EFFECT OF THERMAL INDICES AND RELATIONSHIPS WITH MILK YIELD IN EXOTIC DAIRY COWS USING INVASIVE AND NON-INVASIVE MARKERS

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BY

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA,
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DEPARTMENT OF ANIMAL SCIENCE

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ZARIA

JUNE, 2017

DECLARATION

I declare that this my thesis entitled "**EFFECT OF THERMAL INDICES AND RELATIONSHIPS WITH MILK YIELD IN EXOTIC DAIRY COWS USING INVASIVE AND NON-INVASIVE MARKERS**"has been carried out by me in the Department of Animal Science under the supervision of Professor (Mrs) Grace Iyeghe-Erakpotobor, and Dr. (Mrs) O. M Daudu. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree ordiploma at any University.

Roseline Fugah Raymond _____ _____

Signature **Date**

CERTIFICATION

This thesis titled "**EFFECT OF THERMAL INDICES AND RELATIONSHIPS WITH MILK YIELD IN EXOTIC DAIRY COWS USING INVASIVE AND NON-INVASIVE MARKERS**By Roseline Fugah Raymond meets the regulations governing the award of degree of masters of Science in Animal Science of the Ahmadu Bello University, Zaria and is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

I humbly and sincerely dedicate this research work to God Almighty,my beloved husbandMr. Samaila Phillibus Asaryaand Dear Son Micah Samaila Phillibus Asarya, my late Father Mr. Raymond Yaro Fugah and my beloved Mother Mrs. Maryam Raymond.

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LIST OF ABBREVIATIONS

ALT –Alanine aminotransferase

AST – Aspertate aminotransferase

Ta – Ambient temperature

ANOVA – Analysis of variance

B.p.m – Beats per minute

R – Correlation

ET – Ear temperature

GLM –General linear model

^{o}C – Degree Celsius

R^2 - Coefficient of determination

REG – Regression

RH – Relative humidity

RR – Respiratory rate

RT – Rectal temperature

SAS – Statistical analysis software

ST – Skin temperature

T3 – Triidothyronine

T4 - Thyroxine

THI – Temperature Humidity Index

Abstract

A study was conducted to evaluate the effects of thermal indices and relationship with milk yield in dairy cows using invasive (glucose, total cholesterol, triiodothyronine (T_3), thyroxine (T4), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and inorganic phosphate) and non-invasive (Rectal temperature (RT), respiration rate (RR), ear temperature (ET) and skin temperature (ST) markers in the Sahel Savannah of Nigeria. Fifteen (15) clinically healthy dairy cows of three breeds; five each from Holstein Friesian purebred, Simmental and Brown Swiss, aged 5-8 years from Sebore Farms were used for the experiment. The animals were maintained on similar feeding programme under hot-dry and cold-dry season. Data collected includes thermoregulatory variables: Rectal temperature, respiration rate, ear temperature and skin temperature), blood biochemical variables (glucose, inorganic phosphate, total cholesterol, triiodothyronine (T_3), thyroxine (T_4), aspartate aminotransferase (AST), and alanine aminotransferase (ALT), daily milk yield and milk component traits (protein and fat percentage). All the thermoregulatory parameter values differed significantly ($P<0.05$) between the seasons with the exception of ear temperaturewhich was statistically similar ($P>0.05$). Rectal temperature, respiratory rate and skin temperature were significantly ($P<0.05$) higher during hot-dry season compare to cold-dry season.Glucose, ALT and AST differed significantly ($P<0.05$) across the breeds of dairy cows while cholesterol, phosphorus, T_3 and T_4 were statistically similar ($P>0.05$). The serum ALT was higher in Simmental (47.74±1.33iu/l) and Brown Swiss (46.59±1.33iu/l) which differed significantly ($P<0.05$) from Holstein Friesian cows (39.1±1.33iu/l).However, all the biochemical parameters differed significantly ($P<0.05$) between the cold-dry and hot-dry seasons with the exception of T_3 which was statistically similar ($P>0.05$).Milk yield was significantly ($P<0.05$) higher during the cold-dry season (8.03±0.24kg)

than the hot-dry season (6.93±0.21kg) while fat (P<0.05) was significantly higher during the hot than the cold season. Holstein Friesian had the highest average daily milk value (8.16kg) while Simmental cow had the lowest milk volume (7.08kg). Simmental and Brown Swiss cows had the highest percentage of fat (4.07%) which were statistically different (P<0.05) from Holstein Friesian (3.88%). Protein showed no (P>0.05) significant difference between breeds. Daily milk yield was significant, low and negatively correlated with temperature (R=-19, P=0.0005) while moderate, significant and negative correlations was observed with THIand RH (R=-0.24, P=0.0005 and R=0.26, P=0.0061) Fat had significant, low and positive relationship with temperature (R=0.17, P=0.0019) while low and positive relationship existed with THIand RH(R=0.19, P=0.76 and R=0.12, P=0.059). Protein had low and negative correlations with all the environmental factors with the exception of temperature which was positively associated (R=0.003, P=0.9501).Daily milk yield was significant and highly correlated with phosphorus (R=0.52; p=0.02) and T_4 (R=0.94; p=0.05) but significant, high and negatively correlated with T_3 (R=-0.51).Cholesterol was significant, high and negatively correlated with DMY (R=-0.28). AST was significant, moderately and negatively correlated with DMY (R=-0.26). ALT was negatively correlated with DMY. The regression analysis for prediction of milk yield showed that all the invasive markers combined together best explained daily milk yield (R^2= 0.52) in Brown Swiss dairy cows compare to Simmental and Holstein Friesian which were weakly predicted (R^2= 0.18). The regression analysis for prediction equation using non-invasive markers, showed that all the non-invasive markers combined together explains only (R^2= 0.05) of milk yield when the breeds were pooled. It was concluded that invasive parameters could be a veritable tool in predicting daily milk yield of different genotypes of dairy cows under the Sahel Savannah condition of Nigeria.

CHAPTER ONE

1.0 INTRODUCTION

Heat stress plays a significant role in cattle performance and likely will be of even greater importance in the future as climate change continues. Cattle are produced in a wide range of environments, some of which present thermal challenges to productive performance, even survival in extreme cases. High temperatures can have negative consequences for milk production, and for reproduction, welfare and health, in dairy cattle. Especially high yielding dairy cattle would be susceptible because their thermo-neutral zone is rather limited as compared to low yielding cows (Kadzere *et al.,* 2002). As a result of heat stress, losses of 600 to 900 kg milk per cow per lactation has been reported with regard to milk production (West, 2003).Selection of cattle adapted to warm environments represents one strategy to mitigate the effects of heat stress. There are a number of environmental factors that contribute to heat stress; these include high temperature, high humidity and solar radiation. In tropical regions, animals must be able to balance heat production and heat gain from their environments with dissipation of heat through the skin and respiratory surfaces; simultaneously, they must avoid excessive thermal energy incoming from the environment (Da-Silva *et al.,*2003).

The productivity and health of these animals are being affected by adverse meteorological conditions prevailing in the tropical Africa, predisposing them to hyperthermia (heat stress) and hypothermia (cold stress) (Da-Silva *et al.,*2003). In the tropical conditions of Nigeria, heat stress is common during dry season, occurring between November and May and with a mean monthly rainfall of less than 51mm (Igono *et al*., 1982; Walter, 1969). The harmattan season is characterized by marked fluctuations in ambient temperature (AT) with high AT in the afternoon

hours of the day and relatively low temperature of about 10 ^{O}C in the evening and early morning hours of the day. The season is associated with a dry cold and dust-laden wind that blows from Sahara desert and low relative humidity (RH) (Ayo *et al.,* 1998a). The hot-dry season is also characterized by high ambient temperature, relative humidity and long duration of sunshine. Of all the stress factors adversely affecting dairy production in the tropical environment, ambient temperature manifesting in hypothermia and hyperthermia and humidity changes are the most crucial. It has been shown that high ambient temperature and high relative humidity with wide fluctuations in the values result in heat stress which may alter many physiological parameters in livestock (Ayo *et al.,* 1998b; Sinkalu *et al.,* 2009). These may impair homeostatic mechanisms resulting in pathological changes and alteration in body homeostasis (Teeter *et al.,* 2005).

The general homeostatic responses to thermal stress in mammals include a decrease in fecal and urinary water losses, a reduction in feed intake and production, and an increase in sweating, respiratory and heart rates. Most of the adjustments made by the cow involve dissipating heat to the environment and reducing the production of metabolic heat (Kadzere*et al.,* 2002). As milk production increases in dairy cattle, the metabolic heat production rises with the metabolizing of large amounts of nutrients, which makes the high producing cow more vulnerable to high environmental temperatures and humidity than animals that are metabolically less active (Kadzere *et al.,* 2002). High producing dairy cows must dissipate large amounts of heat produced during the metabolism of high dietary energy used for body maintenance and milk synthesis. Several studies reported that heat stress in dairy cattle affects production and reproduction (Garcia-Ispierto *et al.,* 2007; Morton *et al.,* 2007; Bryant *et al.,* 2007). Despite the moderate effects of THI on milk production, some blood parameters related to energy balance and enzyme activities had significant alterations and cows in the middle of lactation had the highest changes

in these parameters (Dikmen *et al.,* 2008). These authors have indicated that the thermoregulatory characteristic of animals associated with this phenotype is probably due to a lower metabolic rate, increased sensible heat or evaporative heat loss, more efficient transfer of heat to the surface, or a combination of these adaptations. They further reported that dry bulb temperature is nearly as good a predictor of rectal temperatures of lactating Holsteins in a subtropical environment as THI. Hormones known to be homeorhetic regulators are also implicated in acclimatory responses to thermal stress. These include thyroid hormones, prolactin, somatotropin, glucocorticoids and mineralcorticoids. Triiodothyronine (T_3) and thyroxine (T_4) are hormones associated with metabolic homeostasis and susceptible to climatic changes (Perera *et al.,* 1986). Johnson *et al*. (1988) also showed decline in thyroid hormones T_3 and T_4 in response to heat stress which is an attempt to reduce metabolic heat production in the cow. Stress response hormones (glucocorticoids) are elevated during initial heat stress exposure and then become depressed with prolonged periods of thermal stress. Wheelock *et al*. (2006) demonstrated that heat stress elevates plasma insulin concentrations in lactating dairy cows and this may be important for glucose disposal in peripheral tissues. The blood biochemical profiles are considered important in evaluating the health status of animals. The estimates of biochemical constituents are the prerequisites to diagnose several pathophysiological and metabolic disorders in cattle (McDowell *et al*., 1992). Numerous authors reported close relationship between blood levels of calcium, phosphate, total protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and reproductive traits in dairy cows (Malik *et al.,*2003).

1.1 Justification

There is paucity of information on the use of thermoregulatory and biochemical parameters as markers of heat stress in dairy cows reared in Northern Nigeria especially the Sahel savannah regions. Information on effect of varying environmental/climatic conditions on thermoregulatory and biochemical parameters is particularly important in Northern Nigeria as dairy milk production is generally low in this area compared to the temperate regions of the World. A study to investigate whether or not thermoregulatory and biochemical parameters could be used to estimate adaptability of exotic dairy cows to varying environmental conditions is then necessary. The vital and biochemical parameters are of significant diagnostic values for the spot assessment of health status of dairy cows. These parameters have been demonstrated to be important indices of health, production and adaptability to prevailing environmental conditions in livestock (Oladele *et al.*, 2001; Oladele *et al.*, 2005; Adenkola and Ayo, 2009).

