG)	10	155000.0±11838.6[a]	$4.7x10^6 \pm 1.7x10^{6a}$	$6.7x10^4 \pm 3.3x10^{4a}$
Fairy good (ZG_P)	10	128000.0±8537.5[ab]	$2.4x10^6 \pm 4.0x10^{5b}$	$5.7x10^3 \pm 1.8x10^{3b}$
Very good (ZG_E)	10	110000.0±15583.9[b]	$1.1x10^6 \pm 2.9x10^{5c}$	$9.7x10^2 \pm 5.2x10^{2c}$
Breed*		***0.005****	***0.604****	***0.042****
Sanga	10	112000.0 ± 6110.1[b]	$2.1x10^6 \pm 3.3\ x10^5$	$6.2x10^4 \pm 3.9x10^{4a}$
FSCB	10	124000.0 ± 15434.4[ab]	$2.9x10^6 \pm 1.1\ x10^6$	$1.2x10^4 \pm 9.8x10^{3b}$
Jersey	10	166000.0 ± 9451.6[a]	$4.0x10^6 \pm 1.9x10^6$	$1.1x10^4 \pm 7.4x10^{3b}$
Parity		***0.000****	***0.138****	***0.428****
1	10	96000.0 ± 4988.9[b]	$1.8x10^6 \pm 3.6x10^5$	$5.3x10^4 \pm 4.1x10^4$
2	10	146000.0 ±12666.7[a]	$2.1x10^6 \pm 3.7x10^5$	$7.4x10^3 \pm 2.3x10^3$
3	10	160000.0 ± 10328.0[a]	$5.1x10^6 \pm 2.1x10^6$	$2.7x10^4 \pm 1.1x10^4$
Body condition score		***0.748****	***0.505****	***0.438****
BCS Point 2.5	10	128000.0 ± 12063.9	$3.3x10^6 \pm 1.1x10^6$	$3.5x10^4 \pm 1.3x10^4$
BCS Point 3	10	142000.0 ± 15040.7	$1.8x10^6 \pm 4.7x10^5$	$1.1x10^3 \pm 1.5x10^3$
BCS Point 3.5	10	132000.0 ± 11623.7	$3.9x10^6 \pm 1.9x10^6$	$4.8x10^4 \pm 4.0x10^4$
SOL_MIL		***0.005****	***0.604****	***0.042****
1 (1 – 30 days)	10	166000.0 ± 9451.6[a]	$4.0x10^6 \pm 1.9x10^6$	$1.1x10^4 \pm 7.4x10^{3b}$
2 (31 – 60 days)	10	124000.0 ± 15434.4[b]	$2.9x10^6 \pm 1.1x10^6$	$1.3x10^4 \pm 9.8x10^{3b}$
3 (61 days and above)	10	112000.0 ± 6110.1[b]	$2.1x10^6 \pm 3.3x10^5$	$6.3x10^4 \pm 4.0x10^{4a}$

**=P-values; N=Number of observation; RG =Range grazing; ZG_P = Partial zero grazing; ZG_E = Exclusive zero grazing; FS=Friesian-Sanga crossbred; BCS=Body condition score (1-5 scale); BSM=Biosecurity measure observed; SCC=Somatic cell count; TBC=Total bacterial count; TCC=Total coliform count; ZG=Zero grazing; ^{a,b,c,d}=Means bearing different superscript in the same column are significantly different.*

The first month (1 – 30 days) of lactation recorded the highest (P<0.01) count. The mid and late (31 – 149 days and 150 plus days) stages of lactation had similar (P>0.05) MSCC .

4.4.2.3 Effect of fixed factors on total bacteria count

Effect of fixed factors on milk total viable or bacterial count (TBC) is also presented in Table 4.16. Location influenced (P<0.01) TBC. Samples taken from Ashanti Region had the highest TBC while samples from Eastern Region recorded the least count. The TBC in raw milk from Ashanti and Greater Accra Regions was similar (P>0.05).

Sanitary management had significant (P<0.05) effect on TBC in raw milk. Cows kept in good sanitary premises had the least TBC whereas cows under poor/fair management had similar (P>0.05) TBC but the values were significantly (P<0.05) higher than those observed in good sanitary environment. Breed, parity, BCS, and stage of lactation did not significantly (P>0.05) influence TBC in this study.

4.4.2.4 Effect of fixed factors on total coliform count

Effect of location, sanitary management, breed, parity, BCS, and stage of lactation on raw milk total coliform count (TCC) is presented in Table 4.16. Location had a significant (P<0.01) effect on TCC. Raw milk from Ashanti region recorded the highest (P<0.01) TCC, followed by those in Eastern and Greater Accra Regions in descending order.

Sanitary management or practices also influenced (P<0.01) TCC significantly. Raw milk sampled from poor/fair sanitary condition dairy herds had higher TCC with the cows kept

at sanitized premises recording the least (P<0.01) TCC. Raw milk samples from poor/fair sanitary premises had similar (P>0.05) TCC.

Breed had significant (p<0.01) influence on TCC. Sanga cows had the highest (P<0.05) TCC but the value was similar (P>0.5) to that of the value observed in Friesian-Sanga crossbred cows. The Jersey cows had the least (P<0.05) TCC which was also similar to the value recorded by Friesian-Sanga cows. Parity and BCS had insignificant (p>0.05) influence on the TCC.

Stage of lactation significantly (p<0.01) influenced TCC in raw milk. The TCC increased (P<0.05) with increasing months of the categorized stages of lactation (Table 4.16).

CHAPTER FIVE

5.0 DISCUSSION

5.1. Health Management of Ticks and Tick-borne Diseases of Cattle

5.1.1 Incidence of ticks species and factors influencing total tick infestation

5.1.1.1 Incidence of tick species across breed of cattle in the study area

The highest incidence of *Amblyomma (A) variegatum* compared to *Boophilus spp, Rhipicephalus spp* and *Hyalomma rufiges* has also been observed by Koney (1992) and Walker and Koney (1999). The current trend in tick incidence shows higher tick abundance in May, June, July and August and lower incidence in January, February and December in Ghana, which is consistent with the findings of Khan *et al*. (2016) in Pakistan. In this study, tick species incidence increased with increasing trend in warmth and rains. Similar findings have been reported by Muhammed *et al*. (2014).

High incidence of *A. variegatum* has previously been reported (Walker and Koney, 1999; Walker *et al*., 2014; Vetrivel *et al*., 2017), except where hand picking and other control measures are employed by herdsmen (Lorusso *et al.*, 2013). The life cycle of the three-host ticks makes it difficult to control even with a weekly control (Spraying or dipping) programme (Okai *et al*., 2005). Hence some Fulani herdsmen refer to *A. variegatum* as the most dangerous tick species (Lorusso *et al.*, 2013). *Amblyomma variegatum* transmits heartwater or cowdrosis and anaplasmosis diseases (Walker *et al.,* 2014) which makes the vector economically important in the dairy development in Ghana.

The high incidence of *Rhipicephalus (Boophilus) decoloratus* may be attributed to its typical one-host life cycle that enhances abundant multiplication (Walker *et al*., 2014).

However, weekly and fortnightly control is possible (Annan-Prah, 2011). *Rhipicephalus (Boophilus) decoloratus* transmits the protozoan *Babesia bigemina*, causing bovine babesiosis in cattle. This tick transmits the *Anaplasma marginale*, that causes bovine anaplasmosis, and *Borrelia theileri* which also causes spirochaetosis in cattle, sheep, goats and horses. Heavy infestations of *Rhipicephalus (Boophilus) decoloratus* are likely to cause damage to hides and to reduce the rate of growth of cattle (Walker *et al*., 2014).

Rhipicephalus species including *Ripicephalus senegalensis* and *Rhipicephalus evertsi evertsi* occurrence followed a similar trend, peaking in the hot-humid, rainy environmental condition. DeClercq *et al.* (2012) also noticed a similar increase in the incidence of tick population with increasing rainy periods of the year in Benin. *Hyalomma rufipes* also transmits *Anaplasma marginale* to cattle causing bovine anaplasmosis or gallsickness and causing benign babesiosis. The ticks of the genus Hyalomma (H) can transmit Congo haemorrhagic fever virus to humans. The vector, *H. rufipes*, would appear to be the most efficient vector of the virus (Walker and Koney, 1999) and, therefore, its zoonotic importance should not be overlooked.

The massive occurrence of tick species with increasing rains and warmth is consistent with the findings reported by Muhammed *et al*. (2014), Walker *et al*. (2014) and Vetrivel *et al*. (2017). It has been noticed that the greatest tick abundance occur in season of maximum moisture and growth of vegetation to shelter the questing ticks (Walker, 2011). In this study, the lowest tick count in the dry season may have resulted from the hazy, dry, and cold weather condition that occurred in December, which condition posed a challenge to

tick multiplication. Extensive burning of range grasses kills the tick, hence drastically reducing the tick questing and reproduction in the dry period.

5.1.1.2 Factors influencing total tick load/count

5.1.1.2.1 Effect of farm and management on total tick count

The effect of farm on total tick count was modified by type of housing, health and management practices adopted by farmers. Farms that practised range grazing/sedentary husbandry system had the highest tick load followed by partial zero-grazing with the exclusive zero-grazing having the least. This result indicates that individual farm and management regimes determine the success of tick control programme. Swai *et al.* (2006) alluded that most infected ticks are obtained from traditionally managed pastoral and grazed smallholder dairy cattle, while the zero-grazed smallholder cattle have almost absent infected ticks on the hosts. Zero grazing prevents animals from getting exposed to the tick-vector thereby markedly reducing the incidence of tick-borne diseases. Major constraints to cattle/dairy production success might be due to herd management systems that compromise risks of ticks and tick-borne diseases (Koney, 1996; Walker, 2011). It has, therefore, been realized in this study that reshaping of management regimes to prevent exposure of hosts to the tick vectors is the best option for tick prevention and control.

5.1.1.2.2 Effect of breed on total tick load in different management regimes

The differences in total tick count with respect to breed effect were masked by management effect. Sanga and WASH/GSH breeds are more resistant to tick infestation than the crossbreds and zebu as an established fact (Gashaw, 2005). However, Jersey cattle recorded the least or no tick count because the breed was managed in insect-proof barn that

might have prevented the exposure of the cattle to tick vectors. Swai *et al.* (2006) also observed similar trend in tick infestation in different management regimes.

5.1.1.2.3 Effect of location on tick infestation

The highest tick infestation recorded in cattle of Ashanti region is due to the fact that, the hot-humid and moist semi-deciduous forest of Ashanti favours tick development and phenology (Walker and Koney, 1999). Tick abundance varies with respect to climate, vegetation and ecological zones in given location (Norval and Lightfoot, 1982). The differences observed in total tick count between Ashanti and Eastern regions with a similar climatic condition might be attributed to differences in the production system embraced by cattle farmers. Eastern region dairy herds are mostly kept under intensive (exclusive) zero-grazing with insect–proof housing where cattle have little or no exposure to tick vectors. Tick abundance is known to be higher in moist humid environment (where Ashanti and Eastern regions are located) than the coastal savannah zones (as noticed in Greater Accra Region). However, this study found the opposite due to the changing in response to multiple factors (management, health consciousness, etc) that may be operating together to bring about the observed differences in tick abundance (Sonenshine, 2018). It is imperative to note that, tick population densities and distributions are greatly influenced by a dynamic environment of biotic and abiotic factors that include changes in temperature, rainfall pattern, human influence on housing design, management regimes and behaviours (Wikel, 2018) in a given geographical location.

5.1.1.2.4 Effect of season on total tick count

Season was one of the determinants of total tick count. Major rainy season recorded the highest total tick count, followed by minor rains and dry season recorded the least count. This finding is similar to DeClercq *et al.* (2012) who observed a significant increase in the abundance of tick population with increasing rainy periods of the year in Benin. The distinct seasonal differences in the total tick count observed in this study might be due to variations in increased temperature, high humidity, moisture and vegetation cover which influence tick phenology (Walker, 2011).

The highest count records in major rainy season is due to favourable weather conditions including moisture, hot-humid, and thick vegetation that enhance the laying, development and eclosion of instar ticks. Seasonally, tick populations increased in rainy and hot dry periods far more than cool to dry seasons as it has also been reported by Swai *et al.* (2006) and DeClercq *et al*. (2012). The rainy season has characteristically consistent precipitations, which coincide with the active grazing period of the year. It has been noticed that the greatest tick abundance occur in season of maximum moisture and growth of vegetation to shelter the questing ticks (Walker, 2011). In this study, the lowest tick count in the dry season may have resulted from the hazy, dry, and cold weather condition that occur in December which posed a challenge to tick multiplication. Extensive burning of range grasses kills the tick, hence drastically reducing the tick questing and reproduction in the dry period. Supporting evidence by Mohammed and Hassan (2007) indicates that field infestation levels vary across different locations and seasons, with the cool-to-dry seasons having lower tick counts. It is documented that, desiccation remains the principal abiotic threat to ticks (Walker, 2011).

5.1.1.2.5 Effect of sex of cattle on totaltick count

Sex of cattle did not significantly (P>0.05) affect total tick count in the study areas indicating sex is not a good determinant of total tick load or infestation in dairy cattle herds.

5.1.1.2.6 Effect of feed supplementation on total tick count

Cattle that were regularly supplemented had a fewer total tick count as compared to those subjected to occasional/partial and no feed supplementation. In the current study, farmers who practised regular feed supplementation also maintained zero-grazing and managerial practices that regularly prevented hosts-vectors associations. Dairy herd health management for reproductive and productive success also demands monitoring of general animals status and nutrition, which boost up the immune system (Solcan, 2009).

5.1.1.2.7 Type of housing on total tick count

Insect-proof barns recorded little or no tick count whereas roofed and open kraal had high total tick count. Insect-proof barns apart from serving as a screen between cattle and the obligate haemo-parasites, also prevents intensively kept cattle from getting into contact with the infected ticks. Strategic and tactical management of ticks should include good barn management for zero-grazed animals, and separate infested cattle from the healthy ones (Annan-Prah, 2011). This study revealed that cattle kept under intensive housing recorded lesser count of ticks as compared with those cattle exposed, which is consistent with the finding reported by Swai *et al.* (2006). Estrada-Pena and de la Fuente (2014) indicated that poor management of farms, uncontrolled movements of domestic animals, abundance of wild animals, and absence of an adequate framework to capture the

ecological plasticity of certain ticks may be attributed to complexity of the tick control measures.

5.1.1.2.8 Level of biosecurity measures employed and tick incidence

Implementation of moderate level of biosecurity principles resulted in a drastic reduction in total tick count, followed by low level observation of the measures with negligence registering a worst tick count. This is consistent with the assertion made by Buhman *et al*. (2007) that developing and maintaining biosecurity though difficult, it is the cheapest, most effective means of disease control available, and no disease prevention programme works without it. Biosecurity's principle of traffic control, isolation and sanitation (Mathis and Hagevoort, 2010), together with good management played crucial role of preventing tick infestation in this study.

5.1.1.2.9 Period/interval of application of acaricide on tick population

Weekly spraying of tick species had the most reduced total tick count in hot-humid rainy seasons. Fortnightly or bi-weekly spraying recorded a fewer ($P<0.01$) tick count, with a monthly and two months spraying having the worst counts. Ideally, chemical control of ticks is dependent on the life cycle of the arthropods. Applications of acaricide, at the normal interval in days for tick species that start and cease at the calendar time specified using local climatic information is the strategic control measure (Walker, 2011).

5.1.1.2.10 Interactions effect of fixed factors on total tick infestation

Significant interaction between breed and season, feed supplementation, type of housing, level of biosecurity practices (LBSP), and interval of applying acaricide observed is an

indication that tick load on breeds (Crossbred, Jersey, Sanga, e.t.c.) or breed performance against tick infestations are not equally influenced by the different environmental factors. Change in the relative performance of two or more genotypes measured in two or more environments would, therefore, aid in improvement and evaluation of farm animal genetic programmes (Annor, 2011); and development of cattle production objectives (Gebreyohannes *et al*., 2014) with swift tick prevention/control approaches. Wikel (2018) observed complex ecology, agents, and host interactions on ticks and tick-borne infections. Sonenshine, (2018) also reported on changes in responses to multiple factors that operate together to bring about the observed differences in tick abundance. Effective measures to check tick infestations might be focused on holistic management of cattle in a given environmental condition that minimizes or prevents the animals' exposure to the vector.

5.1.2 Prevalence of tick-borne diseases

5.1.2.1 Dermatophilosis

The prevalence of dermatophilosis was determined by demonstration of *Dermatophilus congolensis*' characteristic microscopic appearance, septate, and its branching filament that longitudinally as well as transversely become divided to form ribbons of spherical or ovoid cocci, each about 0.5µm in diameter, in multiple rows in transverse row of four or more (Gebreyohannes and Gebresselassie,2013). *Dermatophilus congolensis* characteristic pin point colonies surrounded by small zones are evident after 24 hour incubation at 37 ºC. Larger colonies form after 3-4 day incubation (Radostits *et al*., 2007). The colonies also displayed wrinkled or smooth, and convex, with varying colour from grayish white to bright orange, which is consistent with the finding of Dalis *et al*. (2010).