1.2 Objectives

The objectives of this research were to:

1) Evaluate the variation in thermoregulatory(non-invasive)parameters in acclamatory responses to thermal stress in exotic breeds of dairy cows under Sahel (semi- arid) region of Nigeria

2) Evaluate the variation in bloodbiochemical (Invasive) parameters in acclamatory responses to thermal stress in exotic breeds of dairy cows under Sahel (semi- arid) region of Nigeria

3) To establish the relationship between milk production characteristics and blood biochemical profiles inexotic breeds of dairy cows under Sahel (semi- arid) region of Nigeria

4) To establish prediction equations for total milk yield using invasive and non invasive parameters

1.3 Research Hypotheses

H_0There is no variation in thermoregulatory(non-invasive)parameters in acclamatory responses to thermal stress inexotic breeds of dairy cows under Sahel (semi- arid) region of Nigeria

H_aVariation existed in thermoregulatory(non-invasive) parameters in acclamatory responses to thermal stress inexotic breeds of dairy cows under Sahel (semi- arid) region of Nigeria

H_0There is no variation in bloodbiochemical (invasive)parameters in acclamatory responses to thermal stress inexotic breeds of dairy cows under Sahel (semi- arid) region of Nigeria

H_a Variation existed in bloodbiochemical (invasive) parameters in acclamatory responses to thermal stress inexotic breeds of dairy cows under Sahel (semi- arid) region of Nigeria

H_o There is no relationship between milk production characteristics and blood biochemical profiles inexotic breeds of dairy cows under Sahel (semi- arid) region of Nigeria

H_a There is relationship between milk production characteristics and blood biochemical profiles inexotic breed of dairy cows under Sahel (semi- arid) region of Nigeria

H_o The prediction equation for total milk yield using invasive and non-invasive parameters is weak using coefficient of determination as judgement criteria

H_a The prediction equation for total milk yield using invasive and non-invasive parameters is strong using coefficient of determination as judgement criteria.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Heat Stress in Dairy Cattle

Over the past two decades, global animal production has increased, especially in tropical and subtropical areas; this growth is driven by a strong increase in meat and milk production in developing countries, mainly in the tropical belt and semi-arid to arid areas (FAO, 2010). These Authors indicated that the high production demand is related to demography, an increase in animal protein consumption per capita and rising consumer income. In many cases, the rise in animal production was achieved through intensification based on animals and rations frequently imported from western countries. More than 50% of total world meat and 60% of milk originates from tropical and subtropical areas (FAO, 2010). It is predicted that livestock production in these areas will continue to sustain the world's future meat and milk production.

Efficient productive performance of lactating dairy cattle in tropical/ subtropical and arid environments throughout the world is impacted by a multiplicity of factors such as: the physical environment, social-economic status of producers, available nutrients, adaptability and genetic composition of cattle, intensive or extensive management systems, and available reproductive technology (Vesna *et al*., 2011). Seasonal summer reductions in productive and reproductive performance of lactating cows is associated with decreased thermoregulatory competence of lactating dairy cows, partially due to intensive genetic selection for high milk production (Al-Katanani *et al*., 1999). With higher production, the associated increase in dietary dry matter intake (DMI) enhances heat increment, which when coupled with increases in metabolic heat production to produce milk, aggravates thermal balance during the stressful summer period (Renaudeau *et al*., 2010). There is increasing concern on production losses, because of high

ambient temperature which is justifiable not only for the tropical area but also for countries occupying the temperate zone in which heat stress is an occasional problem during the 2 to 3 summer months and/or during hot spells (Al-Katanani *et al*., 1999). In North America, Australia and Europe, these events caused morbidity, mortality, directly and indirectly reduced performance, resulting in dramatic economic losses and animal welfare concerns(Nienaber and Hahn, 2007). In 2006 a major heat wave moving across the USA resulted in the death of 25, 000 cattle and 700, 000 poultry in California (Nienaber and Hahn, 2007). According to the Intergovernmental Panel on Climate Change (IPCC, 2007), the likelihood of these heat wave events is projected to increase both in number and in intensity. More generally, the increase in global average surface temperature by year 2100 may be between 1.8^{O}C and 4.0^{O}C(IPCC, 2007). These predictions suggest that negative effects of heat stress on livestock production will become more apparent in the future as the world population and food supply continue to increase rapidly especially in tropical and subtropical regions, while the free land gets limited (i.e. desertification, intensive agriculture, ethanol (biodiesel) production, etc.)IPCC, 2007. Genetic selection program carried out in optimally controlled conditions clearly improved production traits (meat, milk or eggs); however, it may enhance the animal's susceptibility to high ambient temperature (Ta) because of the strong relationship between production level and metabolic heat production (Al-Katanani *et al*., 1999). Because of the recognition that high ambient temperature is a current and a future critical problem for livestock production, there has been a great deal of research and development of ways to reduce heat challenge of animals subjected to a short or an extended period of high ambient temperature. (Renaudeau *et al*., 2010).

Heat stress is a condition caused by an animal's inability to dissipate body heat effectively to maintain normal body temperature, a vital process known as thermoregulation (Pirkkala *et al*.,

2001). Cattle not only gain heat from the environment through solar radiation (exposure to sunlight), high ambient temperatures, and humidity, they also produce additional heat internally through fermentation in the rumen during digestion(Webster, 1973). Within the beef and dairy industry, there has been a tremendous focus on management interventions for heat stress in feedlot cattle as a result of large death losses induced by extreme weather events (Busby and Loy, 1997; Mader *et al*., 2006). Regardless of the production system, animal well-being is just as vital in the cowherd as in later stages of production. Consequently, it is important that all options available are considered to improve the well-being of animals (Mader *et al*., 2006).

Heat stress results from a negative balance between the net amount of energy flowing from the animal to its surrounding environment, and the amount of heat energy produced and absorbed by the animal(West, 1994). Essentially, cattle that are producing and absorbing more heat from the environment than they can dissipate will experience heat stress, While cattle can acclimatize to hotter conditions, an individual animal's adjustment period encompasses anywhere from 2-7 weeks (Blackshaw and Blackshaw, 1994). Additionally, animals that exhibiting higher levels of performance tend to generate more heat due to their inherently higher levels of productivity, hence, they experience more heat stress (West, 1994). As emphasis on productivity continues to mount, heat stress mitigation will likely receive even greater attention in the dairy industry.

Cattle respond to environmental conditions differently than humans, and are more sensitive to environments with high temperature and humidity (Webster, 1973). Therefore, cattle are more susceptible to heat stress than humans under the same environmental conditions. Core body temperature of cattle is typically higher than ambient temperature, which helps ensure that heat from the animal flows to the environment (Collier *et al*., 2006). As with all animals, cattle can dissipate heat to the environment through radiation (such as the infrared radiation emitted by

animals that can be seen with infrared cameras), conduction (transfer of heat between objects in physical contact), and convection (transfer of heat between an object and the environment), but these cooling methods become less effective as ambient temperature rises becausewhen temperature and humidity are high, the primary means by which cattle dissipate heat is by evaporation becomes less effective (Blackshaw and Blackshaw,1994;West, 2003). Evaporative cooling (such as sweating and panting) is essential to maintain body temperature during heat-related events, open-mouthed breathing and panting are some of the most obvious signs of heat stress(West, 2003). This is often accompanied by seeking shade, excessive salivation, and foaming around the mouth. When humidity is high, evaporative cooling is compromised, and even these heat stress-related behaviors may not effectively prevent a rise in body temperature (West, 2003). Planning ahead for heat stress mitigation and making necessary adjustments before the onset of symptoms can improve both the performance and well-being of the animal.

2.1.1 Risk factors for heat stress

Ambient temperature and humidity are environmental conditions that collectively impact heat stress, and they are often combined into one metric called the temperature humidity index (THI)(Dikmen and Hansen, 2009). The THI has been shown to be a reliable indicator of heat stress in cattle, animals can often endure higher temperatures if humidity is low, and the risk for heat stress increases dramatically as humidity increases, even at lower ambient temperatures(Dikmen and Hansen, 2009). Adjusting the THI for wind speed and solar radiation increases predictability of heat stress (Mader *et al*., 2006). In addition to these daytime conditions, nighttime conditions (minimumwind speed, minimum solar radiation, and minimum THI) also impact heat stress in cattle because cattle can often dissipate significant heat during the night if temperatures are lower(Mader*et al*., 2006). Characteristics of individual animals can

also position them at higher risk for heat stress. Hide color is a well-known risk factor because dark hair has lower reflectance values and dark skin absorbs a greater proportion of solar radiation (93% thermal absorption for black skin and 43% for non-pigmented skin; Da-Silva *et al*., 2003). Predictably, animals with black hides spend significantly more time in the shade (89% for black hides and 55% for white; (Gebremedhin,*et al*., 2011). Dark-hided cattle are 25% more stressed at temperatures above 25 degrees Celsius when compared to light-hided cattle (Brown-Brandl, *et al*., 2006). Brown-Brandl *et al*. (2006) also identified other risk factors for heat stress aside from hide color, including history of respiratory pneumonia, level of fatness, and temperament. Brown-Brandl *et al.* (2006) also established that excitable animals were 3.2% more stressed than their calm counterparts.

2.1.2 Modes of heat exchange from animal to environment

An animal must continuously interact with its thermal environment through heat exchange processes to remain near its body temperature,a dairy cow has an average body temperature ranging from 25^{o}C to 26^{o}C (Kadzere *et al.,*2002). Where as that of beef cattle ranges from 38.5^{o}C to 38.6˚C, and a rise or fall of 1°C in body temperature in cattle is sufficient to produce detectable changes in a number of physiological processes (McDowell, 1992). To maintain this temperature in such narrow limits requires sensitive and immediate acting mechanisms.

An animal is said to be in its thermo-neutral zone when it is in a temperature range that requires the least thermoregulatory effort, and temperature regulation is achieved by non evaporative physical processes alone (Hillman et al 1985). The thermo-neutral zone is bounded by a lower and upper critical temperature, which is dependent on the interaction between multiple environmental parameters such as wind, humidity, and ambient temperature(Hahn*et al.,* 2009). When an animal is in its thermo-neutral zone, the variance among animals in body temperature is

small, and as the temperature exceeds the species thermo-neutral zone, the variance increases due to differences among animals in their ability to cope with heat or cold stress (Hahn*et al.,* 2009). These differences are manifested through a complex interaction between anatomical, physiological, and behavioural factors which are dependent on the life stage, nutrition, genetics, previous degree of heat or cold stress, and health of the animal creating a dynamic thermo-neutral zone (McDowell, 1992; Hahn, 1999). Behaviour changes, such as seeking shade or sheltering themselves from the wind, are the first mechanism to account for heat lost or gained (Hillman et al 1985). If behavioural changes do not minimize the heat lost or gained, non-evaporative physical processes that involve the exchange of heat between an animal and its environment are used, which include conduction, radiation, and convection (McDowell, 1992). Resistance to conductive (i.e. passage of heat energy from particle to particle) heat transfer is proportional to the temperature gradients within the animal and the outer extremities and the environment (McDowell, 1992; Finch, 1986).

The act of heat flow originating from the core and spreading to the skin is known as tissue conductance (Finch, 1986). As an animal increases in weight, its tissue conductance decreases linearly which makes the animal become more susceptible to heat stress while decreasing its susceptibility to cold stress (Finch, 1986). This is due to smaller sized animals having a larger surface area per unit of body weight making them lose heat more rapidly than larger animals (McDowell, 1992). During cold stress conditions the opposite occurs due to the animal wanting to retain its body heat, while the environment is absorbing it due to the differing temperature gradients. The temperature gradient between the outer extremities and environment are influenced by the rate of air flow across the skin and physical properties of the animal coat (Finch, 1986). Olson *et al.*(2003), found evidence of a major gene affecting hair length in two

South American heat tolerant Bos Taurus breeds, Senepol and Tuli. The gene referred to as "slick hair", produces a very short and sleek coat which allows for increased heat loss. It has been shown that *Bostaurus* animals with darker hair coats have a warmer internal body temperature and body surface temperature than their light colored counterparts (Finch *et al.,* 1984;Brown-Brandl *et al.,* 2006). similar results were found when comparing surface body temperature for black, red, and white hided animals with black and red hided being 11.6°C and9.1°C warmer than white hided animals, during cold conditions a heavier hair coat will impede the arrival of cold air and warm air will remain, which allows for an animal to retain body heat(Brown-Brandl *et al.,* 2006). The ability of an animal to internally direct heat outward or inward via conduction coupled with convection (i.e. heat exchange through a liquid or gas) is accomplished by vasodilation or vasoconstriction of blood vessels near the skin and lungs (Davis *et al*., 2003). The process of removing heat via the bloodstream becomes increasingly important as body heat rises due to a decreased core to skin gradient (McDowell, 1992). Furthermore, an increase in blood supply to the skin causes a concurrent increase in evaporative heat loss via respiration and sweating (Ingram *et al.,* 1967).

An animal first exposed to an adverse environment reacts initially by activation or acceleration of non-evaporative processes to remain at thermal equilibrium, which involves short-term adaptive changes in behavior and physiology, such as seeking shade or increased peripheral blood flow during heat stress (Nienaber and Hahn, 2007). If non-evaporative physical processes fail to keep an animal at thermal equilibrium, evaporative processes take over (Hahn, 1999).

Resistance to evaporative heat transfer (i.e. vaporization of water from body surface and respiratory tract) is a function of the gradient through which the water vapours move (Finch, 1986). Evaporative heat transfer is not dependent on the temperature, which becomes important

when the environment is warmer than the animal's body temperature and would result in the inward flow of heat from the environment to the animal (Davis *et al*., 2003) Animal factors that affect the efficiency of evaporative heat loss from the skin surface are sweat gland density, function and morphology, hair coat density, length, and color and regulation of epidermal vascular supply (Da-Silver *et al*., 2003; Collier *et al*., 2008). A rise in respiratory heat loss through panting is one of the first physical signs of an animal experiencing heat stress (Nienaber and Hahn, 2007). Also, a heat or cold stressed animal's immune system becomes suppressed and their cellular proteins lose their structure and function causing an increased susceptibility to sickness(Da-Silva *et al*., 2003). These negative consequences cause a decrease in overall production efficiency due to energy being used for processes other than growth or immune regulation(Da-Silva *et al*., 2003). Cold or heat stress has deleterious effects on female and male fertility (Hahn, 1999). After 2 to 4 days of heat or cold exposure depending on the individual animal and the degree of heat or cold exposure, mobilization of heat dissipation or retention functions (physiological coping) will have progressed to the point that acclimation is apparent (Hahn *et al*., 2009). Phenotypic acclimation is defined as the "within lifetime phenotypic response" to environmental stress and relies heavily on the endocrine system (Collier *et al*.,2008). The entire process of acclimation takes around 8 days and is dependent on the animal and degree of heat or cold exposure. Once completed, the animal's body temperature fluctuates around a new set point (Hahn*et al*., 2009). Behavioral and physiological processes aid in keeping an animal's body temperature near its set point, but under severe conditions, heat gain or loss is usually greater than an animal can remove or produce to equalize heat lost to heat gained. Due to this difference an animal stores extra or lacks enough body heat until the severity of the stress

decreases. For example, an animal is aided by cooler nighttime temperatures during summer conditions or warmer daytime temperatures during winter conditions (Mader*et al*., 2006).

These night and day low stress intervals during summer and winter conditions, respectively, allow for an animal to remove excess heat accumulated during the day or gain body heat which was lost during the night (Mader*et al*., 2006).

2.1.3Measurements used as predictors for heat stress and cold stress

Multiple factors influence the amount of heat lost or gained in a certain environment, with one of these being the external conditions. An example would be the cumulative effects of ambient temperature, relative humidity (RH), solar radiation and wind speed(Mader *et al*., 2006). Over the years multiple combinations of these effects have been used to create an index value that takes into account multiple external factors in order to accurately predict the heat load for a specified period(Gaughan *et al*.,2008). The THI has been widely used as an indicator of thermal stress in livestock for the past forty years, Other THI derivations have been developed using dry bulb temperature in combination with wet bulb temperature, relative humidity, or dew point (Gaughan *et al*.,2008). Dry-bulb temperature quantifies the air (ambient) temperature while disregarding the temperature due to radiation and moisture whereas Wet bulb temperature, relative to dry bulb temperature, is a measurement of the amount of moisture in the air (Mader *et al*., 2006), Relative humidity (RH) is a measure of how much moisture is present compared to how much moisture the air could hold at that temperature.

THI formulae:

• THI1 = [0.8× TDB] + [(RH / 100) × (TDB − 14.4)] + 46.4 (Mader *et al*., 2006)

• THI2 = 0.72 *(TDB + TWB) + 40.6 (NRC, 1971)

A common method of quantifying THI values is to arrange them into a table to serve as a benchmark to assess the predicted heat severity, referred to as the Livestock Weather Safety Index (Mader *et al*.,2006). The outlined THI formulae can be effectively used as an indicator of an animal's susceptibility to heat stress. Mader *et al.* (2006) used panting score to determine the adjustments to THI for Wind Speed and Radiation. The THI is an index based on environmental conditions and does not account for animal characteristics such as breed, coat color, management practices, or the cumulative effect of heat load and natural cooling (Gaughan *et al*.,2008).

2.2 Genetic Selection for Heat Tolerance

The goal of any selection program for heat tolerance must be to develop cattle that can perform in challenging environments while maintaining high levels of productivity, Simulated dairy production data has suggested that it may bemore effective to select for heat tolerance within a high milk-producing breed than it would be to select for high milk production within a breed that is highly adapted to hot climates, due to the increased number of generations for the adapted breed to reach comparable levels of milk production (Nardone and Valenti, 2000). Although this result may be influenced by the fact that milk production heritability estimates are generally lower than estimates of heritability for heat tolerance, it does indicate that selection for heat tolerance could be an efficient way to increase adaptability and resilience in high producing animals (Nardone and Valenti, 2000).

Heat tolerance is a heritable trait, so genetic selection can be utilized to increase heat tolerance, provided that the phenotypes and tools exist to make these selection decisions (Ravagnolo *et al*., 2000).Two common phenotypes in the literature include respiration rate, measured as breaths per minute, and body temperature regulation, because respiration rate is fairly labor intensive to collect, body temperature regulation has been the preferred method for studies of heat

tolerance(Dikmen, *et al*., 2012). Body temperature regulation heritability and other phenotypes can be collected using body temperature probes either in the ear (tympanic), rectum, or vagina, through surface body temperatures, or internal body temperatures collected utilizing rumen temperature boluses (Hillman*et al*., 1985).

2.3 Physiological Responses to Heat Stress

Any changein the environmental conditions, as is the case duringheat stress, threatens the normal metabolic balanceand usually produces a positive feedback once thetemperature is above the upper critical temperature (UCT)(Hayes *et al*., 2009). In dairy cattle, asmilk production increases, metabolic heat productionrises with the metabolism of large amounts ofnutrients, which makes the high-producing cow morevulnerable to high ambient temperatures and humidity than animals that are less active metabolically,'Metabolism and productivity run parallel' (Coppock*et al*.,1982). High-producing cows are affected more thanlow-producing cows because the Thermo-neutral zone (TNZ) shifts downward as milk production, feed intake, and heatproduction increase, assumingthat the heat dissipation mechanisms of both types ofcows are similar (Coppock*et al*.,1982). Continued genetic progress suggests that milk production will increase and so there willbe detrimental effects of heat stress on the moderndairy cow (Coppock*et al*.,1982). Heat stress increases loss of body fluiddue to sweating and panting and if this continuesunchecked, the fluid loss can reach a critical level,becoming a threat to thermoregulation and cardiovascular function (Silanikove, 1994).The general homeostatic responses to thermalstress in mammals include reduction in fecal and urinary water losses, reduction in feed intake and production, increased sweating, as well as initial increases in respiratory rates and heart rates, which would slow down if heat stress persists(Silanikove, 1994). In response to stress, the dairy cow brings physical, biochemical and physiological

processes into play to try and counteract the negative effects of heat stress and maintain thermal equilibrium meanwhile, Most of the adjustments made by the cow involve dissipating heat to the environment and reducing the production of metabolic heat (Blazquez *et al*., 1994).

2.3.1 Physical responses to heat stress

Responses of the cow to temperatures above theThermoneutral zone (TNZ) are varied, these include raised respiration rates and rectal temperature,panting, drooling, reduced heart rates, and profusesweating, decreased feedintake as well as reduced milk production (National Research Council, 1989; Abdel-Bary *et al*.,1992; Blazquez *et al*.,1994; Omar *et al*., 1996).Physical responses to heat stress in dairy cowsappear to be breed-specific with the *B.indicus* and other tropical breeds being less responsive to thermal stress than *B. taurus* cattle, thedifferences in response to heat stress between cattlebreeds are attributed to varying levels of adaptabilityto hot environments(Finch, 1986). Sharma *et al*. (1983) showedthat, within *B. taurus* dairy cattle breeds, the Jerseywas less sensitive to thermal stress than the Holstein-Friesian.

2.3.2 Sweating rate

In dairy cows, there are two types of sweating that occur: both are appreciably involved in heat dissipation. The first type is insensible sweating or perspiration that leaves the body at all times, unless the relative humidity is 100%. Another type, thermal sweating, occurs as the principle evaporative cooling mechanism of the cow when ambient temperatures rise(Montgomery *et al*., 1984). The heat required to convert water into vapor is referred to as the latent heat of vaporization. The proportion of metabolic heat that is dissipated from the cow by evaporation increases with rising environmental temperatures and a decreasing temperature gradient between the animal and air (Montgomery *et al*., 1984). The morphology and functioning of the sweat glands of cattle during hot climatic conditions has been extensively investigated (Montgomery *et*

al., 1984). Blazquez *et al*. (1994) reported that increased blood flow to the skin is positively correlated to the sweating rate. Earlier, Kibler and Brody (1952) found similar sweating rates for *B. taurus* and *B. indicus* breeds; however, Allen (1962) showed that *B. indicus* and Zebu cattle had significantly higher sweating rates than breeds from temperate regions. Ferguson and Dowling (1955) and Allen (1962) ascribed elevated sweating rates of *B. indicus* and Zebu cattle to their higher density of sweat glands. Schmidt-Nielsen (1964) reported that as the environmental temperature rose, *B. taurus* cattle showed an appreciable increase in evaporation between 15 and 20^{o}C, with a maximum rate of evaporation being reached before 30^{O}C. Cattle in temperate and tropical regions possess the same type of sweat glands, one to each hair follicle (Findlay and Yang, 1950). However, tropical breeds have a higher density of hair follicles (1698/ cm for Zebu) than is the case in *B. taurus* breeds (1064/ cm for Shorthorn) (Dowling, 1955). Furthermore, Dowling (1955) reported that Zebu have sweat glands that are located much closer to the skin surface than is the case in temperate breeds of cattle.