The 27.37 % prevalence of dermatophilosis observed in this study was higher than 14.20 % reported by Prasad *et al*. (2015). Dermatophilosis is more prevalent in animals maintained under rural household conditions (22.27 %) than intensive dairy farming system (1.92 %) where cattle are not exposed to thorn bushes (Prasad *et al*., 2015). The higher prevalence rate of dermatophilosis in this study is characteristic of the disease in hot humid environment. The prevalent rate is dependent on the intensity of rains in the humid environment. Survey of large number of cattle in Africa revealed prevalence rates approaching 15% with a 100 % infection rate in some herds at the time of peak seasonal prevalence (Radostits *et al*., 2007; Gebreyohannes and Gebresselassie, 2013). In the current study areas, well managed herds under zero grazing with insect proof housing had no tick-borne diseases.

Furthermore, the isolates of *D. congolensis* were molecularly confirmed using PCR by specifically amplifying 500 bp product of 16S rRNA gene of *D. congolensis,* which is consistent with the findings of Han *et al*. (2007) and Prasad *et al*. (2015). The prevalent rate at molecular level was 92.50 % (74/80) indicating a very high infection rate (Radostits *et al*. 2007) in carrier herds that was not detected at the conventional microscopic examinations. The 16S rRNA gene is a highly conserved region in most bacteria and it is of diagnostic significance (Han *et al*., 2007; Shaibu *et al*., 2010; Gebreyohannes and Gebresselassie, 2013). Though the technique was originally used in sheep, it has been successfully used in the amplification of the same segment from skin scabs of dermatophilosis infected cattle and nasal swabs in carrier herds in the current study.

5.1.2.2 Anaplasmosis

The Giemsa stained thin blood smears of 486 samples showed *Anaplasma marginale* organisms as dark or blue black dot at the margins of red blood cells as also indicated in Radostits *et al.* (2007). The current prevalence of anaplasmosis (21.40 %) observed by microscopical examination is comparable to 21.9% of the disease prevalence reported in Morocco (Hamou *et al.*, 2012) but higher than the overall mean prevalence of 4.07 % in cattle reported by Muhammad *et al*. (2014). Very high prevalence rate (66.66% and 75.92%), have been reported in Islamabad and Attock, respectively (Khan *et al*., 2004; 2016). Higher prevalence rate of anaplasmosis usually coincided with hot-humid rainy season when Boophilus and Amblyomma ticks were at the peak of infestation in the current study area. Similar findings have been reported by Khan *et al*. (2016) and Vetrivel *et al.* (2017). Nevertheless, the low level of rickettsiaemia exhibited in carrier cattle and the difficulty to differentiate the organism from other similar structures and staining artifacts renders it a challenge for the detection of persistently infected cattle by microscopy (Noaman and Shayan, 2010). This calls for a more accurate test of which molecular/PCR is among the best (Singh *et al.*, 2012).

An amplicon of 458 bp specific for Major surface protein 5 (MSP5) of *A. marginale* was detected in 33.75% of cattle surveyed. The PCR finding is higher than 21.40 % prevalence obtained in Giemsa stained thin blood smear investigation confirming the observation made by Noaman and Shayan (2010). Singh *et al.* (2012), in primary PCR, observed amplification of 458 bp specific for MSP5 in 45.20 % positive for *A. marginale* infection in studied animals. The MSP5 protein, for which it encodes, is of little structural complexity, equally conserved and it induces high antibody titres (Knowles *et al*., 1996), thus, it is a

strong candidate for bovine anaplasmosis diagnosis. Major surface protein 5, as comparable to MSP1 and MSP4, is present in the genome as a single copy gene which makes the gene an easy useful for diagnostic of bovine anaplasmosis by PCR (Corona *et al*., 2009). The MSP5 differs from MSP1β, MSP2 and MSP2 and MSP3 genes, which are present in multiple copies in *A. marginale* genome (McGuire *et al*., 1991). MSP5 is a good tool for molecular detection due to its high conservation in *A. marginale* species. The MSP5 is a highly conserved 19-kDa protein, which is encoded by a single-copy gene on the genome of *A. marginale.* It has been used in several detection studies utilizing different primer sets (Visser *et al.*, 1992; Torioni *et al.*, 1998). The current PCR finding is lower than 54 %, 45 % and 75 % prevalent rates reported in Guinea savannah, deciduous forest and coastal savannah zones of Ghana (Beckley *et al.*, 2016). The lower prevalence of *A. marginale* obtained in this study is due to management of cattle in the dairy setting that involve tick control measures which reduce the numbers of infective vector infestations in the range grazers. In addition, the zero grazing regimes prevent host-vector contact, with little or no ticks transmitting the pathogens in some farms in the study areas.

5.1.2.3 Heartwater

The 7.41 % prevalence of *Ehrlichia ruminantium* infection fell within the range of 6.25 to 39.30 % reported by Peter *et al*. (2000). The percentage of *Ehrlichia ruminantium* infected Amblyomma ticks at different sites ranges from 1.60 to 15.10 % depending on the site of sampling (Faburay *et al.*, 2007a). A study done in 2011 on 7 sites in south of Nigeria showed 9.60 % of *Ehrlichia ruminantium* tick prevalence (CABI, 2018).

In Burkina Faso, the *Ehrlichia ruminantium* prevalence in ticks by pCS20, nested PCR has been evaluated from 3 to 10 % depending on the year of tick samplings (Adakal *et al.*, 2010; CABI, 2018).

Molecular diagnostic tools allow a better estimation of the prevalence of heartwater than to detect both in organs from suspected dead ruminants and in ticks. In Burkina Faso, the *Ehrlichia ruminantium* prevalence in ticks by pCS20, nested PCR has been evaluated from 3 to 10 % depending on the year of tick samplings (Adakal *et al.*, 2010; CABI, 2018). Molecular techniques for *Ehrlichia ruminantium*; specific DNA, PCR and the probe pCS20 have proven to be useful screening techniques for identification of heartwater in both infected animals and ticks (Suliman, 2011). Gediyon and Teshale (2014) documented that assessment of *Ehrilichia ruminantum* within the ticks using PCR yielded 10 positive cases representing 8.3 % prevalence rate.

5.1.3 Factors influencing the prevalence of tick-borne diseases (TBDs)

This study assessed the various managerial and environmental factors or a combination of factors that can be used to reduce or prevent tick-borne diseases (TBDs) including dermatophilosis, anaplasmosis and heartwater or cowdrosis. Influence of farm, management regimes, feed supplementation, season, types of housing, and level of biosecurity practices were the factors considered.

5.1.3.1 Effect of farm and management practices on the prevalence of TBDs

The influence of farm practices on percentage prevalence of tick borne-diseases is an indication of the need for involvement of individual farms in the quest of combating

outbreaks of TBDs. Farms that maintained good health care, proper sanitation and screened cattle from getting exposed to tick vectors under zero grazing regimes experienced little or no outbreak of TBDs, and vice versa. This finding is consistent with the assertion that, dairy cattle that are zero grazed have minimal or no tick infestation and TBDs (Swai *et al*., 2006). Strategic and tactical management of ticks should include good housing, good barn management for zero-grazed animals, and separation of infested cattle from the healthy ones (Annan-Prah, 2011). The control and reduction of tick populations is possible by employing control programmes with integrated and consistent approaches (Kiss *et al*., 2012; Mondal *et al*., 2013) of which farm effect is a pivot. These should include sustainable farm management strategies and other environmental considerations (de la Fuente *et al*., 2015). Estrada-Pena and Salman (2013) noted that the poor management of farms, uncontrolled movements of domestic animals, and abundance of wild animals impede prevention of TBDs. The observation made in this study is that farmers' role in the control of ticks/vectors and pathogens of dairy cattle in Ghana can only be effective if it fits well with the day-to-day constraints on farmers, and with changes that farmers make in response to economic essentials of adopting intensive rearing of highly productive breeds (Walker, 2011).

The least prevalence rate of the tick-borne diseases (TBDs) recorded in the exclusive zero grazing management regimes is due to biosecurity practices that screen the cattle from getting into contact with the tick vectors transmitting the TBDs. High prevalence of the TBDs observed in increasing intensity of range grazing is consistent with the findings of Gitau *et al*. (1999) in Kenya. Therefore, grazing management that prevents host-vector exposure drastically reduces the prevalence of the TBDs. Similar findings by Rubaire-

Akiiki *et al*. (2006) indicates that zero-gazing in fertile land areas reduces the risk of all tick species on crossbred cattle in Uganda.

5.1.3.2 Effect of feed supplementation on the prevalence of TBDs

The reduced percentage prevalence of dairy cattle given regular feed supplementation could be attributed to the ability of good nutrition to replenish worn out tissues and maintenance of good health (McDonald *et al*., 2011). Though, most farmers who provided regular feed supplementation also observed good biosecurity practices which contributed to the reduced prevalence rate of TBDs, animals that are provided with adequate feeding through supplementation are well composed, hardier with respect to disease (Solcan, 2009) and resilience (Rodriguez, 2017). Poor nutrition produces a fall in haematological and biochemical indices in cattle (Radostits *et al*., 2007; Kaur *et al*., 2017) with accompanying reduction in antibodies that fight against parasitic infection. Poor feeding of cattle is a predisposing factor to TBDs (Radostits *et al*., 2007; Annan-Prah, 2011).

5.1.3.3 Effect of season on the prevalence of TBDs

Seasonal prevalence of dermatophilosis increased with the increasing rains resulting in the highest clinical conditions in the major rainy season (53.97 %), minor rainy (26.19 %) and dry (14.48 %) seasons in descending order. The observed prevalence rate in the dry season is comparable to 15 % reported but infection rate can be 100 % in some herds (Radostits *et al*., 1994). The disease has highest incidence and severity during the humid and high rainfall season (Gebreyohannes and Gebresselassie, 2013). The seasonal occurrence is associated with concomitant increase in tick and insect infestation (Radostits *et al*., 2007).

Prevalence of anaplasmosis and heartwater were highest in the minor rainy season, with major rains and dry seasons following in descending order. Reports on the lowest overall tick loads and TBD are observed during the cool-dry season while the highest occur during the hot-wet season (Marufu, 2008). Similarly, DeClercq *et al.* (2012) observed a significant increase in the abundance of tick population and TBDs with increasing rainy periods of the year under tropical conditions. Different ticks can act as a vector for heartwater pathogen, *Ehrlichia ruminantium* and hence understanding of the distribution of *E. ruminantium* in a given geographical location and season is an important prerequisite for effective control of cowdriosis (Kifle and Sori, 2014). This study noticed that the greatest tick abundance and TBDs occur in season of maximum moisture and growth of vegetation to shelter the questing ticks, which is consistent with the findings of Walker (2011). The percentage of *Ehrlichia ruminantium* infected Amblyomma ticks ranges from 1.6 to 15.1% depending on the season of sampling (Faburay *et al.*, 2007a; CABI, 2018).

5.1.3.4 Effect of types of housing on the prevalence of tick-borne diseases

Good housing facility that can provide a complete security to dairy cattle and prevent them from arthropod vectors is worth consideration. Dairy cattle reared in insect-proof barn had no prevalence of the TBDs. Open kraal recorded higher prevalence than roofed barn because of the poor nature of housing that subjected cattle to stress of standing all night during period of rains. Challenges associated with the sustainable management of the pure exotic breeds for effective production and efficient utilization of their products may be attributed to a number of factors, the principal ones being housing, disease incidence and shortage or unavailability of good quality feed year-round (Oppong-Anane, 1999);

Aboagye, 2002). According to Ngongoni *et al.* (2006) good housing, routine health checks and feed supplementation may account for higher performance at the commercial farms.

5.1.3.5 Effect of level of biosecurity practices on the prevalence of TBDs

Biosecurity constitutes a form of risk management in animal production operations. It highlights and reinforces recommendations applicable to the location, construction, establishments, operation of animal establishments, and prevention of dissemination of infectious disease agents in livestock/poultry and from live animal markets (OIE, 2017). Biosecurity measure/scheme is designed to prevent the spread of disease by minimizing the movement of biological organisms and their vectors (e.g. ticks) onto and within an operational area (Buhman *et al.*, 2007) coupled with supportive management regimes in a given environment.

Prevalence rate of Tick-borne diseases (TBDs) reduced with increasing levels of biosecurity practices. Indeed, there was no outbreak of any of the TBDs in farms with moderately practised biosecurity measures. This indicates that biosecurity is one the most reliable and effective means of disease control measures available and no disease prevention programme works without it (Buhman *et al*., 2007). In this study, farmers who observed low and very low biosecurity measures experienced high prevalence of TBDs. Therefore, observation of biosecurity's components, viz: traffic control, isolation and sanitation (Mathis and Hagevoort, 2010), together with good farm management, could curb TBDs menace in dairying in Ghana.

5.2. Haemato-biochemical Indices of Dairy Cows

5.2.1 Haematology of dairy cows: Effect of breed, physiological state and feed supplementation

5.2.1.1 Effect of breed on haematological indices

5.2.1.1.1 Effect of breed on erythrocyte indices

Significantly highest mean RBC indices in Sanga over Jersey and crossbreds are characteristic of beef or dual-purpose cattle (Tornquist and Rigas, 2010). The differences in mean erythrocyte indices of the breeds are consistent with the report made by Aggarwal *et al*., (2016) that haemoglobin and HCT/PCV differed among Tharparkar, Sahiwal, Karan Fries and Murrah cattle breed. The determination of haemato-biochemical parameters in different bovine breeds allows the physiological status interpretation in order to characterize the productive future of these individuals (Prisacaru, 2014), stress and welfare levels (Radkowska and Herbut, 2014).

5.2.1.1.2 Effect of breed on leucocytes

The observed differences in concentrations of leucocyte indices among Sanga, Friesian-Sanga crossbred and Jersey cow were within the healthy reference ranges and, therefore, may not be a challenge to the cows' health. Nevertheless, cattle infected with flukes showed no increase in basophil numbers (Conboyand Stromberg, 1991). In this study, neutrophil, lymphocytes, monocytes and eosinophil values (%) were significantly modified by breed. This implies that effect of breed should be considered when evaluating haematological parameters for health assessment.

5.2.1.2 Effect of physiological state on haematological indices

5.2.1.2.1 Effect of physiological state on erythrocyte indices

Erythrocyte indices such as MCH and MCHC have been reported to increase in cyclic cows as compared to non-cyclic heifer (Ijaz *et al.,* 2003). In this study, HCT was higher in heifer and cycling dry cows than those observed in gestation periods, though, the values fall within the reference range of Radostits *et al.* (1994). Haematocrit value of < 24 % is considered as anaemic whereas ≥ 24 % is considered as normal as described by Kessell (2015). This is an indication that pregnant cows in the study area with HCT values of < 24 % could be anaemic and require nutritional intervention to forestall the anaemia. Haematocrit/packed cell volume is involved in the transport of oxygen and absorbed nutrients (Isaac *et al*., 2013). Increased HCT shows a better transportation of blood and thus results in an increased primary and secondary polycythemia. A low haematocrit with a low MCV and with a high RDW suggests a chronic-iron-deficient anaemia resulting in abnormal haemoglobin synthesis during erythropoiesis (Etim *et al*., 2013). Low hematocrit requires increased production of red blood cells, so dietary modifications may include increased protein and iron. Also, Elevated glucose levels cause RBCs to swell and may cause a falsely elevated hematocrit (Lockwood, 2015).

The observed increase in MCV, MCH, RDW, and PLT in cycling cow than in non-cycling heifers, though within the ranges reported by Roland *et al*. (2014), has also been noticed by Ijaz *et al.* (2003). Physiologically, higher RBC, haematocrit and haemoglobin levels can play a role in better physical activity and some parasitic diseases (Meliani *et al.*, 2015).

Also, the elevated erythrocytes indices during gestation have been observed to be due to maternal adaptation to pregnancy in order to meet the requirements of a growing foetus. Foetal growth results in greater oxygen demands. This is consistent with findings of Kopp and Hetesa (2000) and Chineke *et al*. (2006). The greater need for oxygen is compensated by the endocrine system that stimulates the release of erythropoietin by renal tissue (Patel *et al*., 2016). In the present study, differences in RBC, HGB, MPV, PDW and PCT were insignificant with respect to physiological state.