2.3.3 Rectal temperature

Rectal temperature is an indicator of thermalbalance and may be used to assess the adversity ofthe thermal environment which can affect the growth, lactation, and production of dairy cows (Johnson, 1980a). A rise of 1^{o}C or less in rectal temperature is enough to reduce performance in most livestock species (McDowell *et al*., 1976), which makes body temperature a sensitive indicator of physiological response to heat stress in the cow because it is nearly constant under normal conditions. Shalit *et al*. (1991) recorded rectal temperature of lactating cows as 0.9 ^{O}C higher than in pre-partum cows, although environmental conditions were similar. It appears that there are notable differences between breeds in their abilities to regulate rectal temperature, the

mean rectal temperature is higher in *B. taurus* than in *B.* i*ndicus* cattle (Finch, 1986) and as a result, *B.* t*aurus* cattle are more sensitive to heat stress than their *B.* i*ndicus* counterparts.

2.3.4 Respiration rate

There is no evidence of breed differences in therespiratory response to low temperatures, but at hightemperatures, Kibler and Brody (1954) showed thatJersey cows had much higher respiration rates thanHolsteins, which was attributed to the Jersey's betterability to dissipate heat compared to Holsteins. Highambient temperatures induce physiological adjustments, including increased respiration rate (Coppock*et al*.,1982). Johnston *et al*. (1959) reported increases from 20 breaths /min under cool conditions to100 breaths or more per minute at 32 ^{O}C and above. In studies involving high-producing dairy cows in a subtropical environment, Berman *et al*. (1985) found that the respiratory frequency started rising above 50–60 breaths /min at ambient temperatures higher than 25 ^{O}C. The importance of relative humidity in the study of heat stress was demonstrated by a decline in milk production between 32^{O}C with 20% relative humidity (RH) and 32 ^{O}C with 45% RH (Johnson and Vanjonack, 1976). This difference in RH reduced respiratory and surface evaporation, which resulted in a rise in rectal temperature, reducing feed intake and milk production.

2.3.5 Thermoregulatory responses to heat stress

Farm animals are homeotherms as they can keep relatively constant body core temperature within narrow limits despite wide variations in climatic environment(Collin *et al*., 2001). Thermoregulation is the balance between heat production and heat loss mechanisms that occur to maintain a relatively constant body temperature (Tb) (Igono *et al*., 1988). Under high thermal conditions, animals reduce heat storage by reducing metabolic heat production and improving heat losses by latent and sensible pathways. (Renaudeau *et al*., 2010). An animal can lose heat by

evaporation, conduction, convection and radiation. Although for evaporation the main driving force is the level of humidity in the surrounding air, for convection, radiation and conduction it mainly depends on the thermal gradient between the animal surface area and the surrounding air (radiation and convection) and objects (Curtis, 1983). In both cases, the body surface area that is in contact with the surrounding environment plays a crucial role in the efficacy of the heat loss process. When submitted to high temperatures, animals adjust their blood flow to favor heat loss, for instance by increasing blood flow toward skin (Collin *et al*., 2001). However, it has been recently shown that an optimal building air renewal and/or indoor ventilation while ambient temperature is above the animal thermoneutral zone (TNZ) during the growth period of broilers and turkeys (Yahav*et al*., 2008) or during the period of egg production in laying hens can significantly increase heat loss by convection. It decreases heat loss by evaporation and contributes to a better production rate(Yahav*et al*., 2008). In addition, it must be considered that in very humid tropical areas, any evaporation process will be less efficient and mainly sensible heat loss may reduce heat load, however, there is no doubt that heat loss by water evaporation is an efficient means of heat transfer to the hot environment (Renaudeau *et al*., 2010). Animals can evaporate water from the skin and through the respiratory tract. The early response of an animal is to increase its respiratory ventilation rate and thus the respiratory evaporative heat loss. First, a rapid shallow breathing called thermal polypnea leads to an increase in the amount of air passage through the upper region of the respiratory tract(Renaudeau *et al*., 2010). When temperature continues to rise, this thermal polypnea shifts to a slower deeper panting phase (thermal hyperpnea) characterized by an increase of alveolar ventilation rate (Renaudeau *et al*., 2006). This thermal hyperpnea improves the evaporative heat loss increasing the respiratory minute volume, but also results in respiratory alkalosis in the blood and may lead to a moderate-to-

severe dehydration (from unpublished results).Skin evaporation can occur by both passive and active processes, in passive conditions, water diffuses through the skin with a direction that depends on the vapor pressure gradient and the rate of ventilation (Leterrier *et al*., 2009). In some cases where the rate of ventilation is very high, it may induce a passive water loss from the skin (Yahav*et al*., 2005). When ambient temperature (Ta) increases above the UCT, the animal can no longer control its body temperature (Tb) and this severe hyperthermia can be lethal (Amand *et al*., 2004). In chicken, death occurs approximately when body temperature (Tb) reaches 48^{o}C above the regular physiological temperature (Amand *et al*., 2004). However, when pigs, chickens or calves are exposed for a long period to a moderately high temperature, they will first present a phase of increasing body temperature (Tb) that will then decline to a dynamic steady state corresponding to an acclimated state (Collin*et al*., 2002; Renaudeau*et al*., 2010).

2.3.6 Metabolic responses to heat stress

Christopherson and Kennedy (1983) and Lu (1989) reported reduced metabolism in cattle under heat stress, which they found to be associated with reduced thyroid hormone secretion and gut motility, resulting in increased gut fill. Mitra *et al*., (1972) found plasma growth hormone concentration and growth hormone secretion rate to decline with hot temperatures (35^{O}C). Igono *et al*. (1988) showed that the concentrations of growth hormone in the milk of low, medium, and high producing cows declined when the THI exceeded 70 and suggested a suppressed production of growth hormone in order to lower metabolic heat production. McGuire *et al*. (1991) found that plasma growth hormone reductions that occurred with heat stressed cows did not occur in thermoneutral conditions for cows fed amounts that were similar to those consumed during heat stress. Also working with lactating cows, Johnson *et al*. (1988) measured decline in thyroid hormones triiodothyronine (T_3) and thyroxine (T_4) in response to heat stress, which they

attributed to attempts to reduce metabolic heat production in the cow. Earlier, Alvarez and Johnson (1973) suggest that cows respond to heat stress by engaging homeostatic processes to maintain homeothermy.

Hyperthermia is an important stress factor known to increase blood cortisol levels, this is expected since hypothalamo-pituitary-adrenocortical (HPA) axis is activated in response to stressors such as heat and inflammation thyroid function may be altered by hyperthermic conditions (Mustafa *et al*., 2008).

2.3.7 Biochemical responses to heat stress

Seasonal and environmental changes may influence haematological values in domestic animals, Some species have evolved endogenous annual rhythmicity as an adaptive mechanism to react in advance to regular environmental changes associated with the seasons (Piccione *et al*., 2009).Exposure of cows to hot environment stimulates thermoregulatory mechanisms and produces reduction in the rates of metabolism, feed intake and productivity (Abdelatif and Alameen, 2012). In order to maintain homeothermy, an animal must be in thermal equilibrium with its environment, which includes radiation, air temperature, air movement and humidity (Kadzere *et al*., 2002).

In the higher standards of milk production, the priority in modern breeding is keeping dairy cows in high lactation and healthy, the control of their feeding and metabolic status is equally important for the health of the herd in the health control system (Kida, 2002). Lactation has a great impact on biochemical parameters in the blood of cows, reflecting on metabolic demands so the need for their systematic monitoring and the role of each individual parameter were shown byKida (2002).

The activity of aminotransferases in blood is very important, Aminotransferases act as a catalyst in connecting the metabolism of amino-acids and carbohydrates (Cebra *et al*., 1997). Accordingly, changes in their activity in the blood can be a consequence of their increased activity in cells (primarily liver), but also a reflection of cell structure damage (Meyer and Harvey, 1998).

In the liver aspartate aminotransferase (AST) and alanine aminotransferase (ALT) show high activity and are most often determined if there is a suspicion of acute and chronic liver disease (Cebra *et al*., 1997). Determining AST activities in dairy cows is most often connected with fatty liver syndrome (Cebra *et al*., 1997), low appetite and the appearance of ketosis in dairy cows during early lactation. Increased AST activity in the serum is a sensitive marker of liver damage, even if the damage is of a subclinical nature (Meyer and Harvey, 1998). Unlike AST, ruminant liver cells do not show high ALT activity, and the increased activity of that enzyme in the serum during liver damage, even in necrosis is insignificant, (Ahmed and Abdalla, 2012), reported that activities of AST was higher during summer while that of ALT was lower compare to winter values. They also reported that serum levels of cholesterol were higher during summer compare to respective winter values. Plasma glucose levels decrease in winter where as inorganic phosphate level was lower in summer, serum calcium level was higher in summer. (Ahmed and Abdalla, 2012).

2.4 Characterization of the Climatic Environment

The animal's climatic environment is complex, especially in outdoor conditions, in such conditions, ambient temperature alone cannot be a representative measure of thermal environment and relative humidity (RH), solar radiation and wind speed must also be considered (Mader *et al*.,2006). However, scientists attempt to define it in an index value representing the influence of sensible and latent heat exchanges between the animal and its environment(Renaudeau *et al*., 2010). Heat exchange could be accessed directly from physiological measurements (rectal, cloacal and skin temperatures, respiratory rate, panting and heat production) or indirectly from animal performance (growth rate, egg and milk production) that are related to the animal's ability or in ability to efficiently cope with acute or chronic hot conditions (Renaudeau *et al*., 2010). Various indexes derived from meteorological measurements have been developed and recently reviewed by Hahn *et al*. (2009). These indexes range from a simple measurement of ambient temperature to an index that takes into consideration the effects of air temperature, RH, solar radiation and wind speed. For example, the effective temperature for grazing animals was calculated from ambient temperature (dry bulb temperature) and direct and indirect radiations (Yamamoto *et al*.,1994), and a wet/dry bulb temperature or temperature humidity indexes (THIs) estimated for pigs, poultry and ruminants from ambient temperature Ta (dry bulb temperature) and RH (wet bulb temperature) Hahn*et al*., (2009). These climatic indexes have limitations because they reflect the average conditions in the facility and not the micro environment around an animal (Hahn*et al*., 2009). In temperate countries, short-term changes in physiological, behavioral and immunological functions are required to survive acute stressful events such as summer heat waves (Nienaber and Hahn, 2007). The severity of these short thermal challenges depends on the magnitude intensity and

duration of heat wave events and the possibility of recovering during the cool night time period(Nienaber and Hahn, 2007). The long-term solutions to alleviate heat stress in livestock thermoregulatory responses underlying heat acclimation increase the physiological strains, which in most cases are accompanied by reduced performance (Renaudeau *et al*., 2008). These responses occur within the lifetime of the animal and include a reduction of metabolic rate, changes in the cardiovascular system, efficient alteration in heat loss (vasomotor response: vasodilation response), changes in behavior response and in the general morphology of the animal. In cattle, most acclimation occurs three to four days after the onset of a heat challenge (Nienaber and Hahn, 2007). Similar data were obtained in pigs. However, the time course for the total acquisition of thermal acclimation takes several weeks and varies with the magnitude of the heat challenge (Renaudeau *et al*., 2010).

2.4.1 Ambient temperature and relative humidity

The environmental condition of the animal is made up of the ambient temperature, relative humidity, solar radiation, wind etc. Elevated environmental temperature, solar radiation, and relative humidity leads to hyperthermia or heat stress (Collier *et al*., 2006). Increase in ambient temperature (Ta) makes heat transfer by conductive, convective, and radiative exchanges less effective because the reduction of the required minimal thermal gradient between skin and air temperature for exchange (Hillman *et al*., 1985).Combination of environmental conditions that will cause the effective temperature of the environment to be higher than the temperature range of the animal's thermal neutral zone leads to heat stress. Renaudeau *et al.* (2008) reported that when rectal temperature is greater than 39.2 ^{O}C and breaths exceed 60/min, cows are on the verge of experiencing significant heat stress; this is the point at which the cow will heat up exponentially if exposed to further increases in temperature and humidity. Collier *et al*. (2006)

reviewed extensively the dynamics of environmental management and subsequent impacts on the lactating dairy cow. A benchmark reference point for lactating cow status is a surface skin temperature of $35^{O}C$, below this temperature all routes of heat exchange are possible, and the micro environment to sustain a skin temperature at or below $35^{O}C$ prevents reductions in milk yield (unpublished). The magnitude of environmental stress can be measured directly by changes in hormones and/or in blood flow, heart rate, body temperature as well as indirectly through the animal response in terms of productivity (Renaudeau *et al*., 2010).

2.4.2 Temperature humidity index (THI)

Temperature Humidity Index (THI) has been used to integrate environmental temperature and relative humidity i.e., THI = Td – (0.55 -0.55RH/100) (Td -58), where Td is the dry bulb temperature in Fahrenheit and RHis relative humidity expressed as a percentage. Lactating cows are thought to experience no stress when THI is less than 72 and severe stress when THI exceeds 88 (Armstrong, 1994). These guidelines may shift somewhat depending on amount of milk produced, degree of air movement, and direct solar radiation. Zimbelman *et al*. (2009) reported that dairy cows producing more than 35 kg/d of milk need additional cooling when average THI is 68 for more than 17 hr/d. Ahmed and Abdalla (2012), reported that the THI value during summer (80.92) is considered to induce heat stress in cows; the value during winter (65.75) is considered to be comfortable for dairy cows. The THI is usually used as an indicator of thermal stress in animals; THI of 72 and below is considered as cool; 78-88 as moderate heat stress (Armstrong., 1994).

2.5 Consequences of Thermoregulatory Responses on Animal Performance.

Selection for milk yield reduces the thermoregulation ability during heat stress which magnifies the seasonal depression in productivity caused by climatic stress (Al-Katanani*et al*., 1999; Chaiyabutr*et al*., 2008). The zebu (*Bos indicus*) breeds have higher degree of thermotolerance compared with temperate (*Bos taurus*) breeds because of lower metabolic rate and greater sweating capacity (Blackshaw and Blackshaw, 1994; Hansen, 2004). The metabolic profile test that was adopted by Payne (1970) has been used for assessing metabolic status and diagnosis of metabolic disorders in dairy herds (Kida, 2002). The changes in blood constituents can reflect the physiological condition as well as nutritional and health status of cows. Acclimation to thermal stress also imposes physiological and metabolic adjustments associated with reduction of performance and compromising of health (Bernabucci*et al*.,2010).Physiological and metabolic adjustments resulting from the thermoregulatory responses to a thermal stress have negative consequences on animal productivity and health(Chaiyabutr*et al*., 2008). In *ad libitum*fed animals, the reduction in heat production related to consumption and metabolic utilization of feed is an essential mechanism to maintain body temperature (Tb) within a physiological safe range (Renaudeau*et al*., 2006). The related low energy and nutrient intakes mainly explain the reduction in meat, milk and egg production under heat stress.

2.5.1 Consequence ofthermoregulatory responses on ruminant performance.

Both grazing and feedlot cattle suffer from heat stress during periods of high ambient temperature and humidity, which affects rate and efficiency of production (Birkelo *et al*., 1991). Worldwide, ruminant animals are often partially or completely reared outdoors with a constant exposure to natural climatic conditions and in such conditions, heat stress is caused by a combination of environmental factors (temperature, RH, solar radiation, air movement and precipitation) (Birkelo *et al*., 1991). In ruminant species, a THI or black globe heat index is often

used to assess the degree of thermal stress for a given environment (Collier and Beede, 1985; Wiersma and Armstrong, 1989). Dairy cattle are particularly vulnerable not only to extreme thermal environmental conditions but also to a rapid change in climate whereas Pastured cattle have the ability to seek shade, water and air movement to cool themselves, whereas radiant heat from dirt or concrete surface is increased for feedlot cattle(Wolfenson, 2009). The susceptibility to heat stress of feedlot cattle mainly depends on the intensity and duration of the thermal challenge and on animal-related factors (breed, BW, growing phase, sanitary and nutritional status)Bernabucci *et al*. (2010). In a review by Bernabucci *et al*. (2010), it was reported that the threshold Ta at which DM intake of beef cattle starts to decrease is about 30^{O}C for a RH below 80%. Above 80%, the corresponding value for the threshold (ambient temperature) Ta is 27^{O}C. For growing steers,fed ad libitum with concentrate and housed in a climatic chamber, feed consumption was reduced by 20% from 18^{O}C to 30^{O}C, and by 25% from 30^{O}C to 34^{O}C (Brown-Brandl *et al*., 2003). Generally, the digestibility of feed is improved under hot conditions because of the reduced feed intake (Morand-Fehr and Doreau, 2001). This higher digestibility coefficient seems to be related to a slower rate of digesta passage in the total gastrointestinal tract (Christopherson and Kennedy, 1983). High ambient temperature can also have a strong negative effect on milk yield and quality (Gregory, 2010). Seasonal high ambient temperature is associated with low reproductive performance in dairy and beef cows (Wolfenson, 2009). According to Amundson *et al*. (2006), a reduction in pregnancy rate is noticed when THI is equal or exceeds 72.9 for beef cows in pasture. On average, conception rate drops by 24% during summertime (Bernabucci *et al*., 2010). This decrease in fertility is caused by an impaired ovarian function, lower expression of estrus, oocytes health problems and lower embryonic development (Wolfenson, 2009). Meyerhoeffer *et al*., (1985) reported that the deterioration of bull fertility

related to heat stress could also partly explain the summer infertility of the cow. Modern high-producing dairy cows are more vulnerable to heat stress because of their very high metabolic rate associated with milk production (Kadzere *et al*., 2002). According to Kadzere *et al*. (2002), feed intake in lactating cows begins to decline at a threshold ambient temperature of 25^{O}C to 26^{O}C. The extent to which temperature affects feed intake in dairy cows depends on the ambient temperature level(Collier *et al*., 1981). Milk production is negatively affected by high ambient temperature, the point on the lactation curve at which the cow experiences heat stress is also important for milk production. Collier *et al*. (1981) demonstrated that milk yield decline was detectable at Black Globe Temperatures above 25^{O}C. Research has also indicated that the effects of a given temperature on milk production are maximal between 24 and 48 hours following heat stress (Collier *et al*., 1981). It has also been reported that ambient weather conditions two days before milk yield measurement had the greatest correlation to decreases in milk production (West, 2003). Recently, the THI for lactating dairy cows was recalculated by Zimbleman*et al*. (2009). The revised THI takes into account new formation from controlled and large herd studies under a variety of climatic conditions using data from high-producing dairy cows. The new THI stress threshold for high-producing cows (35 kg milk/day) is 68(Zimbleman*et al*., 2009). In a comprehensive review (Bernabucci *et al*., 2010), heat-induced reduction of milk production is 14% in early lactation and 35% in mid-lactation in dairy cows. The negative effect of heat stress on milk production is primarily explained by a reduced nutrient intake and a decrease of nutrient uptake by the portal-drained vein (McGuire *et al*., 1989). The reduced milk production in hot season is accompanied by a decrease in milk protein contents related to a decrease in casein fraction (Bernabucci*et al*., 2002). Sheep and goats appear to be less susceptible to heat stress than other domesticated ruminant species as a result of their unique characteristics such as water

conservation capability, higher sweating and respiratory rate and lower basal heat production (Kadzere *et al*., 2002), Whereas cattle are considered stressed above a THI of 72, the threshold for heat stress in sheep is 82 (Marai *et al*., 2007). Sheep evolving in a semi-arid environment can easily handle high ambient temperature (Ta), but have great difficulty with the combination of high ambient temperature (Ta) and humidity (Marai*et al*., 2007). Sheep are also panting animals and develop respiratory alkalosis under conditions of high Ta and concomitant high respiration rates (Marai *et al*., 2007). Heat stressed cows generally exhibit altered blood acid–base chemistry as a result of the shift in cooling from sensible heat losses to evaporative cooling (Finocchiaro *et al*., 2005). In addition, heat-stressed dairy cattle can lose a large amount of potassium (K) via sweat, whereas feed consumption is reduced and K requirement for milk synthesis is high and this could result in K deficiency with detrimental consequences on health and performance (Beede and Collier, 1986).Gaughan *et al*. (2009) reported a higher occurrence of mastitis in dairy cows during periods of heat stress and they hypothesized that hot conditions could favor the development of pathogens responsible for mastitis and/or have negative effects on the animals' immune response. Another direct effect of heat stress includes thermal-related death mainly during heat wave events (West, 2003).

CHAPTER THREE

3.0MATERIALS AND METHODS

3.1 Experimental Site

The study was conducted inthe Dairy Unit of Sebore Farm in Mayo-Belwa, Adamawa State. Sebore Farms in Adamawa State is located at an altitude of 200 to 300 metres, between latitude $9^0\ 20^{'}$ and $9^0\ 33^{'}$N and longitude $12^0\ 30^{'}$ and $12^0\ 50^{'}$ E. It is bordered by Borno State to the North West, Gombe to the West and Taraba to the South West and has an Eastern border with Cameroun Republic. It has average daily minimum and maximum temperatures of 23.2 and 35.2 ^{0}C respectively. The average annual rainfall is 718.1 millimetres and relative humidity, 44.2 %. It occupies an area of 39,742.12 square kilometres. It is generally characterized by many rivers; the major one being the River Benue whose source is from the highlands of the Cameroun and flows southwards to join the River Niger.The predominant climate is Sahel, rainy season last for only three to four months (June-September). The rest of the year is cold-dry characterized by a lack of heat stress conditions with a relatively cool, dry with continental air mass that originates from the Sahara Desert associated with dry, cold and dusty North-East trades (harmattan) and hot-dry (March-May) characterized by high ambient temperature, RH and heatstress conditions. (Ileoje, 2001; National Bureau of Statistics, 2010; Anonymous, 2014).

3.2 Source of Experimental Animals andManagement

Fifteen clinically healthy dairy cows made up of five each from Holstein Friesian purebred, Simmental, and Brown Swiss, aged 5-8 years from Sebore Farms wereused for the experiment. The animals were maintained onsimilar feeding programme under hot-dry andcold-dry conditions. Animals were kept in sheds located close to the milking parlor with appropriate facilities for feeding and watering. The non-grazing nutritional regimen comprised two types of feeds hay and silage with molasses, the feeds were offered twice daily at 10.00 a.m. and 5.00

p.m. and lactating cows were offered supplemental concentrate mixture twice daily, before milking at 8.00 a. m. and at 4.00 p.m.The experiment was conducted for a period of 22 weeks).