5.2.1.2.2 Effect of physiological state on leucocyte indices

The observed differences in white blood cells (WBC), the differentials including neutrophils, lymphocytes, eosinophils and basophils were insignificant in non-cycling heifer and CC. This means that heifer and CC had little or no effect on leucocyte indices. Non-cycling heifer, CC, G_E, G_M, and G_L also had little effect on percentage monocytes

The higher level of WBC, neutrophils, lymphocytes, eosinophils and basophils in G_E might be attributed to stress and changes in hormonal levels in G_E. Nevertheless, with the exception of WBC, all leucocyte indices fell within the reference ranges of Kalaitzakis *et al*. (2011) and Roland *et al.* (2014) which is an indication of physiological adaptation and not as a result severe infection (Tornquist and Rigas, 2010; Reece *et al*., 2015). Higher than normal range (1 – 6 %, Etim et al., 2014) of monocytes level indicates stress among the cows in gestational stages, though monocytes in circulation were insignificant across the physiological states. Lymphocytes and eosinophil levels fell within reference ranges of Roland *et al.* (2014) and, therefore, might not be attributed to healing phase of infectious

diseases, during chronic antigenic stimulation due to infectious agents, neoplasia, and hypoadrenocorticism as in lymphocytosis (Fowler, 1998; Keller *et al*., 2006).

Eosinopenia may be a component of a stress response in ruminants (Tornquist and Rigas, 2010). In addition, the significant basophilia observed in this study may be due to allergy resulting from hormonal changes in GE or allergic reaction due to tick infestations (Tornquist and Rigas, 2010).

5.2.1.3 Effect of feed supplementation on haematology of dairy cattle

5.2.1.3.1 Effect of feed supplementation on erythrocyte indices

Provision of feed supplementation to dairy cow resulted in improvement of haematocrit (HCT), MCV, MCHC, RCD CV and platelet indices within the normal reference ranges reported by Radostits *et al*. (2007), Tornquist and Rigas (2010) and Reece (2015a). The improved normal levels of these erythrocyte indices may be attributed to the ability of feed supplement to elicit significant improvement in blood metabolites that enhanced good health. Normal physiological levels of HCT in dairy cows that had feed supplementation indicate dietary supply of protein and iron. The low level of HCT in dairy cows which had no feed supplementation means that those cows were anaemic and might result in or influence haemo-concentration. A low haematocrit with a low MCV and with a high RDW CV suggests a chronic-iron-deficient anaemia resulting in abnormal haemoglobin synthesis during erythropoiesis (Etim *et al*., 2013). In anaemic cows, nutritional intervention may be required to correct the deficiencies since malnutrition is one of the most important contributing factors determining the thresholds and width of reference intervals of haematology (Wood and Quiroz-Rocha, 2010; Krimer, 2011). In addition, inadequate diet

might predispose dairy cows to stress up, poor body condition, and negative energy balance leading to inefficient reproductive and productive performances.

Provision of feed supplementation had little influence on RBC, HGB, MCH, PWD and PCT levels. The PWD levels were, however, lower than the reference range of 56.0-80.0 % reported by Radostits *et al*. (2007) and Constable *et al.* (2017). The PCT levels fell within the normal range (Constable *et al.*, 2017) in cows supplemented with feed but were higher than normal reference range in cows given occasional or no feed supplement. The PDW and MPV increase in sepsis with appearance of large and heavy platelets in circulation (Golwala *et al*., 2016).

5.2.1.3.2 Effect of feed supplementation on leucocyte indices

The higher granulocyte levels, especially, neutrophil and eosinophil components that fell within the normal physiological range (Tornquist and Rigas, 2010; Reece, 2015a) were observed in cows supplemented with feed than those that had no feed supplement. This is an indication of enhanced immune system. The lower levels of lymphocytes, monocytes and basophils within the normal reference range (Constable *et al*., 2017) of the cows supplemented with feed means that the cows are in good health. It has been reported that good nutrition boost up the immune system and resilience (Solcan, 2009; McDonald *et al.*, 2011). Higher levels of MON and BAS in cows that had no feed supplement are normally found in stressed animals or infections. Basophilia has been reported in cattle with tick infestations (Tornquist and Rigas, 2010).

5.2.2 Serum biochemical indices of dairy cows

5.2.2.1 Effect of breed on serum biochemical indices

5.2.2.1.1 Effect of breed on serum hepatic enzymes

The mean serum concentrations of hepatic enzymes including ALT, AST, and γGT were slightly influenced by breed. The mean serum values of the hepatic enzymes also fell within normal reference range of 35 – 126 u/L reported by Radostits *et al.* (2007). The significant disparities observed in mean serum ALP concentrations may be attributed to the differences among breeds and physiologic conditions of individual cows other than health implications. Differences in serum ALP have also been reported in Tharparkar cattle and Sahiwal breed in India (Aggarwal *et al*., 2016). An increase in ALP level may quarry the possibility of membrane damage, because ALP is a membrane bound enzyme (Rao, 2006; Ruothalo, 2008). High levels of serum ALP activity is usually observed in liver damage, cancer and heart infections (Jaroslaw *et al*., 2009). A decrease in serum ALP may be an indication of the healthier state of the plasma membranes (Adeyemi *et al.*, 2015).

5.2.2.1.2 Effect of breed on serum metabolites

The differences in blood biochemical indices, especially total protein and cholesterol, with respect to breed, have also been reported (Prisacaru, 2014). Serum protein indices give information about kidney damage, liver damage, and nutritional health (Stojević *et al*., 2005). The mean variations in total protein and globulin among breeds fall within the normal ranges, which indicate a good health. Also the elevated concentration of urea/BUN in Jersey cows could be assigned to the supplementation of brewers' spent grain/malt which is high in protein. Otherwise the increase could have been instigated by renal disease/failure; dehydration due to low fluid intake; decreased renal perfusion due to heart

failure, hypovolemic shock, and severe hypotension; gastrointestinal bleeding; ageing; and catabolic state, such as trauma, severe infection and starvation (Higgins, 2016), conditions which were not indicated by assessment of other metabolites and enzymes in this study.

Although serum cholesterol components significantly differed among the breeds, mean levels were within the normal reference range. This is an indication of absence of secondary endocrine, hepatic or renal diseases. It is, moreover, worth noting that, disorders of cholesterol metabolism are rare in domestic animals (Evans, 2011). There was also absence of ketonaemia among the breeds since the observed significant differences in serum β-HBA concentration were within normal range. Breed had slight or no influence on mean creatinine, triglycerol, glucose, and NEFA, hence, the breed cannot be fully accounted for by the observed differences alone.

5.2.2.1.3 Effect of breed on serum electrolytes

The significant influence of breed on mean serum chlorine, potassium, and phosphorus ions/cations concentrations is as a result of breed differences, though the observed variations fell within the normal reference ranges (Radostits *et al*., 1994). Normal serum mineral concentrations in cows mean that there would be less mineral deficiencies and less concern about metabolic disorders from these sources. Breed however, had little or no effect on Na^{+}, Ca^{2+}, HCO_3^{-}, and Mg^{3+}.

5.2.2.2 Effect of physiological state on serum biochemical indices

5.2.2.2.1 Effect of physiological state on serum hepatic enzymes

Although physiological state had significant influence on serum ALT, AST, and γGT, the values were within normal reference ranges of Meyer *et al*. (1992) and Radostits *et al*. (2007). The normal serum levels of ALT of cows of varied physiological states indicate that the cows are free from acute liver damage, whereas normal level of AST means that the animals are devoid of damaged liver cells (Nelson and Cox, 2003), infiltration mass and degeneration of hepatocytes (fatty liver) (Meyer and Harvey, 2004; Lubojacka *et al*., 2005). In this study, γGT levels were normal across physiological states except in G_M where the value was more than normal. The elevated level of γGT might be an indication of impending poor liver function when it is associated with elevated AST. Serum γGT is an essential indicator of hepatic lesions and function (Stojević *et al*., 2005) and cows with severe fatty liver have higher levels of γGT and AST (Sevinc *et al*., 2001). Elevated serum ALP in non-cycling heifer might be attributed to the enzyme function in bone growth and organs involved in active transport mechanism (Ophardt, 2003).

5.2.2.2.2 Effect of physiological state on serum metabolites

Protein profile

The observed significant differences in total protein concentration across the physiological states fell within the normal reference range of 66 – 78 g/L (Radostits *et al*., 1994). Total protein gives information about kidney damage, liver damage, and nutritional health (Stojević *et al*., 2005). Nevertheless, normal serum total protein level is an indication of no dehydration (Radostits *et al*., 1994), healthy liver and good health. The significant differences in the protein profile noticed in G_L and other physiological states might be due

to the changes in physiological condition and demand of foetus on dam triggering lower protein profile in G_L, although it was within the normal reference range (Garcia *et al*., 2017). Similar findings have been reported by Zvorc *et al*. (2000) and Yaqub *et al*. (2013).

Decreasing albumin concentration can reflect hepatic insufficiency (Whitaker, 2000). Hypoalbuminaemia is more common in chronic liver disorders such as cirrhosis and usually reflects severe liver damage and decrease albumin synthesis (Fauci *et al*., 2008). The normal levels of albumin observed in this study means less health concerns. Globulin increased in response to an inflammatory process (Kaneko, 2008; Fauci *et al.*, 2008). The increased levels of globulin in heifers, CC, G_E, and G_M may be due to synthesis of antibodies against secondary infections as it has also been indicated by Fauci *et al*. (2008).

Urea/BUN

The serum urea concentration across the physiological state fell within the range of 2.0 – 8.0 mmol/L (Radostits *et al*., 1994). Concentrations of urea have been noted to have effect on reproductive disorders including repeat breeding. Repeat breeding syndrome is a failure cows to conceive from three or more routine services without detectable abnormalities, leading to large economic loss due to unsuccessful inseminations, increased calving interval and culling rates (Gustafsson and Emanuelson, 2002). Urea helps us understand the mechanisms impairing fertility in repeated breeder cows (Mimoune *et al.*, 2017). Heifers fed with high nitrogen supplement usually have highest BUN levels and also have lowest pregnancy rate (Tshuma, 2013). Decreased serum urea concentration of <2.50 mmol/L or BUN of <2.50 mmol/L (<7.0 mg/dL) may be associated with pregnancy, low-protein diet,

overhydration, advanced liver disease (cirrhosis, liver failure), and inherited defect in "urea cycle" enzymes (reduced urea synthesis) (Higgins, 2016).

The observed normal serum urea concentration was an indication that the cows of different physiological states were devoid of dehydration and disturbances of acid-base balance. Animal experiencing diarrhoea can have twice as high mean plasma/serum urea concentration as compared to healthy ones of similar age (Klinkon and Ježek, 2012).

Analytic index for assessing the protein profile is blood urea nitrogen (BUN). It is a good indicator of the energy intake of the cow, and in particular, it is used as an indication of the synchronization between fermentable carbohydrates and rumen degradable protein (RDP) (Van Saun, 2010). In this study, the mean levels of urea and BUN, except in GE and GM, were normal, therefore, cows were free from factors that induce decreased/elevated serum urea or BUN concentration. Decreased BUN level might be associated with pregnancy, low-protein diet, over-hydration, advanced liver disease as seen in cirrhosis, or liver failure, and reduced urea synthesis due to inherited defect in urea cycle enzymes, whereas elevated BUN might be triggered by renal disease/failure, high-protein diet, dehydration due to low fluid intake, excessive fluid loss as evident in diarrhoea and diuretic drugs (Higgins, 2016).

Bilirubin

The total bilirubin concentration observed fell into the normal ranges of 1.71 – 8.55 µmol/L (Meyer *et al.*, 1992) and 0.00 – 32.00 µmol/L (Radostits *et al*., 1994). Direct and indirect bilirubin also fell within the reported normal reference ranges of 0.66 – 2.40 and 0 – 5.10 µmol/L, respectively (Meyer *et al.*, 1992).

Hyperbilirubinaemia due to increase in DBIL almost always implies liver or biliary tract disease. In such condition, both direct and indirect fractions of the bilirubin tend to be elevated (Fauci *et al*., 2008). The increment in total and indirect bilirubin may be related to fat infiltration of the liver (Sevinc *et al.,* 2002). Isolated unconjugated hyperbilirubinaemia (bilirubin elevated but <15 % direct) should prompt a workup (assessment) for haemolysis (Fauci *et al*., 2008). In this study, the differences in serum bilirubin concentrations fell within the normal ranges. This is an indication for little concern about liver or biliary tract infections and/or fat infiltration of the liver.

Creatinine concentration significantly differed with the varied physiological states, in that, heifer had the highest. Mohri *et al.* (2007) also observed that serum creatinine levels decreased with age. Creatinine is excreted with urine; its concentration in serum does not depend on the nutrition. Diagnostically, it is important for the assessment of functioning of the glomerular system in the kidneys, but its concentration increases only during serious damage (Klinkon and Ježek, 2012).

Triglyceride, Cholesterol and lipoproteins

Physiological state had little influence on mean serum triglyceride concentration in the present study. Significant variations in mean total cholesterol levels across the physiological states all fell within the normal reference range of 2.07 to 4.66 mmol/L (80 – 180 mg/dL) as reported by Reece *et al*. (2015). Increased serum cholesterol concentrations reflect increased concentration of the cholesterol rich lipoproteins such as LDL, and HDL. Increase in these lipoproteins commonly occur secondary to endocrine, hepatic, or renal

diseases (Evans, 2011). Normal levels of cholesterol and LDL mean low risks of atherosclerosis, and enhanced cholesterol metabolism. In this study, VLDLs component was insignificant with respect to the physiological statuses considered. Nevertheless, the observed levels of VLDL in CC, cows of G_E, G_M and G_L were higher than the reported values observed in mild to severe fatty liver (Sevinc *et al*., 2001). However, heifers had low VLDL level as seen in fatty liver. This could be attributed to the VLDL transformation to LDL loosing its triglycerides to different organs/hepatic tissues or reduced synthesis of VLDL which is associated with feeding (Sevinc *et al*., 2001).

Ketone/β-hydroxybutyate (β-HBA)

Although serum β-hydroxybutyate concentrations were similar among the different physiological states, the values observed in G_E and G_M were higher than the normal healthy limit of <1.2 mmol/L (Seifi *et al*., 2011; McArt *et al*., 2013), hence implicated in subclinical ketoses. The β-HBA is used as early marker for detection of ketosis in ruminant (Duffield, 2004; Oetzel, 2007). Ketosis can be predisposed by disturbance of the reproductive performance and early embryonic death (López-Gatius *et al*., 2002). A holistic approach to improve on appetite, body condition and reduce weight loss of cows in G_E and G_M could curb further escalation of ketonaemia. Provision of poor nutrition during third trimester of gestation and early lactation predispose cows to ketosis.

Glucose

Mean serum glucose concentrations were within normal ranges of 1.9 – 3.9 mmol/L (Radostits *et al*., 1994) and 2.2 - 5.6 mmol/L (Merck Manual, 2012) though the values were similar across the different physiological state in this study. Increase in the blood glucose concentration may be due to the gluconeogenesis and the glycogenolysis (Szablewski,

2011), which may be stimulated by glucocorticoids and cathecolamins during stressful condition or parturition (Fonseca *et al*., 2004).

Nonesterified fatty acid

Significant elevation in nonesterified fatty acid concentration in cows of GL over the normal range as compared with those observed in heifer, CC, and the first two trimesters of gestation might be due to fat mobilization. According to Oetzel (2004), higher concentration of more than 0.40 mmol/L indicates negative energy balance and an increased lipid mobilization. In the third trimester of pregnancy, there is an exponential development of the foetus that relies on the dam's nutrition and body reserves (Mao *et al.*, 2008). It has been reported that plasma NEFA dramatically increase more than a two-fold between 2 to 3 weeks prepartum and 2 to 3 days prepartum (Vazquez-Añon *et al*., 1994; Bačić *et al.*, 2007). The use of NEFA is a better indicator of NEB compared to β-HBA, although β-HBA is a more useful indicator during postpartum (Garcia *et al*., 2017). Normal NEFA concentration in a healthy cow of positive energy balance is usually around 0.25 mM which is characterized by low lipid mobilization (Fonseca *et al*., 2004).

5.2.2.2.3 Effect of physiological state on serum electrolytes

The physiological states of cows considered in the present study had little or no effect on serum chloride, calcium sodium and magnesium concentrations. These cations and ion fell within the normal reference ranges (Radostits *et al.*, 1994; 2007). Although significant differences were observed with respect to serum potassium concentration, the levels fell within the normal range of 3.9 – 5.8 which is a good indication of no hypokalaemia or hyperkalaemia or metabolic alkalosis. The significant levels of bicarbonate ions across the physiological conditions also fell within the normal reference range of healthy cows (20.0 –

30.0 mmol/L) meaning an absence of ionic gap to trigger an impending acidosis or alkalosis (Reece *et al*., 2015). Mean serum phosphorus levels were also within the reference range (Latimer, 2011), though concentrations significantly differed in such a way that GL had the lowest P concentration. Pal and Bhatta, (2013) also noticed that the last trimester of pregnancy had serum P lower (4.03±0.18 mg/dL) than the values for normal healthy dairy cattle.

5.2.2.3 Effect of feed supplementation on serum biochemistry

5.2.2.3.1 Serum hepatic enzymes

The mean serum concentrations of hepatic enzymes, AST, γGT and ALP, were significantly influenced by increasing levels of feed supplementation. Though, the mean serum values of the hepatic enzymes fell within normal reference range of 35 – 126 u/L reported by Constable *et al.* (2017). The normal concentrations of these enzymes are indication of good health and nutrition. The lower levels of hepatic enzymes in cows that had no feed supplementation might be attributed to inadequate feeding that predisposed the cows to protein, energy and vitamin deficiencies (Merck Manual, 2011). The correct concentrations are required for body building and enzymes. Feed supplements contain varying amounts of the essential nutrients required by grazing animals (Hinton, 2007).

5.2.2.3.2 Effect of feed supplementation on serum metabolites

The significant increase in serum protein concentration within physiological range with increasing intensity of feed supplementation can be attributed to improved microbial protein synthesis enhanced by additional nitrogen obtained from feed supplements. Nutrient imbalances in grazed herbages are corrected by giving supplements that are

balanced in energy and protein to correct the nutritional deficits (McDonald *et al*., 2011). The normal serum protein concentration observed in the current study indicates that the dairy cows were devoid of dehydration (Radostits *et al*., 2007), had healthy liver, good health (Garcia *et al*., 2017), and were not malnourished (Elshahawy and Abdullaziz, 2017; Obese *et al*., 2018). The slightly higher levels of serum albumin and globulin in the supplemented cows than those having no feed supplementation were insignificant.

Increase in total and indirect bilirubin concentrations with increasing intensity of feed supplementation fell within the normal physiological range (Constance *et al.*, 2017). This implies that supplemented cows were free from liver or biliary tract defects/diseases or fat infiltration of the liver.

Blood urea nitrogen (BUN) is one of the analytic indices for assessing protein profile (Higgins, 2016). It is a good indicator of the energy intake of the cow, thus, as an indication of the synchronization between fermentable carbohydrates and rumen degradable protein (RDP) (Van Saun, 2010). In the present study, cows supplemented with feed had higher BUN concentration than those that did not have feed supplementation. The supplemented cows had higher BUN levels than the reported range of 3.6-8.9 mmol/L (Merck Manual, 2012). This might be due to high protein levels in the supplemented feed. High concentration of BUN is usually triggered by renal failure, high-protein diet, and dehydration due to low fluid intake, excessive fluid loss (Higgins, 2016). The effect of feed supplementation on serum urea concentration was not significant in this study.

The insignificant difference in serum creatinine concentrations in dairy cows with respect to varied intensity of feed supplementation corroborates with the findings of Mohri *et al.* (2007) who indicated that creatinine concentration in serum does not depend on nutrition. It is worth noting that, elevated creatinine concentration beyond the physiological range is observed during serious damage of kidneys (Klinkon and Ježek, 2012).

The lower concentrations of triglyceride, total cholesterol, and VLDL in regularly supplemented cows than those that had occasional or no feed supplementation might be due to lower level of fat in the supplements. The VLDL is made up of 92% lipid and 8% protein (Seeley *et al*., 2004) This is also evident in the significantly higher concentrations of HDL—which composes of 30 % phospholipid, 5 % triglyceride, 20 % cholesterol, and 45 % protein (Seeley *et al*., 2004) in regularly supplemented cows. The LDL, comprising of 75% lipid and 25% protein, was not significantly influenced by feed supplementation.

The serum ketone (β-HBA) level is used as early marker for detection of ketosis in ruminant (Duffield, 2004; Oetzel, 2007) usually as a result of glucose insufficiency. However, feed supplementation had little influence on serum β-HBA concentration, probably due to the ability of gazing cows to obtain the basal nutrients from the range grazing. Glucose concentration markedly increased with increasing feed supplementation but the observed values fell within the normal physiological range of 2.2-5.6 mmol/L (Merck Manual, 2012).

Significant differences in nonesterified fatty acid (NEFA) concentrations were observed in cows supplemented with feed and those that were not given supplementation. Normal

concentrations of NEFA below the reported threshold of < 0.4 mmol/L (Oetzel, 2004) were recorded in regularly or partially supplemented cows while elevated concentration was recorded in cows that had no feed supplementation. Normal NEFA concentration is an indication of a healthy cow of positive energy balance whereas higher level of NEFA above the threshold is characterized by fat/lipid mobilization (Fonseca *et al*., 2004). The NEFA concentration is a better indicator of NEB compared to β-HBA (Garcia *et al*., 2017).

5.2.2.3.3 Effect of feed supplementation on serum electrolytes

The importance of nutrition in enhancement of body fluid electrolyte balance cannot be underestimated (Anderson and Rings, 2009; McDonald *et al*., 2011; Constable *et al*., 2017). The significant differences in serum potassium and magnesium levels with respect to different intensity of feed supplementation, however, fell within the normal range of 3.9-5.8 mmol/L and 0.6-1.2 mmol/L respectively for healthy cows (Reece *et al*., 2015). This reflects an absence of ionic gap to trigger an impending acidosis or alkalosis as well as hypomagnesaemia or hypermagnesaemia (Reece *et al*., 2015). Feed supplements provide essential nutrients required by grazing livestock (Hinton, 2007). However, effect of feed supplementation had slight influence on serum sodium, chloride, calcium, HCO3 and phosphorus concentrations, although the levels fell within the reference ranges reported by Radostits *et al.* (2007), Merck Manual, (2012) and Reece *et al*. (2015).

5.3. Reproductive Hormones Profiles of Dairy Cow Using Commercial EIA

5.3.1 Progesterone profile during prepubertal stage to conception in crossbred, Sanga and Jersey heifers

The progesterone concentrations in serum of crossbred, Sanga and Jersey heifers during prepubertal stages (day 45 to the onset of puberty) were below 1 ng/mL is consistent with the findings of Ball and Petters (2004). Cows with lower P_4 levels of < 1 ng/mL is an indication of prepubertal anoestrus (Akers and Denbow, 2013). The first rise in progesterone levels ≥ 1 ng/mL observed in 285, 295 and 415 days (≈ 9.5, 9.8 and 13 months) for Jersey, Friesian-Sanga crossbred and Sanga cows marked impending full pubertal cyclicity as it has also been reported by Prakash *et al.* (1988). The P_4 rise also indicates the age at which heifers can become pregnant after experiencing oestrus and support pregnancy without health concerns (Akers and Denbow, 2013). The differences in first rise in P_4 concentrations among the breed may be attributed to variation in the genotype of the heifers considered in the study. Noakes *et al.* (2001) noticed that the range of age at puberty in cow is between 7 – 18 months (≈ 210 – 540 days). The onset of puberty for crosses between *Bos indicus* and *Bos taurus* ranges from 14.5 months in ¾ *Bos taurus* cross in India to 26 months in Ankole and Jersey cross in Ruwanda. Cunningham and Klein (2007) observed that the range in cow is 8 to 12 months in *Bos indicus*. The early attainment of puberty (9.5 – 13.0 months, ≈ 285 – 390 days) by heifers in the current study and those in literature were due to feed supplementation provided for the heifers. According to Noakes *et al*. (2001), prepubertal development and the onset of puberty might be determined by quality and quantity of feed resource availability for good feed management.

The observed dates of conceptions in Jersey, Crossbred and Sanga heifers determined by P_4 EIA differed among the breeds in ascending order which explain the variation in breed, and management regimes. Regular feed supplementation given to the heifers might have contributed to early rise in P_4 which in turn corresponded to the age of conception. Major factors influencing first conception in heifer also contribute to variations in age at first calving. These factors include breeds, nutrition, and management of animals in a given environment (Obese *et al*., 1999; Aboagye, 2002; Obese *et al*., 2008). Early attainment of puberty with subsequent reduced age at first conception/parturition is an economic trait that determines cost of rearing heifer to productive stage, and generation interval (Wakchaure and Meena, 2010).

Increased in P_4 levels 10 – 15 days accompanied behavioural oestrus in Jersey and Sanga which recur after 20 days. Onset of ovarian cyclicity is usually considered as two consecutive samples with concentrations of progesterone ≥ 1.5 mg/ml (Martins *et al.*, 2012).

5.3.2 Effect of feed supplementation on P_4 concentrations heifers during pre-pubertal stage to conception

The early onset of puberty observed in heifers provided with regular feed supplementation justifies the positive influence of nutrition on body reserves that enhance cyclical pulsatile of reproductive hormones. The first rise in P4 levels of ≥ 1.00 ng/mL that marked impending full pubertal cyclicity led to overt oestrus in 310, 335 and 420 days in heifers that had regular, occasional/partial and no feed supplementation respectively. This confirms findings of Noakes *et al*., (2001) that, the onset of puberty is determined by

quality and quantity of feed resource availability. It has also been observed that reproductive hormone production depends on the energy status of animals (Ibtisham *et al*., 2018). Leptin, produced by adipose tissue, is essential for attainment of puberty or reproductive functionality, and is positively correlated with body condition in ruminants (Bova *et al.*, 2014).

Delay in attainment of oestrus in heifers that had no feed supplementation might be due to inadequate body reserved to stimulate the activity of hypothalamus to trigger gonadotrophic hormones to initiate follicular growth. Plasma progesterone concentrations are also affected by the energy balance of dairy cows. Nutritional inadequacy may lead to several disorders such as poor body condition, ketosis, and hypocalcaemia (Anderson and Rings, 2009; Constable *et al*., 2017) that delay reproductive performance. It is also worth noting that heifers on feed supplementation should to be managed in a order to avoid overweighing

Heifers that had regular feed supplementation recorded the highest concentration of progesterone, followed by partial and no feed supplementation in descending order during the onset of puberty, and in conception. This corroborates with finding of Wathes *et al*. (2007) that reproduction and secretion of hormones in ruminants are closely associated with the availability of energy status of animals.

5.3.3 Oestradiol and progesterone concentrations in cows during oestrous cycles

Oestradiol and progesterone concentrations in serum of the Sanga, crossbred and Jersey cows showed a similar trend during oestrous cycle, as reported by Naik *et al*. (2013) and

Domenech *et al.* (2011). However, hormonal concentrations differed among the breed which might be attributed to genotype and individuality of individual cows. Oestradiol concentrations observed in this study were comparable to values observed by Domenech *et al.* (2011) but higher than those reported by Gupta *et al*, (1998), in oestrus cows, Shukla *et al.* (2000) in crossbred cows and Naik *et al.* (2013) in Punganur cows. The differences in oestradiol concentrations observed might be due to the variations in EIA standards in part and assay differences. The standard used for this study ranged from 0.0 to 1000.0 ng/mL, hence, reflecting the observed oestradiol concentration.

On day zero (0) to day four (4) of oestrous cycle, oestradiol concentrations in serum of the indigenous cows were relatively low along the threshold continuum which may result from ovulation and post ovulatory development of luteal tissues. This is triggered by LH whose concentration is regulated by oestrogen (Reece *et al.*, 2015). Day 5 to day 14 showed pulsatile concentrations of oestradiol in similar trend in all the breeds of cows which can be explained by first and second waves of follicular growth under the influence of FSH which give rise to dominant follicle to secrete oestradiol (Noakes *et al*., 2001; Hafez, 2008). Day 15 onwards marked increasing levels of oestradiol concentrations that peaked between day 17 and 20 depending on the breed. Similar trend in oestradiol concentration has been reported by Naik *et al.*, (2013) in Punganur cows. The preovulatory developing follicle is accounted for by the highest concentration of oestrogen (Ball and Petters, 2004). It was observed that peak oestradiol concentrations among the breeds preceded standing oestrus between 18 – 36 hours which might be following LH surge (Akers and Denbow, 2013). Oestrogen enhances the initiation of sexual receptivity whereas LH facilitates ovulation (Reece *et al.*, 2015).

Result obtained on serum P_4 concentrations during oestrous cycle with commercial EIA is comparable to findings reported by Rao *et al*. (2005), Singh *et al*. (2006) and Naik *et al.* (2013), but it was lower than values reported by Rabiee *et al*. (2002) and Hamit *et al*. (2005) on day 0 to 1 in oestrous cycle. The P_4 concentrations were higher than those recorded by Shukla *et al*. (2000), Mehrotra *et al*. (2005) and Venkatesan *et al.* (2005). These disparities in hormonal concentrations were due to differences in assay standards, sampling, and physiological state of cows (Alvarez *et al.*, 2000; Naik *et al.,* 2013).

Progesterone concentrations among the breeds during oestrous cycles were similar. Progesterone concentration was almost always the lowest during oestrus at day 0 and gradually assumed an increase below 1 ng/mL up to day 3 or 4 after which P_4 significantly increased through day five and reach its maximum concentrations at day 10 to day 12. This trend in P4 concentration have been previously demonstrated in cows during oestrous cycle (Noakes *et al.*, 2001; Ball and Petters, 2004; Naik *et al*., 2013; Reece *et al*., 2015). In the current study, progesterone concentrations sharply declined after day 17 and reached its minimal threshold between days 19 and 25 within which standing oestrus occurs. Increase in progesterone levels after day 4 for 12 to 15 days might be as a result of development of corpus luteum which spikes increasing secretion of P_4 during the luteal phase of the oestrous cycles (Noakes *et al.*, 2001; Hafez *et al*., 2008). LH facilitates ovulation whiles enhancing luteinization of luteal tissues leading to the formation of corpus luteum (Reece *et al.*, 2015). The sharp decrease in P_4 concentrations after day 17 is due to luteolysis (Ball and Petters, 2004;) which is induced by 5-keto-13, 14-dehydro_$PGF_{2\alpha}$ synthesis (by uterus) and pulsatile with 4 to 5 pulses within 24 hours occurring at 6 hours interval to effect

complete luteolysis—regression of CL (Cunningham and Klein, 2007). At this time inflammatory condition of the endometrium caused by bacteria can result in significant synthesis and secretion of $PGF_{2\alpha}$ leading to premature luteolysis and shortening of the oestrous cycle (Cunningham and Klein, 2007).

5.3.4 Progesterone and oestradiol levels in dairy cows during gestational stages

Serum P_4 levels in the current study were higher than values observed by Mekonnin *et al*. (2017). Significant increase in P_4 concentrations from the first to second trimester was due to enhanced production of progesterone by CL induced by specific embryonic protein called *trophoblastin* produced by the developing embryo (day 14 of pregnancy in cows) that led to the maternal establishment of pregnancy. This modifies uterine $PGF_{2\alpha}$ synthesis leading to absence of the pulsatile secretion of $PGF_{2\alpha}$ hence extension of the life span of the CL and the establishment of pregnancy (Cunningham and Klein, 2007). The CL of pregnancy is needed throughout the pregnancy period in cow (Reece *et al*., 2015), and therefore, remains at its maximum size throughout the whole of the period of gestation. The main source of progesterone for the maintenance of pregnancy in the cow is the CL with the placenta producing only small amounts (Noakes *et al.*, 2001). It has been observed in literature that up to about 200 days of gestation, removal of the ovary containing the CL or ablation of the CL either surgically or with the use of $PGF_{2\alpha}$, usually results in abortion (Noakes *et al.*, 2001). Second and third trimester P_4 levels were similar whereas the last 10 days prepartum had greatly reduced P_4 concentration reaching minimal threshold prior to parturition. As witnessed in the current study, complete withdrawal of progesterone has also been reported to occur immediately before delivery (Cunningham and Klein, 2007).

Oestradiol concentrations were similar in the first three trimesters but assumed a sharp increase in the last 10 days. Similar trend in oestrogen levels have been observed by Noakes *et al.* (2001). The escalating increase in oestradiol concentration might be attributed to increasing production of oestrone by foetoplacental unit as the foetus increases in maturity at approximately 3 to 4 week prepartum. The increase in production of cortisol by fetal adrenal cortices, concurrent with maturity of the foetus, initiates the prepartum increase in estrogen production (Reece *et al*., 2015) which might have accounted for massive increase in the oestradiol concentration in the last 10 days. Oestrogen induces oxytocin receptor formation in the myometrium, and it is synergistic with $PGF_{2\alpha}$ in promoting contraction of the uterus (Cunningham and Klein, 2007).

5.3.5 Reproductive steroids, FSH, and LH concentrations in serum/raw milk postpartum

Mean values of oestradiol in serum and milk of this study are comparable to those reported by Mekonnin *et al*. (2017) in crossbred cow in Ethiopia but are however higher than oestradiol levels recorded by Yamanaka *et al.* (2007) in dairy cattle and Domenech *et al*. (2011) in milk in Spain. High positive correlations (0.92) existed between serum and skim milk oestradiol concentrations indicating that there were strong associations and therefore each of them could be used as a measure for oestradiol concentrations in cows. Similar high positive correlations have been reported in oestradiol concentrations in milk and blood of goats in Poland (Gorecki *et al*., 2004) and in dairy cattle (Roelofs *et al*., 2006).