3.3 Data Collection

3.3.1Environmental measurements

Mean values of ambient temperatures and relative humidity measurements were obtained from the Department of Meteorological Services, Yola, Adamawa State. On days when thermoregulatory parameters were measured, air temperature, and relative humidity were recorded with a Kestrel® 3000 Pocket Weather Meter (Nielsen-Kellerman. Boothwyn, PA). The temperature-humidity index (THI) was calculated according to the equation reported by (Ravagnolo *et al*., 2000):

THI = (1.8 x T + 32) – [(0.55 – 0.0055 x RH) x (1.8 x T – 26)] where T = dry bulb temperature (°C) and RH = relative humidity (%).

Ranges for temperature humidity index (THI)

THI over 64 (minimal heat stress),

THI over 72 (moderate heat stress), and

THI $\geq$ 76 (maximal heat stress)

According to the following: At THI of 84 or more, death occurs (West, 2003; Igono *et al*., 1992)

3.3.2Thermoregulatory measurements

Rectal temperature (RT), respiration rate (RR), ear temperature (ET) and skin temperature (ST) were recorded weekly in the morning at 08:00am and in the afternoon at 14:30pm for the study period (22 weeks). RT, ET and ST measurements was made with Hi-Performance Digital Thermometers (GLA M525/550, GLA Agricultural Electronics, San Luis Obispo, CA) using a 21 mm probe. RR was determined as breaths per minute based on observations of the number of

flank movements in a 15 second period as recorded with a thermometer which were standardized to a minute. All measurements were taken in a shaded holding area.

3.3.4Blood analysis

Blood samples (10 mls) from each of the 15 animals were collected by jugular venipuncture once a month using plastic disposable syringes. 2 ml of blood was kept in a tube containing sodium fluoride and after centrifugation, the plasma sample was used for glucose determination. The rest of the blood sample was left at room temperature for 3 hrs, then centrifuged and the serum samples were harvested and immediately frozen at -20 °C for subsequent analysis. Blood serum parameters such as glucose, inorganic phosphate, total cholesterol, triiodothyronine (T3), thyroxine (T4), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were assayed on automated clinical chemistry analyzer BS-1 20 (Mindray MIL, Nanshan, Shenzhen, China).Serum triidothyronine (T3) level was determined using radioimmunoassay kit-M (HTA CO. LTD, China), while serum thyroxine (T4) level was determined using radioimmunoassay kit - IMK-437 (HTA CO. LTD, China). All these were analysed in the biochemical laboratory, Federal Medical Centre Yola (FMCY), Adamawa State.

3.3.5 Milk production parameters

3.3.5.1 Milkyield and milk composition analysis

Cows were milked twice daily (morning and evening)with a milking machine commencing threetofourday postpartum. Average daily milk yield (AMY) was calculated. The daily milk yield of the cows was measured in litres using calibratedmeasuring cylinder, total of their yields was summedon weekly bases. The milk yield records were used to calculate the total milk yield (TMY)Average daily milk yield = Total milk yield/ Number of days in milk.The milk composition analysis was carried out at the Department of Food Science and Technology Laboratory,

Modibbo Adama University of Technology (MAUTECH) Yola, Adamawa State, to determinetotal fat, and protein.For the determination of fat, 10 mlof milk was precipitated with 20% tricarboxylic acid [TCA (15 ml)], filtered through a filter paper (Whatman, No.40) and the precipitate was subjected to ether extraction (Mech *et al*.,2007).Proteincontent was estimated according to the method described by DePeters and Cant (1992).

3.4 Data Analysis

3.4.1 Statistical analysis

Data collected were subjected to analysis of variance (ANOVA),using General Linear Model of (SAS) (2002) while significant difference were separated using Orthogonal pairwise difference method. The design was a completely randomized design (CRD).The effects of invasive and non invasive parameters on milk yield were estimated using the GLM procedure of the statistics analysis software (SAS, 2002) package. These were computed on the basis of interaction with breed groups. Means were separated within breed using Orthogonal pairwise difference method. The degrees of association between all pairs of metric variables were computed for all the animals within each breed groups using CORR procedure of the SAS (2002) statistical package. This was done to evaluate changing magnitude of association among variables. The regression analysis was carried out using the SASREG procedure of the SAS (2002) Package.

Mathematical model:

$Y_{ijkl} = \mu + B_i + Sj + (BS)ij + e_{ijk}$

where:

μ = general mean,

$B_i = i^{th}$ fixed effect of breed

$Sj = j^{th}$ fixed effect of season

(BS)ij = interaction effect between breed and season

e_{ijkl} = experimental error.

3.4.2 Regression model

Stepwise single and multiple regression model was used to find the predictors that best explainsdaily milk yield among invasive and non-invasive markers and this was performed for each breed. The following linear multiple regression models were applied.

$Y = a + b_1 X_1 + b_2 X_2 + b_3 X_3 + \ldots\ldots b_i X_i$ and $Y = a + bX$

Where:

Y = the dependent variable (150day milk yield)

a = the intercept of regression curve on y – axis and is the value of the dependent variable y when all independent variables are Zero.

b_1 = the partial regression coefficient associated with respective independent variable X_1.

X_1= the independent variables (i.e. invasive and non invasive parameters) the regression assumes that the independent variable has no measurement error, and that the above errors about the regression line are equal.

CHAPTER FOUR

4.0 RESULTS

4.1 Environmental Conditions of Study Area during the Cold-Dry and Hot-Dry Seasons.

Weekly ambient temperature (Ta), relative humidity (RH) and calculated Temperature Humidity Index (THI) of the study area during the experiment period is shown in (Table 4.1). The meteorological parameters of the study area indicated that season and circadian rhythm had a significantly ($P<0.05$) effect on environmental parameter, higher values for environmental parameters was recorded during the hot-dry season while lower values was recorded during the cold-dry season. It was observed from the overall result that afternoon values fortemperature, relative humidity and Temperature Humidity Index were higher ($P>0.05$) compare to morning. During the cold-dry season, the meanmorning and afternoon Ta and RH were25.2 and 27.0^{O}C and 34.7 and 35.3%, respectively, THI morning and evening was 70.3 and 72.4 in the cold-dry season. Mean morning and afternoon Ta andRH were 40.6^{O}C and 41.5^{O}C and 46.3 and 47.3%, respectively, THI morning and evening was 91.3 and 92.2.

4.2 Thermoregulatory Response of Dairy Cows

4.2.1 Effect of breed on thermoregulatory response of dairy cows

Figure 4.1 presents the effect of breed on thermoregulatory response of dairy cows. Breed had no effect on thermoregulatory parameters. Rectal temperature, skin temperature, respiratory rate and ear temperature were statistical similar ($P<0.05$) for all breeds of Dairy cows.

Table 4.1: Environmental Conditions of Study Area During The Cold-Dry And Hot-Dry Seasons

Environmental variables	Range	Season	LOS

	Minimum	Maximum	Cold-dry	Hot-dry	
Temperature (Morning)(^{o}C)	21.40	43.10	25.2 ±0.16[b]	40.6±0.15[a]	**
Temperature (Afternoon) (^{o}C)	23.60	44.10	27.0±0.16[b]	41.5±0.14[a]	**
Relative humidity (Morning) (%)	22.00	70.00	34.7±0.86[b]	46.3±0.79[a]	**
Relative humidity (Afternoon) (%)	28.00	71.00	35.3±0.75[b]	47.3±0.68[a]	**
Temperature Humidity Index (Morning) (%)	65.69	96.20	70.3±0.29[b]	91.3±0.25[a]	**
Temperature Humidity Index (Afternoon) (%)	68.50	97.12	72.4±0.28[b]	92.2±0.27[a]	**

[ab]Means with different superscript differs significantly (P<0.05) across the row, **-P<0.01-Highly significant.

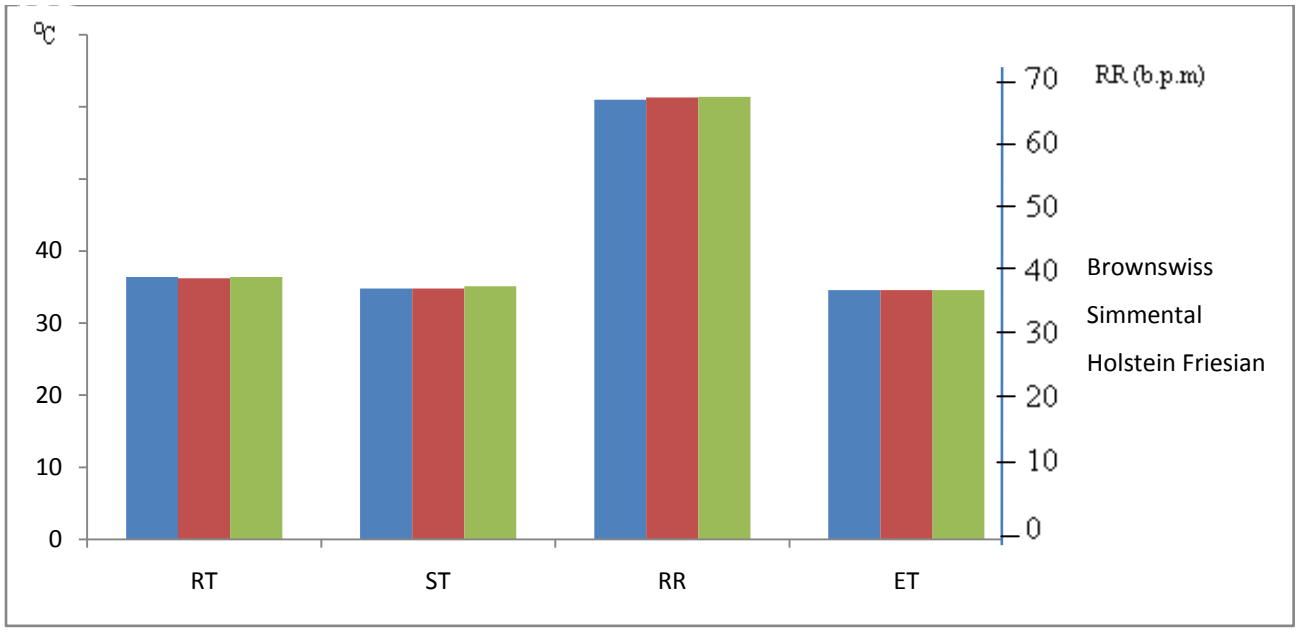


Figure 4.1: Effect of Breed on Thermoregulatory Response of Dairy Cattle

RR (beats/minutes)
RT(^{o}C)
ST (^{o}C)
ET (^{o}C)

4.2.2 Effect of season on thermoregulatory responseof dairy cows.

Table 4.2 shows the effect of season on thermoregulatory parameters of dairy cows. All the parameters differed significantly ($P<0.05$) between the cold-dry and hot-dry seasons with the exception of ear temperature which was statistically similar ($P>0.05$). Hot-dry season rectal temperature (37.8±0.06 ^{o}C) respiratory rate (63.9±0.33^{o}C) and skin temperature (35.9±0.05 ^{o}C) were significantly ($P<0.05$) higher than the cold-dry season respective values (34.9±0.07^{o}C), (58.5±0.33^{o}C)and (33.9±0.05^{o}C)

4.2.3 Interaction between season and breed on thermoregulatory response of dairy cows

Table 4.3 illustrates the interaction between season and breed on thermoregulatory response of dairy cows. Rectal temperature and skin temperature for cold and hot dry season differed significantly ($P<0.05$) across the breeds of Dairy cows but there was non-significant interaction ($P>0.05$) of season and breeds on respiratory rate and ear temperature. Holstein Friesian (35.9±0.12 and 39.9±0.10^{o}C) cows had higher rectal temperature compare to Brown Swiss (34.9±0.12 and 37.8±0.10^{o}C) and Simmental (34.8±0.12 and 37.7±0.10^{o}C) during cold-dry and hot-dry seasons respectively. Higher values was recorded in Holstein Friesian (36.0^{o}C) for skin temperature compare to Brown Swiss (35.8^{o}C) and Simmental cows (35.9^{o}C) during the hot-dry season the same trend followed during the cold-dry season.