Serum and milk progesterone concentrations in crossbred cows were higher than the values of 0.16 and ≤ 1 ng/mL in milk and serum, respectively, reported in anoestrus cows (Mekonnin *et al.*, 2017). There is also great association between serum and milk

progesterone concentrations in crossbred cow using the commercial EIA assay, indicating that both serum and milk can be used as measure for P_4 assessment. Gorecki *et al*. (2004) also observed strong correlation between serum and milk P_4 concentrations.

Significant correlation observed in testosterone concentrations in serum and milk is an indicative that there are high association of reproductive steroids in serum and milk of cow. Testosterone levels in cows, though lower in concentration, are very crucial in the synthesis of oestrogen. Testosterone is converted to oestrogen by the enzyme aromataze (Ball and Petters, 2004; Akers and Denbow, 2013; Reece *et al.*, 2015).

In adult female, high levels of testosterone are generally found in virilization, polycystic ovaries, ovarian tumours, adrenal tumours and adrenal hyperplasia. In male, high levels of testosterone are associated with hypothalamic pituitary unit diseases, testicular tumours, congenital adrenal hyperplasia and prostate cancer (Johnson, 2003). Low levels of testosterone can be found in association with diseases such as hypopituitarism, Klinefelter's syndrome (Ganong, 2003; Johnson, 2003).

High positive correlation (0.93) between serum and raw milk concentration of FSH (Fig. 4.8) means that there is a very strong relationship and each can be used for assessment of FSH profile in cows. There were also low concentrations in FSH from day 1 to day 20 postpartum. A slight pulsatile in serum was observed in day 22 and significant surge in FSH was noticed in day 42 and dropped at day 45 postpartum. The rise in FSH in day 42 might be stimulating follicular waves which may commence subsequent ovarian cyclicity (Akers and Denbow, 2013; Goff, 2015b).

Luteinizing hormone concentrations in serum and milk were moderately and negatively correlated. That means that an increase in LH level in serum moderately results in a decrease in milk LH concentration.

5.3.6 Effect of age on testosterone and oestradiol concentrations in bulls

Age of bull significantly influenced testosterone concentrations, such that, the levels of testosterone increased with increasing age. The overall mean of testosterone concentration in serum across ages considered fell within a wide range of 0.89 to 14.59 reported by Mahmood *et al*. (2013). The observed value was lower than the mean values of 8.6±0.70 and 5.81±0.32 ng/mL reported by Sekoni *et al*. (2010) in Zebu cattle and Mahmood *et al*. (2013) in Cholistani bull, respectively, but was also higher than the values recorded by Sajjad *et al*. (2007) and Javed *et al*. (2000). The variations in the values of testosterone concentration in different studies are due to variations in standards and assay procedures by manufacturer's instructions. It was also noticed in this study that, young bulls of 6 months to 1 year old and those of ≥ 12 years old had similar and lowest testosterone concentration whereas the highest levels were observed in bull of 4 years. The differences in testosterone concentrations may be due to age differences which also reflected in the vigor of libido by bulls of 2 to 4 years. Serum testosterone level has been reported to be correlated with libido (Gulia *et al.*, 2010; Sajjad *et al*., 2007).

Oestradiol concentration was highest in yearling bulls whiles all other bulls of different ages had similar levels. High concentration of 17β-oestradiol in young bull is required for protein anabolism, epitheliotropic activity, and early union of the epiphysis with the shafts

of long bones, where growth of long bones ceases (Reece *et al*., 2015). This may accounts for the highest level of oestradiol in yearling bulls.

5.4 Productive Performance of Dairy Herds

5.4.1 Milk yield of dairy cows

The overall least square mean milk yield (5.4 ± 0.02 l/day) across breed (Sanga, Friesain-Sanga crossbred, and Jersey cows) fall within the range of 4.0 – 9 litres/day in Ghana (Aboagye, 2002), but lower than 7.0 litres at smallholder farm under tropical environment (Wangdi *et al.*, 2014; Fernando *et al*., 2016) and 15.4 – 21.8 litres/day at commercial farms for Jersey and Friesian, respectively, in Zimbabwe (Ngongoni et al., 2006). The overall mean value observed in this study was, however, higher than the mean value obtained in literature for dual-purpose cows in Ghana (Oppong-Anane, 1999; Coffie *et al*., 2015a), and 3.8 litres/day reported by Kamal *et al*. (2009). The differences in means might be attributed to variations in breed, different management regimes and effect of individual farms. It was realised in this study that daily milk yield ranged from 0.6 to 26 litres a day, resulting from extremely varied husbandry/management practices and feeding. Similar observation has been elucidated by Millogo *et al*. (2008).

5.4.1.1 Effect of farm on mean daily milk yield

FAO (2013) stated that good farm management through good housing, adequate nourishment/feeding and disease prevention improves performance, health, milk production and longevity. It was noticed in the current study that, farms (Hallelujah, Ofori, and Nartey farms) that have insect proof housing, sanitized facilities and observed moderate level of biosecurity practices have best mean milk yield. The dominant breed

reared in a given farm might influence the mean milk yield. In the current study, Amrahia farm kept Friesian-Sanga and Sanga (meant for crossbreeding) as dairy herd whereas Hallelujah farms reared Jersey and Friesian-Sanga crosses. Quality and quantity of forage available (Ngongoni *et al.,* 2006) for dairy herd, and dominant breed reared might determine a farm's productive performance of dairy herd (Millogo, 2010).

5.4.1.2 Effect of breed on mean daily milk yield

Jersey cows recorded the highest least square mean milk yield (7.4 litres/day) which was similar to the value (6.6 litres/day) observed in the Friesian Sanga crossbred. This result is comparable to 7.0 units of mean daily milk yield reported for Jersey cows under tropical condition (Wangdi *et al.*, 2014; Fernando *et al*., 2016) but lower than 14.2±0.29 kg observed in zero-grazed Friesian cows in highland regions of Cameroon (Gwaza *et al.*, 2007). The least square means for the dairy cows fall within the ranges of 4 – 9 and 6 – 11 litres of mean milk yield for cows of 6 to 9 year and Friesian-Sanga/Sokoto Gudali crosses, respectively (Aboagye, 2002). Sanga cow had the lowest mean milk yield but it was higher than the mean value obtained for dual-purpose cattle (Oppong-Anane, 2013; Coffie *et al.*, 2015a).

The observed differences in milk yield among breeds might be due to their genetic potential and environmental effects (Aboagye, 2002). According to Annor (1996) crossing of low milk production potential breeds (e.g. WASH, and N'dama) with the high milk production ones (e.g. Jersey and Holstein Friesian) leads to a dramatic improvement in the quantity of milk yield, presumably due to heterosis and breed

complementarity (Lopez-Villalobos and Garrick, 2002). This might have accounted for the better mean milk yield of Friesain-Sanga crossbred over the Sanga.

5.4.1.3 Effect of parity of dam on mean daily milk yield

The observed result for the effect of parity of dairy cow on milk yield is consistent with the trend of the findings reported by Epaphras *et al.* (2004) and Darfour-Oduro *et al*. (2010) in local cattle, Coffie *et al*. (2015) in dual-purpose cows and Afzal *et al.* (2007) in sheep. The present finding showed that, mean milk yield increases as parity increases for one to five and then declines at parity six. This may be explained by further development of reproductive organs/udder after parity one. Bhuiyan *et al.* (2004) stated that at parity one (1), the udder and teat structural growth have not yet fully developed to assume their peak functions. Cows' udder and teat development increases with increasing parity.

5.4.1.4 Effect of Stage of lactation on mean daily milk yield

The result on effect of stage of lactation shows that, milk yield decreased with increasing months of the stage of lactation. Similar trend in milk yield with respect to stage of lactation has been reported by Vijayakumar *et al*. (2017). The increasing milk yield with early stages of lactation might be due to calf continuous demand for milk triggering milk letdown efficiency. This may be attributed to milk yield increasing gradually from the date of calving till its peak in the early stage of lactation (Vijayakumar *et al*., 2017).

5.4.1.5 Effect of body condition score at calving

Differences realized in average milk production with respect to different body condition score (BCS) suggest that lower BCS (≤2) may not be recommended for profitable dairying. Though, reducing BC at calving with proper nutritional management limit negative energy balance (NEB), the procedure requires high-quality diets (Rodriguez-Martinez *et al*., 2013), and may not be applicable in areas where dairy nutrition is a challenge. Samarutel *et al.* (2006) observed that, cows with lower body condition score at calving cannot achieve their genetic milk yield potentials and days in milk due to lack of body reserves that may support increasing milk yield at the beginning of lactation. In the present study, mean daily milk yield of dairy cows increased with increasing BCS from point 2 to point 4 on five BCS scale. Similar trend in mean daily milk yield has been noticed in indigenous dual-purpose cows (Coffie *et al.*, 2015a). According to Loker *et al.* (2010), good BCS may be selected for without a large negative impact on milk production. The current result on the mean milk yield with respect to BCS (Table 4.19) buttresses the findings of many researchers who recommend that a cow entering the dry period should have a BCS between 3.25 and 3.75 points for better performance (Gumen *et al*., 2003; Lopez *et al*., 2005; Watters *et al.*, 2010; Gergovska *et al*., 2011).

5.4.1.6 Effect of udder size on mean daily milk yield

Assessment of mean daily milk yield with respect to udder size showed that, udder size is one of the indicators of high milk yield. Dairy cows with large udder had highest daily milk production, followed by medium and small udder sizes. Similar findings have been reported in literature (Bhuiyan *et al*., 2004; and Deng *et al*., 2012). This feature can be used as a determinant of milk yield potential. Deng *et al*. (2012) noticed that, the

morphological characteristics of the udder is one of the genetic features in dairy cattle and it is one of the fundamental criteria for selection. Bhuiyan *et al*. (2004) reported that size and shape of udder are very important conformational traits which could play a vital role for the suitability of milking and economical milk production when considered for selecting dairy cows. Ahmed and El-Barbary (2000) stated a selection based on a control table that gives 50 % of the score for udder traits. Further, Seykora and Hansen (2000) noted that, the udder is 40 % of the judging score card and often becomes the deciding factor in close placing, perhaps due to the udder size role in determining mean milk production potential. Good udder and teat anatomy and milk flow rate are positively correlated with the daily milk yield (Bardakcioglu *et al*., 2011; Deng *et al*., 2012).

5.4.1.7 Effect of teat size on daily milk yield performance

Milk yield from dairy cows increased with increasing teat size. This observation was also made by Kukovics *et al*. (2006) and Bardakcioglu *et al*. (2011). Holmes *et al*. (1984) stated that large teat size facilitates the ease at which milking is done. According to Kukovics *et al*. (2006) effect of teat size is prominent for breed and its effect on milk yield seemed to be general, and can be useful in selection of the milk trait. However, Boettcher *et al*. (1998) noted that excessively pendulous teats reduce milking speed.

5.4.1.8 Effect of season on mean daily milk yield

The highest milk yield was recorded in the major rainy season, followed by the minor rains, with the dry season recording the least. This observation agrees with the findings of Epaphras *et al.* (2004) and Hatungumukama *et al*. (2006). This can be explained by the availability and good quality of pasture. In the dryer and hotter periods, as a result of

feed inadequacy and low nutritive value of the natural pastures (Coffie, 2014), milk yield tends to be low (Epaphras *et al*., 2004; Coffie *et al*., 2015a).

5.4.1.9 Effect of feed supplementation on mean daily milk yield

Similar results of the effect of feed supplementation on milk yield were observed in local cattle and local exotic crosses in Ghana (Annor, 1996; Aboagye, 2002; Coffie *et al*., 2015a; Obese *et al.*, 2018), Burkina Faso (Millogo *et al.,* 2008) and Bangladesh (Kamal *et al*., 2009). Nutrition is an important environmental factor that is very crucial in animals' productive performances and health. According to FAO (2013), adequate nourishment through supplementation provides benefits including less health issues by building on cow's immunity, and more milk production. Better daily yields exhibited by cows supplemented with feed might be attributed to the enhancement in nutrients that would occur during the lean seasons and also improvement of utilization, as it has been also observed by Epaphras *et al.* (2004) and M'hamdi *et al*. (2012).

5.4.1.10 Effect of frequency of milking on mean daily milk yield

Milking twice a day recorded higher mean milk yield than the cows milked once day. The trend observed in the milking frequency is consistent with the findings reported by Knight, 1992; Campos *et al.*, (1994), and Assan (2014) in goat and Vijayakumar *et al*., 2017 in cow. It has been realized in the previous studies that increasing milking frequency from twice to thrice a day has been shown to increase galactopoiesis by 10 to 20% in goat (Knight, 1992; Campos *et al.*, 1994) and 10% to 20% increase in milk production in cow (Vijayakumar *et al*., 2017). This trend in increasing milk yield with increasing number of milking may be attributed to milking sensations that trigger

mammary gland and secreting cells activities to produce more milk. The quantity of milk produced involves changes in the level of activity of mammary secreting cells, and also to the cell proliferation/ and death balance (Boutinaud *et al.*, 2004).

5.4.2 Lactation Length

The overall mean lactation length (LL) of the three breeds was 271.4 ± 2.96 days, and ranged from 80 to 371 days across the three breeds. The overall mean is higher than LL reported by Darfour-Oduro *et al*. (2010) for Sanga (164.1 days) and Friesian-Sanga (201.1 days) cows in Ghana and of 248 days stated by Bajwa *et al*. (2004) in Pakistan. It also falls within ranges reported by Aboagye (2002), and Kugonza *et al*. (2011) in local breeds in Africa on-station. The overall mean was far lower than averages of 305, 305, and 327 days observed for Friesian-WASH, Jersey-Gudali and Friesian-Gudali crosses, respectively, in Ghana (Annor, 1996). The crossbred cows might have inherited longer lactation periods from Zebu or foreign breeds in addition to the local breeds' adaptability to humid climate. This might have been achieved presumably through heterosis and breed complementarity (Aboagye, 2002; Lopez-Villalobos and Garrick, 2002).

5.4.2.1 Effect of farm management on lactation length of dairy cows (in days)

The mean values of lactation length for the effect of the farms under study fell below the mean LL of 321.28 observed by Fernando *et al*. (2016) and the range of 314±61 to 321.7±2.26 days reported for Jersey cows (Rao and Rao, 1997). The means, except in Karimah farm, also fall within the range of 234±11.7 to 333±17.6 days recorded for Jersey crosses in Ghana (Aboagye, 2002). Significant differences observed in LL for the

effect of farm might be due to distinct differences in housing units, feeding, routine disease control and feed supplementation in the various farms (Ngongoni *et al.,* 2006). The mean values of LL noticed in Ofori and Hallelujah farms (304.9 ± 12.64 and 303.6 ± 7.37 days, respectively) reflected the ability of the farms meeting the optimal calving interval of 365 – 395 days (12 – 13 months) for dairy cows/breeds (Vanholder *et al.,* 2005, Yousefdoodt *et al.*, 2012; Mimoune *et al*., 2017). Higher observation LL greater than 305 days is an indication of hindering the optimum calving interval and again depicting inappropriate dry off time due to extended period of milking (Fernando et al., 2016). Shorter LL noticed in Karimah farm can be associated with inadequate feeding practices, insufficient dry period, and low level of biosecurity practices that might have predisposed cows to parasitic and infectious challenges. Similar findings of shorter LL have been reported in India (Javed *et al*., 2003; Mondal *et al,*. 2005), under tropical condition (Sattar *et al*., 2004) as well as in Ghana (Aboagye, 2002; Coffie *et al.*, 2015b). In the present study, it was realized that farm effect on productive parameters of dairy can be adequately manipulated to achieve set production objective (s).

5.4.2.2 Effect of breed on lactation length of dairy cows

The least square means for the effect of breed had little influence on LL in the current study. All the breeds obtained a similar LL suggesting that LL might be dictated by factors other than breed alone in dairy herds of the study area. This observation in the present study differed from findings reported by Aboagye *et al.* (1994), Aboagye (2002), Oppong-Anane (2013) and Coffie *et al*. (2015b) in Zebu, Taurine breeds and their crosses. In this study, milk yield potentials were almost always determined by

management practices, especially, feed supplementation without which the breed potentials become overshadowed by the environmental factors.

5.4.2.3 Effect of parity on lactation length of dairy cows

Significant variations in LL at different parity are in agreement with observations made by Watters *et al*. (2010). It was observed in this study that LL increased with increase in parity and peaked at parity 3. The LL values started declining at parity 5 and worsened at parity 6. Other researchers have also noticed that, parity/lactation 1, 4 and 5 have lower milk yields as lactation length advances (Epaphras *et al.*, 2004; Darfour-Oduro *et al*. 2010; Watters *et al.*, 2010). Lower milk production is associated with shorter LL (Fernando *et al*., 2016). Total milk yield per lactation is determined by the length of lactation (M'hamdi *et al*., 2012).

5.4.2.4 Effect of season on lactation length

Lactation length significantly increased with decreasing dryness and enhanced feed availability. In the rainy season, inspite of the proliferations of secondary and inciting factors of disease, a well-managed dairy cows have access to quality and quantity of forage for high productive purposes. Lactation length increased with enhanced galactopoietic process (Knight, 1992; Campos *et al.*, 1994) through good nutrition. M'hamdi *et al*. (2012) also noted that both lactation length and milk yield increased with decreasing dryer and warmer seasons.

5.4.2.5 Effect of body condition on lactation length

The best performances in terms of LL occurred at parities 3 to 4 which invariably agree with the findings of Watters *et al.* (2010). It has been recommended that a cow should enter the dry period with a BCS between 3.25 and 3.75 (Watters *et al.*, 2010). Ensuring that the animal does not lose more than one point in BCS is important because of the increased chances of the cow being anovular—absence of ovulation (Watters *et al.*, 2010; Gergovska *et al*., 2011). Gergovska *et al*. (2011) also noted that milk yield and lactation period of cows with low body condition score (≤2.5 scores) have 1400 kg lower and shorter LL than that of cows with BCS greater than 3.5 points.

Cows with BCS 3 had better lactation length (Table 4.2) and unitary loss in body condition may not adversely affect their performances. Indeed, cows with BCS 4 recorded the best LL because they might have utilised most of their body reserves in early lactation for relatively lengthy lactation period. Similar findings by Gergovska *et al*. (2011) indicated that Friesian and Brown Swiss cows that lost more body condition had highest milk yield at optimal lactation duration. It is common for a dairy cow to lose more than one BCS, therefore ensuring that a cow does not lose more than one score is important because of the increased chances of the cow experiencing anovular—absence of ovulation (Watters *et al.*, 2010). A herd of cattle in good body condition (BCS ≥3) will produce more milk in longer LL, and will be less susceptible to metabolic disorders, disease, mastitis and reproductive problems (Patton *et al*., 1988). The two extremes BCS 1 and 5 had no records in the current study presumably due to health issues as reported by Keown (2005) that under-conditioned cows are subject to health problems, whilst over-conditioned cows are prone to calving difficulties, fatty liver

syndrome and possible death. The current findings, therefore, suggest that the optimum range of BCS for lengthy LL lies between ≥3 to ≤ 4. It can also be asserted that BCS of 3 to 4 of the dual-purpose cows have enough body reserves for longer lactation length. This corroborates with the finding of previous study reported on dual-purpose cattle (Coffie *et al.*, 2015b).

5.4.2.6 Effect of udder and teat sizes on lactation length

Large udder size recorded the longest LL due to its ability to produce sustained milk. Heavy dairy cows have remarkable udder that enhances milk production for good lactation period.

5.4.2.7 Effect of supplementation on lactation length

It is not surprising to realise longer lactation duration for cows that were given regular and occasional supplementations than those provided with no supplement. This result in the current study is comparable to the findings of Epaphras *et al.* (2004) and M'hamdi *et al.* (2012). The yields of farm animals are the result of the combined effects of genotype and environmental conditions which include nutrition and all other management routines (Afzal *et al*., 2007; and Darfour-Oduro *et al*., 2010). Feed supplementation of lactating cows substantiates inadequate nutrients in feed or fodder in the lean seasons and thereby increases daily milk yield and LL. According to Looper (2012) and M'hamdi *et al*. (2012), provision of good feeding level (3.5 to 4.0 percent of their body weight daily as dry matter) for dairy cows boosts up lactation performance. It is imperative to note that, a well-managed feed supplementation is one of the environmental factors which can mask genetic potential for production.

5.4.2.8 Effect of milking frequency on lactation length

Frequent milking is practised to increase milk production and hence LL is sustained. This initially prevents a down regulation of cellular differentiation (Stelwagen *et al*., 1994) and subsequently curbs a total loss of mammary cell number through apoptosis (Boutinaud *et al*., 2004). As milk yield increase with increasing number of milking per day, LL is sustained (Fernando *et al*., 2016; Vijayakumar *et al*., 2017).

5.4.3 Milk composition

5.4.3.1 Effect of location on percentage milk composition of raw milk

Variation in percentage milk protein, total solids and solids-non-fat observed in the current study at different location can be as a result of changes in both the types of feed available and climatic conditions in Ashanti, Eastern and Greater Accra Regions. Hot weather, high temperature and humidity decrease dry matter intake and increase feed sorting, resulting in lower forage and fibre intake (Looper, n.d.; Looper, 2012). The nutritive value in grasses in forest areas of Ashanti Region is far better than those in Greater Accra Region in the coastal savannah zone. Kumaresan *et al.* (2008) and Looper (2012) observed that cows' milk protein increased with increasing feed intake and frequency, high non-fibre carbohydrates and crude protein.

5.4.3.2 Effect of breed on percentage milk composition of raw milk

Protein composition of Sanga and Friesian-Sanga crossbred were similar (Table 4.21) and were far higher than means of 3.5 % and 3.8 % reported for exotic breeds (Fox and Mcsweeney, 1998; Aboagye, 2002). However, percentage protein component of Jersey (3.8 %) was comparable to those reported in literature (Heinrichs *et al.,* 2005; Looper, 2012).

The higher percentage protein component in fresh milk in the current study might be due to higher nutrient levels with relatively minimal fibre content in feed as noticed in the proximate fractions in dominant forage fodder in the region (Coffie, 2014). Kumaresan et al. (2008) and Looper (2012) stated that cows' milk protein increased with increasing feed intake and frequency, high non-fibre carbohydrates and crude protein.

Additionally, the considerable variations among breeds' milk protein component might be attributed to the individual breed potentials, although environmental effects (management, feeding, and disease condition e.g. mastitis) can play an influential role in modifying milk components. This is in agreement with the assertion made by Varga and Ishler (2007) that besides breed, nutrition and blood amino acids (primary precursor of milk protein), rumen microbial amino acids are easily and efficiently converted into milk protein by the cow.

Percentage fat components of Jersey cow was the highest whereas that of Sanga and Friesian-Sanga crossbred cows were similar. The means of fat components, nevertheless fall within the range of 1.77 to 5.98 % (Heinrichs *et al.,* 2005). The differences in means of percentage fat can be explained by the differences in breed and individuality of cows studied. Jersey cow is known for its higher fat component in raw milk (Jensen, 1995; Heinrichs *et al.,* 2005)

There was similar influence of effect of breed on percentage lactose, cholesterol, solids-non-fat, and water components. However, percentage composition of TS recorded for Sanga, Friesian-Sanga crossbred and Jersey fell within the range of 10.5 to 14.7 % (O'Mahony, 1988; O'Connor, 1995) but higher than mean of 12.6 % (Heinrichs *et al.,*

2005). The TS observed in Jersey in the current study (14.7 %) is comparable to the values of 14.7 % in Zebu, 5.0 % in Jersey cows elsewhere (Jensen, 1995; O'Connor, 1995; Fox and McSweeney, 1998). The differences in breed (Sanga, Friesian-Sanga crossbred and Jersey cows) might have accounted for these variations in percentage TS, though environment influences play a vital role. Heinrichs *et al*. (2005), Looper (2012) and Schroeder (2012) reiterated that although breed and inheritance contribute greatly (55 %), 45 % of changes in milk component are influenced by environmental factors.

Significant effect of breed on ash component is due to the breed differences and individuality of cows. The observed values in the current study are comparable to 0.72, and 0.83 recorded by Fox and McSweeney (1998) and Guetouache *et al*. (2014). The ash contents of raw milk are a reflection of the mineral compositions of the milk (Ajai *et al*., 2012). The percentage components of solids, non-fat solids, and dissolved salt which are in turn influenced by nutrition and environmental changes may be attributed to the variations in raw milk ash in the current study. O'Connor (1995) noticed that milk ash composition is influenced by a number of factors including breed, individuality of the cow, stage of lactation, feed, infection of the udder and season of the year. In addition, certain milk salts, such as sodium and potassium chlorides are sufficiently soluble to be present almost in the dissolved phase. Percentage compositions of raw milk ash have also been reported to be relatively constant at 0.7 to 0.8 %, but the relative concentrations of the various ions vary considerably (O'Connor, 1995).

5.4.3.3 Effect of parity of cow on percentage milk composition of raw milk

On the basis of the current finding, parity of a cow had little or no effect on milk compositional yield. This finding supports the observation made by Strzałkowska *et al*. (2010) that milk component especially cholesterol is not affected by parity. Insignificant differences for the effect of parity on percentage milk compositions in dual-purpose cattle have also been observed in Ghana (Coffie *et al.*, 2015c)

5.4.3.4 Effect of body condition score on percentage milk composition

Body condition score greatly influenced percentage protein, total solids, water and ash components such that higher compositional yields were noticed in cows with BCS 3 and 3.5. This might apparently be due to better energy reserves and balance in cows of BCS 3 and 3.5 (Loker *et al*., 2010; Coffie *et al*., 2015a). It has been reported that level of production or yields of fat, protein, non-fat solids and total solids are highly related with body condition and milk yield (Looper, 2012).

Amount of water is controlled by the amount of lactose synthesized by the secretory cells of the mammary gland (Guetouache *et al*., 2014). It is then not surprising that BCS can be a good determinant of milk water component. Water activity, together with temperature and pH, is one of the most important parameters which determine the rates of chemical, biochemical and microbiological changes which occur in foods (Fox and McSweeney 1998). Abd El-Salam and El-Shibiny (2011) noticed that 87 % of milk component is water, in which the other constituents are distributed in various forms. The BCS, however, had little or no effect on fat, lactose, cholesterol, and SNF components in the current study.

5.4.3.5 Effect of stage of lactation on percentage milk composition

Milk compositional yield responded to the stage of lactation such that percentage protein, fat, TS, and ash components were higher in the first 30 days, 61st day and beyond than those observed in between 31 to 60 days. The results obtained from these components followed a similar trend which concur with the findings of Heinrichs *et al*. (2005), Strzałkowska *et al*. (2010), Looper (2012) and Simões *et al.* (2014) that the concentration of milk fat and protein are highest in early and late lactation and lowest during peak milk production through mid-lactation. In the current study, peak milk production frequently occurred 18 days through the second stage of lactation. Different ranges have been used for the stages of lactation in literature for early, mid and late lactations. O'Connor (1995) and Heinrichs *et al*. (2005) noted that at 0-3 and 30-55 weeks of lactation, the concentration of milk solids are higher than 4 - 25 week (mid lactation). Usually, an increase in milk yield is followed by a decrease in the percentages of milk fat and protein, while the yields of these constituents remain unchanged or increase (Looper, nd.; Looper. 2012). The three categories of stages of lactation considered in this study had little influence on percentage lactose, cholesterol, SNF, and water compositions.

5.4.3.6 Effect of feed supplementation on percentage milk composition of raw milk

Significantly higher percentage protein, TS, SNF, water and ash compositions of raw milk in cows provided with feed supplementation followed by occasional and no feed supplementation in descending order, can be explained by the swift response of milk compositional yield of dairy cows adequate feed supply in term of quality and quantity. Similar observations have been made (Ngongoni *et al*., 2006; Looper, 2012). Heinrichs

et al. (2005) and Kumaresan *et al*. (2008) noticed highest values of total solids and SNF (including protein) contents of Jersey milk during the coldest/winter months with provision of feed supplements.

5.4.3.7 Effect of season of calving on percentage milk composition

Milk compositional yields of protein, TS, SNF, water and ash were highest in season where there were the best qualities of feed and in adequate quantities. The trend observed in the present study is consistent with the findings of Ngongoni *et al*. (2006) and Loker *at al*. (2010). This variation is concerned with changes in both the types of feed available and climatic conditions. Hot weather and high humidity decrease dry matter intake and increase feed sorting, resulting in lower forage and fiber intake (Looper, n.d.; Looper, 2012).

5.4.4 Milk quality assessment

5.4.4.1 Effect of fixed factors on milk temperature, pH and specific gravity

Location, breed, parity, body condition, stage of lactation, feed supplementation and season of calving were poor determinant of milk temperature and specific gravity in raw milk**.** On the contrary, breed, and stage of lactation had significant effect on pH. Jersey cow had significantly higher pH value which was similar to the value observed in Sanga cow's raw milk. Friesian-Sanga had the lowest pH (6.4±0.06) which slightly fall below the normal range of pH between 6.5 – 6.7 (O'Connor, 1995).

The pH values higher than 6.7 indicate mastitic milk whereas values below pH of 6.5 indicate the presence of colostrum or bacterial deterioration. A pH lower than 6.5,

therefore, indicates that considerable acid development has taken place which is normally due to bacterial activity (O'Connor, 1995).

5.4.4.2 Milk somatic cell count of dairy cows

Milk somatic cell count (MSCC), which is a general indicator of the mammary gland health, subclinical mastitis and as a standard measure for determining the quality of stored raw milk (Tsenkova *et al.*, 2001) was influenced by sanitary management of premises, breed of cows, parity of dam and stage of lactation. The overall mean for composite MSCC observed (134000 ±7498.7 cells/mL) in the current study was higher than 100,000 cells/mL for uninfected cows, and fall below the optimum MSCC threshold set at 150,000 and 200,000 cells/mL (Petzer *et al*., 2017).

The current result indicates that, MSCC decreased with increasing levels of good sanitation. The worst MSCC of poor sanitary production area might have resulted from septic infections of udder and contaminations of milk. Similarly, Dehinenet *et al*. (2013) noticed that MSCC count is higher in poorly managed cows, mastitic problem, unhygienic milking and poor milk handling conditions. According to Harmon (1994), elevated SCC and compositional changes are due to an inflammation of the mammary gland that results from the introduction and multiplication of pathogenic microorganisms in the mammary gland which is a complex series of events leading to reduced synthetic activity. Elevated SCC above 200,000 cells/mL is a clear indicator that an animal has experienced or is developing (or recovering from) an infection (Bradley. and Green, 2005). Norman *et al.* (2015) and Kelci *et al*. (2017) reiterated that, good sanitation, bedding for lactating cows,

control of flies, correct milking procedures, hygienic milking equipment, and good nutrition in a given season/month of the year can reduce the level of MSCC.

Breed differences significantly determined levels of MSCC. The noted variation is as a result of differences in the breeds' ability to withstand the hot-humid weather condition in the study area, which is dependent on the innate physiology of individual cow (Bradley and Green, 2005), with different physiologic vitals (Cunningham and Klein, 2007; Reece *et al*., 2015). Jersey cows are less adapted and may experience more stress with respect to high temperature and humidity as compare with Sanga and their crossbred counterparts.

Increased MSCC with increasing parity was observed in the current study. According to Bradley and Green (2005) increase in SCC in milk of multiparous cows may be often, but not exclusively, due to an increased likelihood of infection with age.

Stage of lactation influence MSCC in that cows in early stage of lactation experienced highest count, followed by mid and late stage of lactation. This variation can be linked to the stress associated with labour and the period of negative energy balance in the first stage of lactation. Physiological stress normally increase SCC in the first two weeks after calving, usually at a minimum in second month of lactation and gradual increase in later lactation accentuates when an infection is present (Bradley and Green, 2005). Location and body condition score had little effect on MSCC.

5.4.4.3 Effect of fixed factors on total bacterial count in raw milk of dairy cows

Farms located in the Ashanti and Greater Accra regions had similar but higher TBC. The observed trend is due to the dominant management of dairy herds on range and partial zero grazing regimes with minimum level of biosecurity practices, especially, on sanitation, as compared with farms in the Eastern Region with fair to good hygienic standards. Otherwise, the farms in the Ashanti and Eastern regions lie in the hot-humid environment with more secondary and inciting factors of diseases might have experience higher count. The implication is that good management with good sanitation, proper handling of milk and its equipment can offset or reduce environmental impact on contaminations emanating from sepsis. The main sources of bacterial contamination of raw milk emanate from within the udder, outside the udder, and from the surface of equipment used for milk handling and storage (Wallace, 2008). The bacteriological quality of raw milk, therefore, justifies the contamination of milk with bacterial organisms at different stages of the value chain (Reda *et al*., 2014).

Means of TBC observed in the current findings were higher than the bacterial count limit for the EU ($1.0x10^5$ cells/mL) (Centres for Epidemiology and Animal Health—CEAH, 2014; Reda *et al*., 2014). By standards, the overall mean TBC ($3.0x10^6$ $\pm 7.4x10^5$ cells/mL) in the findings of the present study can be graded fair since the count is between $1x10^6$ and $5x10^6$ CFU/mL of milk. Whereas TBC exceeding $5x10^6$ CFU/mL (as observed in Ashanti Region) is graded as poor quality (Sherikar *et al*., 2004; Reda *et al*., 2014). Effect of breed, parity, BCS, and SOL were not good determinants of TBC in raw milk.