Table 4.2: Effects of Season on Thermoregulatory Parameters of Dairy Cows

	Season		
Parameters	**Cold dry**	**Hot dry**	**LOS**
Rectal temperature (^{o}C)	34.9±0.07[b]	37.8±0.06[a]	**
Respiratory rate (beat/min.)	58.5±0.33[b]	63.9±0.33[a]	**
Skin temperature (^{o}C)	33.9±0.05[b]	35.9±0.05[a]	**
Ear temperature (^{o}C)	34.2±0.23	35.1±0.21	NS

[ab]Means with different superscript differs significantly (P<0.05) across the row, HF-Holstein Friesian.

Table 4.3: Interaction between Season and Breed on Thermoregulatory Response of Dairy Cows.

Breed	**Brown Swiss**		**Simmental**		**Holstein Friesian**		
Season	**Cold dry**	**Hot dry**	**Cold dry**	**Hot dry**	**Cold dry**	**Hot dry**	
Parameter							**LOS**
RT (^{o}C)	34.9±0.12^{c}	37.8±0.10^{a}	34.8±0.12^{d}	37.7±0.10^{b}	35.9±0.12^{c}	39.9±0.10^{a}	*
ST (^{o}C)	33.8±0.10^{d}	35.8±0.09^{c}	33.8±0.10^{e}	35.9±0.09^{b}	34.2±0.10^{f}	36.0±0.09^{a}	*
RR (breath/min.)	58.6±0.57	63.4±0.52	58.5±0.57	64.2±0.52	58.5±0.57	64.2±0.52	NS
ET (^{o}C)	34.1±0.39	35.0±0.36	34.2±0.39	35.0±0.36	34.3±0.39	35.2±0.36	NS

^{abcde} = Means with different superscript differs significantly (P<0.05) across the row, HF-Holstein Friesian, BS-Brown Swiss, RT- rectal temperature, ST-skin temperature, RR-respiratory rate, ET-ear temperature, LOS- level of significance

4.2.4 Circadian rhythm

4.2.4.1 Effect of circadian rhythm on thermoregulatory response of dairy cows

Figure 4.2 depicts the effect of circadian rhythm on thermoregulatory response of dairy cows. Rectal temperature, skin temperature, respiratory rate and ear temperature were statistical similar ($P<0.05$) in the morning and afternoon periods.

4.2.4.2 Effect of breed and circadian rhythm interaction on thermoregulatory response of dairy cows

The effect of breed and circadian rhythm on thermoregulatory response of Dairy Cows is shown in (Table 4.4). Rectal temperature, skin temperature, ear temperature and respiratory rate for both morning and afternoon periods were statistically similar ($P<0.05$). Rectal temperature values ranged from 36.3±0.18 ^{o}C in Simmental to 36.5±0.18 ^{o}C in Holstein Friesian, and 36.4±0.18 for Brown Swiss in the morning period.

4.2.4.3 Effect of season and circadian rhythm interaction on thermoregulatory response of dairy cows

Table 4.5 presents the effect of season and circadian rhythm on thermoregulatory response of dairy cows.The mean values for thermoregulatory parameters; rectal temperature, skin temperature, respiratory rate and ear temperature were significantly ($P<0.05$) influenced by season and circadian rhythm. Higher values 59.1 b.p.m, 34.8, 34.8, and 35.4^{o}C for RR, RT, ST and ET respectively were recorded in the afternoon period compare to 57.9 b.p.m, 34.9, 33.1 and 32.9^{o}C for RR, RT, ST and ET respectively recorded in morning time of the day during cold-dry season. Whereas during the hot-dry season, non significant effect of season and circadian rhythm on thermoregulatory parameters were recorded in the morning and afternoon.

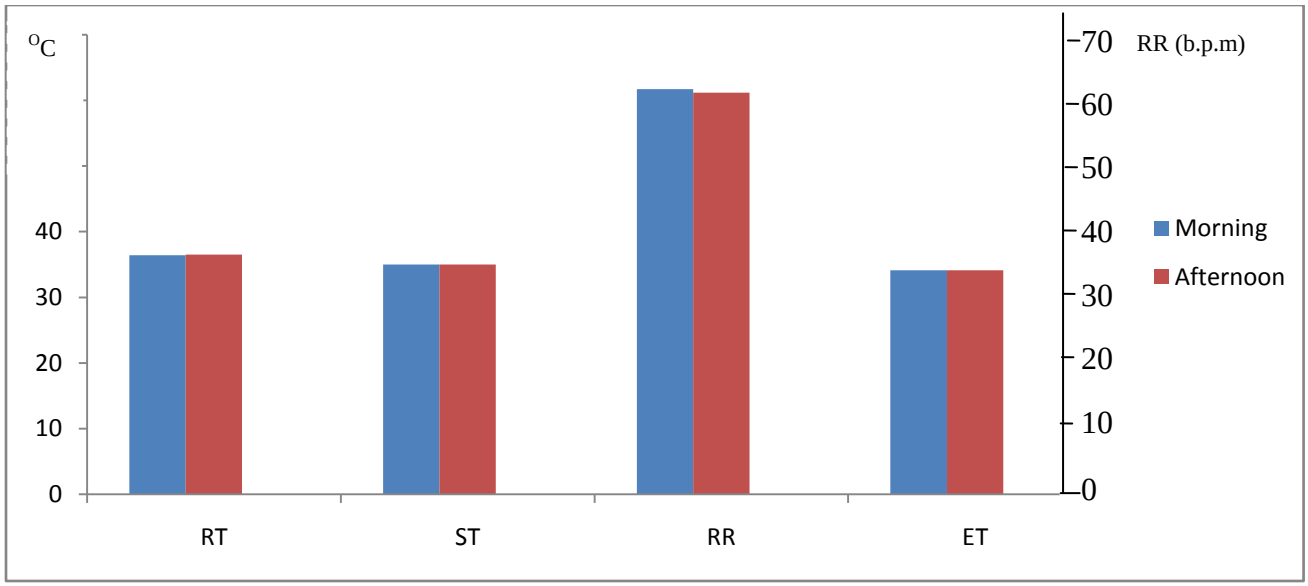


Figure 4.2: Effect of Circadian Rhythm on Thermoregulatory Response of Dairy Cows

RR (beats/minutes)
RT (^{o}C)
ST (^{o}C)
ET (^{o}C)

Table 4.4: Effect of Breed and Circadian Rhythm Interaction on Thermoregulatory Response of Dairy Cows.

Breeds	**Brown Swiss**		**Simmental**		**Holtein Friesian**		
Circadian Rhythm	**Morning**	**Afternoon**	**Morning**	**Afternoon**	**Morning**	**Afternoon**	
Parameter							**LOS**
Rectal temperature (^{o}C)	36.4±0.18	36.5±0.18	36.3±0.18	36.4±0.18	36.5±0.18	36.5±0.18	NS
Skin temperature (^{o}C)	34.9±0.13	34.9±0.13	35.0±0.13	34.9±0.13	35.2±0.14	35.2±0.13	NS
RR (beat/minutes)	61.5±0.59	60.9±0.59	61.7±0.59	61.5±0.59	62.0±0.6	61.2±0.59	NS
Ear temperature (^{o}C)	34.0±0.37	35.2±0.37	34.1±0.37	35.2±0.37	34.1±0.38	35.4±0.37	NS

RR-respiratory rate; BS-Brown Swiss; NS-Not significant, HF- Holstein Friesian, LOS- level of significance

Table 4.5: Effect of Season and Circadian Rhythm Interaction on Thermoregulatory Response of Dairy Cows.

Season	**Cold-dry**		**Hot-dry**		
Circadian rhythm	**Morning**	**Afternoon**	**Morning**	**Afternoon**	
Parameters					**LOS**
Skin temperature (^{o}C)	33.1±0.08^{b}	34.8±0.08^{b}	35.8±0.07^{a}	35.9±0.07^{a}	**
Respiratory rate (beat/min.)	57.9±0.45^{c}	59.1±0.45^{b}	63.3±0.42^{a}	64.6±0.42^{a}	**
Ear temperature (^{o}C)	32.9±0.31^{b}	35.4±0.31^{a}	35.0±0.28^{a}	35.1±0.28^{a}	**
Rectal temperature (^{o}C)	34.9±0.31^{b}	34.8±0.09^{b}	37.7±0.08^{a}	37.9±0.08^{a}	**

^{ab}Means with different superscript differs significantly (P<0.05) across the row, HF-Holstein Friesian, LOS- level of significance

4.3 Biochemical Response of Dairy Cows

4.3.1 Effect of breed on biochemical response of dairy cows

The results for serum biochemical parameters of Brown Swiss, Simmental and Holstein Friesian Dairy Cows is presented on (Table 4.6). There was significant ($P<0.05$) difference in serum glucose of Brown Swiss, Simmental and Holstein Friesian cows; being in the order of Brown Swiss>Holstein Friesian>Simmental. ALT and AST differed significantly ($P<0.05$) across the breeds of dairy cows while cholesterol, phosphorus, T_3 and T_4 were statistically similar ($P>0.05$). The mean value for AST was significantly ($P<0.05$) higher and similar in Simmental (47.74±1.33iu/l) and Brown Swiss (46.59±1.33 iu/l) while Holstein Friesian cows had lower (39.1±1.33iu/l) value. Brown Swiss had higher ($P<0.05$) ALT and glucose (27.32±0.77 iu/l and 3.56±0.09 mmol/l) than Simmental (21.3±0.77iu/l and 2.56±0.09mmol/l) and Holstein Friesian (19.72±0.77iu/l and 3.05±0.09mmol/l). The cholesterol concentration ranged from (102.0±3.74mg/dl) in Holstein Friesian cows to (109.7±3.74mg/dl) in Simmental cows. Phosphorus concentration ranged from (2.00±0.47mg/dl) in Holstein Friesian cows to (2.13±0.47mg/dl) in Brown Swiss and Simmental cows. T_3 ranged from 0.84±0.05 in Brown Swiss to 0.92±0.05 in Friesian cows while T_4 ranged from 25.7±1.03 in Brown Swiss to 28.0±1.03 in Simmental cows.