5.4.4.4 Effect of fixed factor on total coliform count in milk

The acceptable level of total coliform counts in raw milk has been set as <103 CFU/mL in Kenya (KEBS, 2007; 2010), and grade 'A' raw milk has <103 CFU/mL for the total coliforms in milk (Gran *et al.*, 2003). The current study recorded higher value of TCC ($2.9x10^4$) than the acceptable European Union (EU) limit of <100 CFU/mL for coliform counts in raw milk (Jay *et al*., 2005) but fell within the range reported for TCC in milk samples in Zimbabwe (Chimuti *et al*., 2016; Wanjala *et al.*, 2018). The significant effect of location, sanitary management practices, breed and stages of lactation on TCC in milk can be attributed to compromise of sanitation, personal hygiene and milking equipment. The presence of high TCC indicates poor milking hygiene originating in environmental contamination such as bedding, soil, poor water, faeces, inadequate cooling of milk (Douglas, 2003; Wallace, 2008).

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary of Findings

6.1.1 Health management of ticks and tick-borne diseases of cattle

6.1.1.1 Incidence of tick species and factors influencing total tick load/infestation

- ***Incidence of tick species***
  - *Amblyomma variegatum* recorded the highest incidence across breeds of cattle, followed by *Rhipicephalus (Boophilus) decoloratus*, *Rhipicephalus spp.* and *Hyalomma rufipes* in descending order in the hot-humid and Accra plains of Ghana.
  - Higher tick incidence occurred in May, June, July (major rainy season) and August (minor rainy season) whereas lower tick population was noticed in January, February and December (Dry season).
  - *Amblyomma variegatum and Rhipicephalus spp* incidence peaked in May whiles *Boophilus decoloratus and Hyalomma rufipes* abundance peaked in July and another slight increase in October.
  - Tick prevention and control management programmes could take into account the phenology (effect of climate on periodic biological phenomina) of the tick species with respect to month and season of the year.

- ***Factors influencing tick load/infestation***
  - Effect of breed, farm and geographical location on the tick load was overshadowed by management regimes and level of biosecurity practices observed by farmers.

- Dairy herds kept under exclusive zero grazing had the least tick load, followed by partial zero gazing and range grazing in descending order.
- Observation of moderate level of biosecurity led to the lowest tick count, with low- and very low biosecurity practices following in descending order.
- Varied management regimes in different locations influenced tick load such that, farms in the Ashanti Region recorded the highest tick infestation, followed by those in Greater Accra whereas Eastern Region had the least tick challenge.
- Tick load in the dairy herds decreased greatly with decreasing intensity of rains.
- Dairy herds maintained in insect-proof housing had the lowest tick load. Open kraal and roofed barn recorded a similar tick infestation challenge.
- In tick infested herds, weekly application of acaricide yielded the lowest tick count, followed by fortnight and monthly control regimes.
- Sex of cattle was a poor determinant of tick infestation in the dairy herds.
- The approach to tick prevention/control in dairy herds in Ghana should be a holistic consideration of factors determining the tick load and their interaction effect.

6.1.1.2 Prevalence of tick-borne diseases (TBDs) and factors influencing their occurrence

- ***Prevalence of TBDs***

- Dematophilosis was the most prevalent among the TBDs considered in the study area followed by anaplasmosis and heartwater/cowdriosis in descending order (through culturing and microscopy).
- A PCR assay showed a prevalent rate of 92.50 %, 33.75 % and 10.00 % for *Dermatophilus congolensis*, *Anaplasma marginale* and *Ehrlichia ruminantium*

respectively; molecular (PCR) diagnostic tools detected carrier and sub-clinical cases of the infections (TBDs).

- ***Factors influencing prevalence of tick-borne diseases***

  - Farms that maintained good health care, proper sanitation and screened cattle from getting exposed to tick vectors under zero grazing regimes experienced little or no outbreak of the TBDs and vice versa.
  - Cattle that were managed under range grazing had the highest percentage prevalence of the TBDs, followed by partial zero-grazing while exclusive zero grazing management regime recorded no prevalence of the three TBDs investigated.
  - Generally, seasonal prevalence of TBDs increased with increasing rains resulting in the highest clinical conditions in the major rains, followed by minor rainy season and dry season having the least.
  - Dairy cattle reared in insect-proof barn and good level of biosecurity practices had no incidence of the TBDs.
  - Open kraal recorded the higher prevalence of TBDs than roofed barn.
  - Prevention of TBDs in dairy herds should take into consideration the factors that limit vectors-hosts exposure.

6.1.2 Haemato-biochemical indices of dairy cows; Effect of breed and physiological state

6.1.2.1 Haematology of dairy cows

- ***Effect of breed on haematological indices***

  - Red blood cells, HGB and HCT were highest in Sanga, but Friesian-Sanga and Jersey cows had similar values.

- Jersey had the highest values of MCV and MCH, followed by Friesian-Sanga crossbreds and Sanga in descending order.
- Breed, however, had little or no effect on MCHC, RDW, PLT, MPV, PDW, and PCT indices.
- Granulocytes (GRAN) and basophil (BAS) levels were highest in Friesian-Sanga crossbreds, followed by Jersey and Sanga cows in that order.
- Breed had little effect on percentage count of white blood cell (WBC), neutrophils (NEU), lymphocytes (LYM), monoccytes (MON) and eosinophils (EOS).

Effect of physiological state of cows on haematological indices

- Physiological state influences haematology of dairy cows. Erythrocyte indices such as MCH and MCHC increased in cyclic cows as compared to non-cyclic heifers.
- Also, erythrocyte indices were generally elevated during gestation (especially early gestation).
- Haematocrit (HCT) was higher in heifer and cycling dry cows than those observed in gestational periods.
- Higher levels of WBC, neutrophils, lymphocytes, eosinophils and basophils were noticed in G_E.
- Nevertheless, with the exception of WBC, all leucocyte indices fell within the reference ranges reported in literature.
- Lymphocytes and eosinophil levels fell within normal reference ranges.
- Non-cycling heifer, cycling cows (CC), cows in early gestation (G_E), mid gestation (G_M) and late gestation (G_L) had little or no effect on percentage monocytes.
- Heifer and CC also had little or no effect on leucocyte indices.

6.1.2.2 Serum biochemistry of dairy cows: Effect of breed and physiological state

- ***Effect of breed on serum biochemical indices***

- Breed had marked influence on ALP concentration such that Friesian-Sanga had the mean highest serum concentration followed by Sanga and Jersey cows in descending order.
- Serum concentration of ALT, AST and γGT were similar across the breeds.
- Sanga had the highest serum concentrations of total protein (TP) and globulin levels, although, the values were within the normal reference range.
- Friesian-Sanga crossbred and Jersey cows had similar mean TP and globulin levels.
- Direct bilirubin concentration was highest in Sanga followed by the crossbred and Jersey in descending order.
- Mean urea and BUN levels were highest in Jersey cows, with the Sanga and the Freisian-Sanga crossbred cows having similar concentrations.
- Jersey cows recorded the highest concentration of cholesterol and HDL, followed by crossbred and Sanga cows in descending order.
- Breed had little effect on mean serum creatinine, triglyceride and VLDL levels.
- Ketone (BHBA) concentration decreased with decreasing level of beef genotype.
- Breed did not significantly influence mean serum glucose and NEFA.
- Jersey recorded the highest concentration of chloride, followed by Friesian-Sanga crossbred and Sanga cows in descending order.
- The highest concentration of potassium was recorded in Sanga but values observed in crossbred and Jersey cows did not differ significantly.

- Phosphorus concentration was highest in Sanga whereas crossbred and Jersey cows had slight difference in P concentration.
- Breed had little influence on serum Na^{+}, Ca^{2+}, HCO_3^{-}, and Mg^{3+}.

- ***Effect of physiological state of cows on serum biochemical indices***

- Although there was significant influence of physiological state on serum ALT, AST, and γGT, the values fell within normal reference ranges.
- The γGT levels were normal across physiological states except in cows in G_M where the value was more than normal; thus, the elevated level of γGT might be an indication of impending poor liver function when it is associated with elevated AST.
- Elevated serum ALP in non-cycling heifer might be due to the enzyme function in bone growth and in organs involved in active transport mechanism.
- Total protein and albumin concentrations were highest in heifers, followed CC, cows in G_E, G_M and G_L in descending order.
- Globulin concentration was lowest in cows in G_L but heifer, CC, cows in G_E and G_M had similar levels.
- The highest total bilirubin (TBIL) concentration was recorded in CC but heifer and cows in G_E, G_M and G_L had similar levels.
- The highest direct bilirubin (DBIL) was noticed in G_E and the value was similar to those observed in CC and G_L.
- Cycling cows had the highest serum indirect bilirubin, followed by G_M. Non-cycling heifer, cows in G_E, G_M and G_L had similar IBIL levels.
- The highest urea concentration was observed in CC while the lowest level was recorded in heifer.

- Blood urea nitrogen (BUN) was highest in cows of G_M and the value was similar to the value observed in G_E.
- Creatinine concentration was high in CC and the value was similar to those in heifer, cows in G_E.
- Creatinine levels slightly decreased with increasing stage of gestation period.
- Nevertheless, creatinine concentrations fell within the normal reference range indicating normal functioning of the glomerulus of the kidneys across breeds and physiological states.
- Triglyceride was not markedly influenced by the physiological state of cows considered in this study.
- Total cholesterol concentration generally increased with increasing period of gestation.
- The lowest cholesterol level was observed in heifers.
- The CC recorded the highest concentration of low density lipoprotein (LDL).
- The LDL level also increased with advancing period of gestation.
- High density lipoprotein and very low density lipoprotein were not markedly affected by the physiological state of the cows.
- Although serum β-hydroxybutyate concentrations were similar, the values observed in G_E and G_M were higher than the normal healthy limit of <1.2 mmol/L, hence implicated in subclinical ketoses.
- The CC had the lowest glucose concentration whereas heifer had the highest, which value was insignificant to those observed in cows of G_E, G_M and G_L.

- The NEFA concentration in G_L was higher than the normal range, an indication of negative energy balance, as compared with normal levels observed in heifer, CC, G_E and G_M.
- Physiological state of cows had little or no effect on sodium, chloride, calcium, and magnesium cation/ions concentrations.
- Potassium levels were similar in heifer, G_E, and G_L but the values were higher than those observed in CC and G_M.
- The lowest serum bicarbonate concentration was recorded in CC.
- The levels of HCO_3^- were similar in heifer, cows in G_E, G_M, and G_L but significantly higher than the value observed in CC.
- Cows in G_L had the lowest serum phosphorus (P) concentration whiles heifer, CC, G_E, and G_M had little or no effect on P levels.

6.1.3 Reproductive hormonal profiles of dairy cow with commercial EIA

6.1.3.1 Progesterone profile of crossbred, Sanga and Jersey heifers during prepubertal stage to conception

- Progesterone (P_4) concentrations during pre-pubertal stages were below 0.96 ng/mL and the time (in days) the P_4 concentration rise to the levels of ≥ 1.0 ng/mL was dependent on breed.
- The first rise in serum P_4 concentration ≥ 1 ng/mL in Sanga, crossbred, and Jersey cows occurred at the 415th, 295th, and 285th day, respectively from birth.
- The first overt oestrus/"heat" in Sanga, crossbred, and Jersey cows occurred in 435th, 340th and 320th day, with corresponding mean P_4 concentrations prior to "heat" of 9.41, 7.15 and 9.83 ng/mL, respectively.

- Conceptions were observed in Sanga, crossbred, and Jersey cows between 470 – 500 days, 420 – 500 and 380 – 500 days with corresponding P_4 levels of 15.46 – 25.39, 10.13 – 21.04 and 15.46 – 31.50 ng/mL, respectively.

6.1.3.2 Oestradiol and progesterone concentrations in cows during oestrous cycles

- Oestradiol threshold concentrations in Sanga, Freiesian-Sanga crossbred and Jersey cows during oestrous cycle (using commercial EIA) ranged from 15.12±4.10 to 125.28±8.43 pg/mL, 18.98±2.12 and 187.30±9.30 pg/mL and 15.12±4.10 and 138.44±9.24 pg/mL, respectively.
- Mean serum progesterone levels during oestrous cycle in Sanga Freiesian-Sanga crossbred and Jersey cows ranged from 0.42±0.19 to 10.0±1.98 mg/mL, 0.41±0.01 and 9.81±1.85 ng/mL and 0.51±0.01 to 10.87±0.45 ng/mL, respectively.

6.1.3.3 Progesterone and oestradiol levels in dairy cows during gestational stages

- Progesterone (P_4) significantly increased from the first trimester and plateaued in the second and third trimesters of gestation.
- Progesterone concentrations superseded oestradiol (E_2) during the three trimesters until the last 10 days to parturition where E_2 levels became higher than P_4.
- There was a significant decrease in P_4 concentration in the last 10 days before parturition while oestradiol levels massively increased.
- In the first 10 days after parturition, progesterone concentration remarkably reduced to a level averaging 1.63 ±0.24 ng/mL whereas oestradiol level was 38.81±4.91 pg/mL.

6.1.3.4 Reproductive steroids, FSH, and LH concentrations in serum/raw milk postpartum

- There were also significant positive correlations of 0.92, 0.90, 0.71, 0.93 between serum and milk concentrations of oestradiol, progesterone, testosterone, and FSH, respectively, during 45 days postpartum period.
- Serum and raw milk concentrations of LH were significantly, moderately and negatively correlated (- 0.38) in this study.

6.1.3.5 Effect of age on testosterone and oestradiol concentrations in bulls

- Testosterone concentration increased with increasing age from one year and peaked at age four.
- Testosterone concentrations in young bulls, yearlings and those of twelve years and above were similar.
- The highest level of oestradiol was observed in 1 year bulls, whereas bulls of up to 6 months, and 2, 3, 4 and 12 years had similar oestradiol concentrations.

6.1.4 Productive performance of Ghanaian dairy cow

6.1.4.1 Milk yield of dairy cows

- Farms that practised zero grazing with insect proof and regular feed supplementation had the best mean milk yield.
- Jersey cows had the highest mean milk production, followed by Friesian-Sanga, with the Sanga recording the least.
- Milk yield at the first parity was relatively low and thereafter, assumed a sustained increase from second to fifth parities and then declined at the sixth parity.

- Mean daily milk yield decreased with increasing months of the stage of lactation recorded. The highest mean milk production per stage of lactation was observed in the first month of lactation (1 – 30 days), followed by 31 – 149 days and 150 and above days in descending order.
- Better mean milk productive performances were recorded by BCS 3 and 3.5 with the BCS 4 having the best record of the mean milk production.
- Large udder and teat sizes had the highest milk yield, followed by medium and small udder and teat sizes in descending order.
- Season of high quality and quantity of feed availability (major and minor rainy seasons), regular feed supplementation and milking twice a day had good mean milk yield.

6.1.4.2 Lactation Length of dairy cows

- Good housing and regular feed supplementation had lengthier lactation length (LL).
- Lactation length lengthened with increasing parity up to 5 but was shortest in parities 1 and 6.
- The best LLs were observed in BCS 3 – 4 on the 5 scale scoring. Extended LL was observed with increasing rains, medium to large udder size, regular feed supplementation and milking twice a day.
- Intermediate teat size had the longest LL whereas small and large sizes had similar LL

6.1.4.3 Milk composition of dairy cows

- Location Influenced percentage protein but had little effect on fat, lactose, cholesterol, total solids, solid-non-fat, water and ash. Cows in Ashanti Region recorded the highest protein and total solids in milk whereas those in Eastern and Greater Accra Regions had similar values.
- Breed had marked effect on protein, fat, TS and ash composition of fresh milk. Jersey cow had the least protein component. Percentage fat was significantly highest in Jersey cow. Total solids were higher in the milk of Sanga than those observed in the crossbred and Jersey cows.
- Body condition score 3 had highest protein followed by BCS 4 while BCS 2.5 recorded the least value of protein component. Percentage composition of TS, water and ash increased with increasing BCS points. Body condition score had little influence on fat, lactose, cholesterol, and SNF in this study.
- Lactation stage of above 150 days had the highest percentage protein value but it was similar to the value observed in 1 – 30. Lactating cows of the stage of 31 – 149 days had the lowest percentage protein component. Stage of lactation category 1 – 30 days had significantly higher percentage fat whereas 31 – 149 and above 150 days had similar values but lower in fat component.
- The TS and ash components were also highest in above 150 day, followed by 1 – 30 days with 31 – 149 days recording the lowest percentage fat. The three categories of stages of lactation had insignificant effect on percentage lactose, cholesterol, SNF, and water compositions.
- Feed supplementation influenced percentage protein, TS, SNF, water, and ash in that these components increased greatly with increasing intensity of feed

supplementation. Feed supplementation, however, had little effect on percentage fat, lactose, and cholesterol composition in milk.

- Rainy and minor rainy seasons had similar protein composition but higher than that observed in dry season. Percentage TS, SNF, water and ash components also increased greatly with decreasing intensity of dryness but with increasing availability of feed.

6.1.4.4 Milk quality assessment

- ***Milk temperature, pH and specific gravity***
  - The overall means for raw milk temperature, pH and specific gravity among the dairy cows were 2.6 ± 0.43, 6.5 ± 0.03 and 1.026 ± 0.00. All the fixed factors had little effect on raw milk temperature and specific gravity.
  - However, raw milk pH was influenced by breed and stage of lactation such that Jersey and Sanga cows had higher pH values as compared with Friesian-Sanga cow.

- ***Somatic Cell Count***
  - The overall mean for composite MSCC was 134000 ±7498.7 cells/mL.
  - Jersey cows had the highest MSCC followed by Friesian-Sanga crossbred cows. Sanga recorded the lowest MSCC.
  - Parity one had the lowest somatic cell count whiles parity two and three had similar counts.
  - The MSCC greatly decreased with increasing stage of lactation.

- ***Total bacterial count (TBC) and total coliform count (TCC)***

  - Effect of breed, parity, BCS, and SOL were not good determinants of TBC in raw milk.
  - Management differences at different locations had marked influence on TBC and TCC, such that, cows sampled from Ashanti Region had the highest TBC while samples from Eastern Region recorded the least count.
  - Cows kept in good sanitary premises had the least TBC, TCC, and vice versa.
  - The Jersey cows had the least TCC but the value was similar to the one recorded by Friesian-Sanga cows.
  - The TCC increased with increasing stages of lactation.
  - Parity and BCS had insignificant influence on the TCC.
  - In comparing microbial levels in mean TBC and TCC in milk of current study and UK standard, the quality of milk is of low standard.

6.2 Conclusions

6.2.1 Prevalence of ticks and tick-borne diseases, and factors influencing them of dairy cattle

The most prevalent tick species was *Amblyomma variegatum*, followed by *Boophilus decoloratus*, *Rhipicephalus spp.* and *Hyalomma rufipes* in descending order in the hot-humid and Accra plains of Ghana. Dermatophilosis was the most prevalent tick-borne disease (TBD), followed by anaplasmosis and heartwater/cowdriosis in descending order. A PCR assay showed a prevalent rate of 92.50 %, 33.75 % and 10.00 % for *Dermatophilus congolensis*, *Anaplasma marginale* and *Ehrlichia ruminantium* respectively.

Dairy herds maintained in insect-proof barn/housing with adequate feed supplementation recorded the least tick load/infestation as well as TBDs. Dairy cattle herds reared under exclusive zero grazing and good level of biosecurity practices had no prevalence of ticks and the TBDs studied. Range grazing recorded higher prevalence of ticks and TBDs than partial zero grazing and roofed barn. Upon tick infestation, weekly application of acaricide resulted in the reduced tick infestations and incidence of TBDs.

6.2.2 Effect of breed, physiological state and feed supplementation on haemato-biochemical indices of cows

Breed had marked influence on haemato-biochemical indices of dairy cows such that Serum ketone (βHBA) concentrations decreased with decreasing level of beef genotype. ALP, total protein (TP), globulin, direct bilirubin, urea/BUN, cholesterol, HDL levels, chlorine, potassium, and phosphorus ions/cations concentrations differed with breed. Breed had little influence on mean serum glucose and NEFA.

Physiological state influenced erythrocytes indices such that values were generally high during gestation period (especially early gestation). Haematocrit (HCT) values were within the normal physiological range in heifer and cycling cows whereas the values were low in gestation periods. Higher levels of WBC, neutrophils, lymphocytes, eosinophil and basophils were noticed in the first trimester of gestation (G_E). The γGT levels were normal across physiological states except in G_M where the value was more than the normal physiological levels. Non-cycling heifer had slight elevation in serum ALP levels. NEFA levels were higher in mid and last trimesters of gestation. Total protein and albumin concentrations were highest in heifers, followed by CC, G_E, G_M and G_L in descending

order, although, the values fell within the normal reference range. Urea concentration was higher in CC while lower level was recorded in heifer. Blood urea nitrogen (BUN) was highest in cows of G_E and G_M. The differences in serum bilirubin concentrations fell within the normal range. Physiological state of cows was a good determinant of serum HCO_3^- and potassium levels but had little effect on sodium, chloride, calcium, and magnesium cations/ions. Cows in G_L had the lowest serum phosphorus (P) concentration. Haemato-biological indices generally improved with regular feed supplementation.

6.2.3 Reproductive hormones of dairy cattle: Assaying of gonadotrophic and reproductive steroid hormones with commercial EIA

The use of commercial enzyme immunoassay (EIA) for profiling of reproductive steroid and gonadotrophic hormones in serum and milk yielded results that were similar to those reported in literature using bovine specific and other commercial reagents. Progesterone (P_4) concentrations during pre-pubertal stages ranged from 0.21 to 0.96 ng/mL. Physiological onset of impending cyclicity marked by P_4 rise of ≥ 1.0ng/mL occurred at the 415th, 295th, and 285th day in Sanga, crossbred and Jersey cows respectively. The first overt oestrus/"heat" in Sanga, crossbred, and Jersey heifers occurred in 435th, 340th and 320th day with corresponding mean P_4 concentrations prior to "heat" of 9.41, 7.15 and 9.83 ng/mL, respectively in dairy herds setting. Feed supplementation led to early onset of cyclical activity, and conception with increased in P_4 levels with increasing level of feed supplementation.

Oestradiol threshold concentrations in Sanga, Freiesian-Sanga crossbred and Jersey cows during oestrous cycle (using commercial EIA) ranged from 15.12±4.10 to 125.28±8.43

pg/mL, 18.98±2.12 to 187.30±9.30 pg/mL and 15.12±4.10 to 138.44±9.24 pg/mL, respectively. Mean serum progesterone levels during oestrous cycle in Sanga, Freiesian-Sanga crossbred and Jersey cows ranged from 0.42±0.19 to 10.0±1.98 mg/mL, 0.41±0.01 to 9.81±1.85 ng/mL and 0.51±0.01 to 10.87±0.45 ng/mL, respectively.

There were significant positive correlations of 0.92, 0.90, 0.71, 0.93 between serum and milk concentrations of oestradiol, progesterone, testosterone, and FSH, respectively, during 45 days postpartum period. Serum and raw milk concentrations of LH were significantly, moderately and negatively correlated (- 0.38) in this study. Testosterone concentration increased with increasing age from one year old to four old in bulls. The highest level of oestradiol was observed in one (1) year old bulls, whereas bulls of up to 6 months, and 2, 3, 4 and 12 years old had similar oestradiol concentrations.

6.2.4 Factors influencing productive performances and milk quality

Good farm housing (zero grazing; insect proof for maiden exotic breeds), productive breed, parity 2 to 5, good body condition score 3 to 4, well set big udder with medium teat sizes, good feed supplements and milking twice a day gave a better milk yield with normal composition in appreciable lactation period/length.

Quality assessment of milk and udder health through milk somatic cell count yielded an overall mean for milk somatic cell count (MSCC) of 134000 ±7498.7 cells/mL. Jersey cows had the highest MSCC followed by Friesian-Sanga crossbred cows. Sanga recorded the lowest MSCC. The MSCC increased with increasing parity from 1 to 3.

The overall total bacterial/viable count (TBC) and total coliform count (TCC) were 3.0 $x10^6 \pm 7.4\ x10^5$ CFU/mL and $2.9x10^4 \pm 1.4x10^4$ CFU/mL respectively. Cows kept in good sanitary premises had the least TBC and TCC, and vice versa. Early stage of lactation (1 - 30 days) had significantly reduced TCC. In comparing microbial levels in mean TBC and TCC in milk of current study and UK standard, the quality of milk is of low standard.

6.3 Recommendations

- It is recommended that, good management of tick prevention in dairy herds should be a holistic consideration of environmental factors that screen dairy herds from getting exposed to tick-vector.
- Choice of good dairy breed combined with good housing—preferably insect-proof, zero grazing, adequate feeding through supplementation and good level of biosecurity practices should be considered in the dairy industry of Ghana.
- For assessment of dairy cattle health using haematology and serum biochemistry of dairy cattle, effect of breed and physiological state should be deemed imperative.
- Commercial enzyme immunoassay (EIA) could be used for assaying of reproductive steroid and gonadotrophic hormones in serum and milk of Ghanaian dairy cattle.
- Milk quality could be improved when milking is done in a cleaned and sanitized premises together with udder cleaning, hand washing with clean running water and mopping udder and hands prior to milking.
- Further studies should be conducted to determine effect of the various geographical zones on haemato-biochemical indices of cattle in Ghana.

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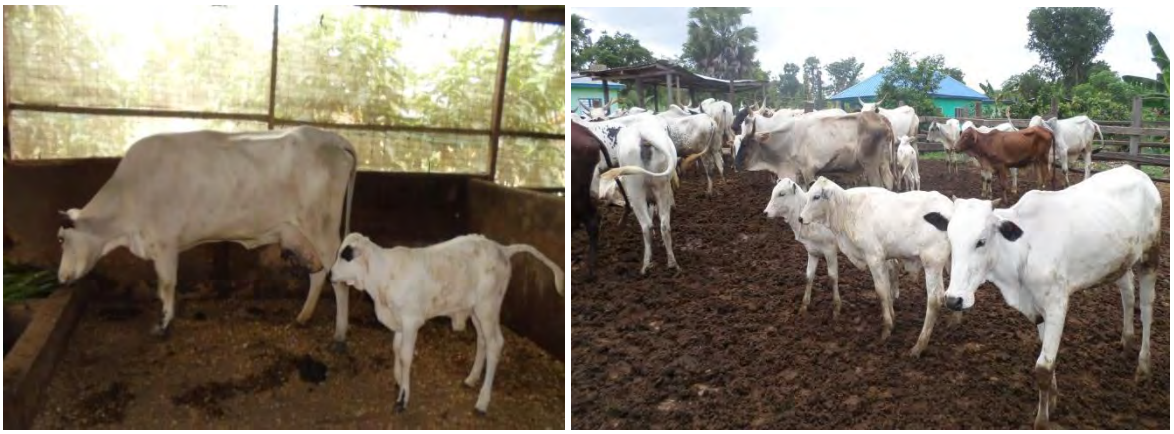
APPENDICES

Appendix A: Breeds of cattle used for the study

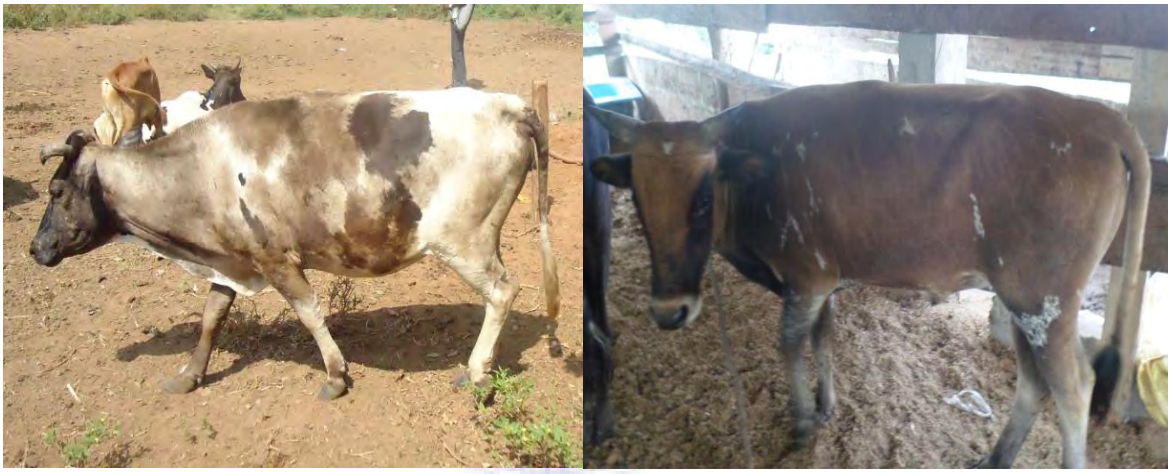


Appendix A 1: Sanga cattle in their production environment

Appendix A 2: Friesian-Sanga crossbred cattle in the study area

Appendix A 3: Jersey cattle in the study area

Appendix A 4: Zebu Cattle in the studi areas



Appendix A 5 West African Shorthorn (WASH) or Ghana Shorthorn (GSH) cattle

Appendix B: Scoring of percentage risk due to level of biosecurity practices (LBSP)

The scoring of percentage risk due to level of biosecurity practices (LBSP) by farms based on major biosecurity components are presented on Appendices. B1 and B2.

Appendix B 1: Showing scoring of percentage risk due to level of biosecurity practices (LBSP) by farms

BS Principles Considered (Weight=W)	Key Performance Area (KPA)	Score (S) based on KPI	Embik	REA	UEW-M	ZA/ RASHID	SUHUM	KARIMA	AMRAHIA	HARUNA	M. ISMAIL
1. Traffic Control (W=24.00 %)	1. Farm land area	1 to 4	2	2	2	2	2	3	3	4	4
	2. Production Area	1 to 4	1	1	1	1	1	1	1	3	4
	3. Type of Kraal/Barn	1 to 4	2	2	2	2	1	2	3	3	4
	4. Structure Design	1 to 3	1	1	1	2	1	1	1	3	3
	5. Visitor	1 to 3	1	2	1	2	1	3	3	3	3
	6. Log book	1 to 2	2	1	2	2	2	1	2	2	2
	S_A Obtained by farm		1.50	1.50	1.50	1.83	1.33	1.83	2.17	3.00	3.33
	S_W 1; $S_W = S_A * W$		0.36	0.50	0.50	0.61	0.44	0.61	0.72	1.00	1.11
2. Isolation (W=24.00 %)	1. Health Personnel	1 to 4	2	2	1	2	2	2	1	3	3
	2. Quarantine	1 to 3	2	2	3	1	1	2	1	3	3
	3. Design	1 to 3	2	1	2	1	1	2	2	3	3
	4. Animals	1 to 3	1	1	1	2	1	2	2	2	2
	5. Workers Movement	1 to 4	2	2	1	3	1	3	2	4	4
	S_A obtained by farm		1.80	1.60	1.60	1.80	1.20	2.20	1.60	3.00	3.00
	S_W 2		0.43	0.53	0.53	0.60	0.40	0.73	0.53	1.00	1.00
3. Sanitation (W=24.00 %)	1. Change room	1 to 2	2	2	2	2	2	2	2	2	2
	2. Foot bath	1 to 3	2	2	2	2	2	2	2	3	3
	3. Footwears/protective gear	1 to 3	3	1	3	3	3	3	2	3	3
	4.Staff	1 to 4	2	2	2	3	2	3	1	4	4
	5. Postmortem/Necropsy	1 to 4	2	2	1	3	2	3	2	3	4
	S_A Obtained by farm		1.69	1.51	1.57	1.93	1.46	2.09	1.69	2.69	2.78
	S_W 3		0.56	0.50	0.52	0.64	0.49	0.70	0.56	0.90	0.93

BS=Biosecurity; KPI=Key performance indicator; S_A =Average score; S_W=Weighted Score; W= % weight

Appendix B 2: Showing scoring of percentage risk due to level of biosecurity practices (LBSP) by farms based on major biosecurity principles, and management, Key performance areas (KPA) and Key performance indicators (KPI)—truncated, (*continuation from Table Appendix B 1*)

	KPA	KPI Score	Embik	REA	UEW-M	ZA	SUHUM	KARIMA	AMRAHIA	HARUNA	M. ISMAIL
4. Management (W= 28.00 %)	1. Feed Supplementation	1 to 3	1	1	2	2	1	2	3	3	3
	2. Pasture availability	1 to 3	2	2	3	2	1	3	3	3	3
	3. Breedinding management	1 to 4	1	2	1	2	2	4	4	4	4
	4. AI with improved breed semen	1 to 2	1	1	2	2	2	2	1	2	2
	5. AI kits	1 to 2	2	2	1	2	2	2	1	2	2
	6. First aid kits	1 to 2	1	1	1	1	1	2	1	2	2
	7. Management of cattle at herd level	1 to 3	1	1	1	3	1	3	2	3	3
	8. Grazing system	1 to 3	1	1	1	2	1	2	3	3	3
	S_A Obtained by farm		1.29	1.38	1.5	2	1.38	2.5	2.25	2.75	2.75
	S_W 4		0.36	0.39	0.42	0.56	0.39	0.70	0.63	0.77	0.77
S_{WRP} calculations	S_{WRP} = $(S_w1+S_w2+S_w3+S_w4)/4*100$	%	43.00	38.40	38.92	46.34	33.28	68.00	45.46	72.37	75.92
REMARKS	Risk due to biosecurity practice lapses		M risk	L risk	L risk	M risk	L risk	H risk	M risk	H risk	H risk
	Level of biosecurity practices observed by farm		Low	Medium	Medium	Low	Medium	Very Low	Low	Very Low	Very Low

S_A =Average score; S_{WRP} = *percentage risk weighted Score due to Level of biosecurity practices observed; H=High level of biosecurity observation; L=Low; M=Medium/moderate; S_W=Weighted score; W= % weight.*