4.3.2 Effect of season on biochemical response of dairy cows

Table 4.7 presents the effect of season on concentration of glucose, cholesterol, inorganic phosphorus, ALT, AST, T_3 and T_4 in Brown Swiss, Simmental and Holstein Friesian cows. All the parameters differed significantly ($P<0.05$) between the cold-dry and hot-dry seasons with the exception of T_3 which was statistically similar ($P>0.05$). Glucose, cholesterol, ALT, AST, inorganic phosphorus had significantly higher concentration during hot dry season than the cold

dry season while T_4 concentration was significantly ($P<0.05$) high during the cold (47.9±0.84) compare to hot-dry season (5.30±0.84).

4.3.3 Interaction between season and breed on biochemical response of dairy cows.

Table 4.8 shows the interaction between season and breed on blood biochemical parameters. There was significant ($P<0.05$) breed by season interaction in all parameters measured (glucose, cholesterol, inorganic phosphorus, ALT, AST and T_4) with the exception of T_3 which was statistically similar ($P>0.05$). During the hot-dry season, Brown Swiss had significantly ($P<0.05$) higher concentration of glucose (4.40±0.14mmol/l), ALT (33.9±5.29iu/l) and AST (72.44±1.87iu/l) compare to Holstein Friesian and Simmental breeds where as Simmental cows on the other hand, had the highest concentration of inorganic phosphorus (2.30±0.07ng/ml) compare to Brown Swiss and Holstein Friesian. The highest cholesterol concentration (121.4±5.29mg/dl) was recorded in Holstein Friesian cows compare to Brown Swiss (116.7.4±5.29mg/dl) and Simmental (110.6±5.29mg/dl) during the hot-dry season. Simmental had the highest T_4 concentration (50.7±1.45µg/dl) compare to Holstein Friesian (46.9.7±1.45 µg/dl) and Brown Swiss (45.9±1.45 µg/dl) during cold-dry season.

Table 4.6: Effect of Breed on Biochemical Response of Dairy Cows

Parameter	Breed			LOS
	Brown Swiss	Simmental	Holstein Friesian	
Glucose (mmol/l)	3.56±0.09[a]	2.56±0.09[c]	3.05±0.09[b]	**
Cholesterol (mg/dl)	105.2±3.74	109.7±3.74	102.0±3.74	NS
ALT (iu/l)	27.3±0.77[a]	21.3±0.77[b]	19.7±0.77[b]	**
AST (iu/l)	46.6±1.33[a]	47.7±1.33[a]	39.1±1.33[b]	**
Phosphorus (mmol/L)	2.13±0.47	2.13±0.47	2.00±0.47	NS
T_3 (ng/ml)	0.84±0.05	0.89±0.05	0.92±0.05	NS
T_4 (µg/dl)	25.7±1.03	28.0±1.03	26.1±1.03	NS

[ab]Means with different superscript differs significantly (P<0.05) across the row, LOS- level of significance; **-P<0.01-Highly significant; NS-Not significantly;

Table 4.7: Effects of Season on Biochemical Response of Dairy Cows

	Seasons		
Parameters	**Cold-Dry**	**Hot-Dry**	**LOS**
Glucose (mmol/l)	2.6±0.08[b]	3.5±0.08[a]	**
Cholesterol (mg/dl)	95.1±3.06[b]	116.2±3.06[a]	**
ALT (iu/l)	18.8±0.63[b]	26.8±0.63[a]	**
AST (iu/l)	21.1±1.08[b]	67.9±1.08[a]	**
Inorganic Phosphorus (mmol/L)	1.9±0.04[b]	2.3±0.04[a]	**
T_3 (ng/ml)	0.89±0.04	0.87±0.04	NS
T_4 (µg/dl)	47.9±0.84[a]	5.3±0.84[b]	**

[ab]Means with different superscript differs significantly (P<0.05) across the row, LOS- level of significance. **-P<0.01-Highly significant; NS-Not significantly;

Table 4.8: Effects of Season and Breed Interaction on Biochemical and Response of Dairy Cows.

Breed	**Brown Swiss**		**Simmental**		**Holstein Friesian**		
Season	**Cold dry**	**Hot dry**	**Cold dry**	**Hot dry**	**Cold dry**	**Hot dry**	
Parameters							**LOS**
Glucose (mmol/l)	2.73 ± 0.14^{abc}	4.40 ± 0.14^{a}	2.66 ± 0.14^{c}	2.47 ± 0.14^{abc}	2.54 ± 0.41^{abc}	3.56 ± 0.14^{b}	**
Cholesterol (mg/dl)	93.7 ± 5.29^{d}	116.7 ± 5.29^{b}	108.9 ± 5.29^{d}	110.6 ± 5.29^{c}	82.7 ± 5.29^{d}	121.4 ± 5.29^{a}	**
ALT (iu/l)	20.8 ± 1.09^{b}	33.9 ± 5.29^{a}	18.5 ± 1.09^{c}	24.1 ± 5.29^{b}	17.0 ± 1.09^{d}	22.4 ± 5.29^{b}	**
AST (iu/l)	20.7 ± 1.87^{e}	72.4 ± 1.87^{a}	23.9 ± 1.87^{d}	71.6 ± 1.87^{b}	18.5 ± 1.87^{f}	59.6 ± 1.87^{c}	**
Phosphorus(mmol/L)	2.01 ± 0.07^{b}	2.25 ± 0.07^{b}	1.95 ± 0.07^{b}	2.30 ± 0.07^{a}	1.73 ± 0.07^{b}	2.26 ± 0.07^{b}	**
T_3 (ng/ml)	0.97±0.06	0.72±0.06	0.73±0.06	1.04±0.06	0.98±0.06	0.87±0.06	NS
T_4 (µg/dl)	45.9 ± 1.45^{b}	5.41 ± 1.45^{c}	50.7 ± 1.45^{a}	5.18 ± 1.45^{d}	46.9 ± 1.45^{b}	5.34 ± 1.45^{c}	**

[abcd]Means with different superscript differs significantly (P<0.05) across the row, **-P<0.01-Highly significant; NS-Not significant; HF-Holstein Friesian, LOS-level of significance.

4.3.4 Milk yield and composition of exotic dairy cows

Milk yield, percentage fat and milk protein of Brown Swiss, Simmental and Holstein FriesianDairyCows is presented in table 4.9. Milk yield and percentage fat were significant ($P<0.05$) for all the breeds. Holstein Friesian cows had the highest average daily milk yield (8.16 kg) followed by Brown Swiss (7.19 kg) and Simmental cows (7.08 kg). Simmental and Brown Swiss cows had similar and highest percentage of fat (4.07 and 4.04%) respectively which was higher ($P<0.05$) than Holstein Friesian cows (3.88 %). Milk Protein showed non significant ($P>0.05$) difference among breeds.

4.3.5 Milk yield and composition during cold-dry and hot-dry seasons

Table 4.10 presents daily milk yield, fat and protein as affected by seasons. Milk yield was significantly affected by season. Daily milk yield was significantly ($P<0.05$) higher during the cold-dry season (8.03±0.24 kg) than the hot-dry season (6.93±0.21 kg). Whereas daily fat yield was higher during the hot-dry season (4.09) compare to cold-dry season (3.91), while milk protein was not affected by season.

Table 4.9: Milk Yield and Composition of Exotic Dairy Cows.

Parameters	Breed			LOS
	Brown Swiss	**Simmental**	**Holstein Friesian**	
Daily Milk yield (kg/d)	7.19 ± 0.28[b]	7.08 ± 0.28[b]	8.16 ±0.28[a]	**
Milk Fat (%)	4.04 ±0.05[a]	4.07 ±0.05[a]	3.88± 0.05[b]	**
Milk Protein (%)	3.66 ± 0.11	3.65 ± 0.11	3.74± 0.11	**NS**

[ab]Means with different superscript differs significantly (P<0.05) across the row, HF-Holstein Friesian; NS-Not significant, LOS- level of significance

Table 4.10: Milk Yield and Composition during Cold-dry and Hot-dry Seasons.

	Season		
Parameters	**Cold-dry**	**Hot-dry**	**LOS**
Daily milk yield (kg/d)	8.03±0.24[a]	6.93±0.21[b]	**
Milk Fat (%)	3.91±0.04[b]	4.09±0.04[a]	**
Milk Protein (%)	3.68±0.09	3.68±0.09	**NS**

[ab]Means with different superscript differs significantly (P<0.05) across the row, HF-Holstein Friesian. NS-not significant, LOS- level of significance

4.3.6 Interaction between season and breed on milk yield and milk composition of exotic dairy cows

Season and breed interaction on milk yield and composition (fat and milk protein percentages) of Dairy Cows is shown on (Table 4.11). The Milk yield and fat content were significantly ($P<0.05$) affected by the season and breed interactions while protein content was statistically similar ($P>0.05$)across the groups.Holstein Friesian breed had the highest daily milk yield (8.68 kg) during the cold dry season followed by Brown Swiss (7.86 kg) and Simmental (7.54 kg). Simmental had the highest fat content (4.21%), followed by Brown Swiss (4.10 %) and Holstein Friesian (3.96 %) during the hot dry season. Highest daily milk yield was recorded during the cold-dry season compare to hot-dry where as highest fat percentage was observed during the hot-dry compare to cold-dry. Daily milk protein percentage had non significant breeds and seasons interaction effect.

4 .4Correlation Analysis between Temperature, Serum Biochemical Parameters, Milk Yield and Composition in Dairy Cows.

4.4.1Correlations between milk yield, protein, fat and environmentalconditions of dairy cows.

Table 4.12 shows the correlations between milk yield and environmental conditions. Daily milk yield was significant, low and negatively correlated with temperature ($R=-19$, $P=0.0005$) while moderate, significant and negative correlations was observed with temperature humidity index ($R=-23$, $P=0.0013$ and $R=-0.24$, $P=0.0005$) and relative humidity ($R=-0.24$, $P=0.0251$ and $R=-0.26$, $P=0.0061$) in the morning and afternoon respectively. Fat had significant, low and positive relationship with temperature ($R=0.17$, $P=0.0019$) while low and positive relationship existed with temperature humidity index ($R=0.18$, $P=0.60$ and $R=0.19$, $P=0.76$) and relative humidity ($R=0.12$, $P=0.059$ and $R=0.15$, $P=0.177$) in the morning and afternoon respectively. Protein

had low and negative correlations with all the environmental factors with the exception of temperature which was positively associated (R =0.003, P=0.9501). Temperature had significant, high and positive relationship with all the environmental factors.

4.4.2 Correlation between serum biochemical parameters and milk yield of dairy cows.

The correlations between milk yield and serum biochemical parameters of Dairy Cows is shown on (Table 4.13).Daily milk yield was significant and highly correlated with phosphorus (R=0.52; p=0.02) and T_4 (R=0.94; p=0.05) but significant, high and negatively correlated with T_3 (R=-0.51, P=0.02). Cholesterol was positive significant and highly correlated with phosphorus (R =0.51, P=0.02) and T_4 (R =0.88, P=0.05) but significant, high and negatively correlated with T_3 (R=-0.69, P=0.0007) and DMY (R =-0.28, P=0.2316). ALT was positive significant and highly correlated with phosphorus (R=0.43, P=0.0608) and T_4 (R = 0.85, P=0.05) but significant, moderately and negatively correlated with DMY (R=-0.26, P=0.2751). AST was negatively correlated with T_3 and DMY (R=-0.28, P=0.2298) but positively and significant correlated with T_4 and phosphorus.

Table 4.11: Interaction between Season and Breed on Milk Yield and Milk Composition of Dairy Cows

Breed	**Brown Swiss**		**Simmental**		**Holstein Friesian**		
Season	**Cold Dry**	**Hot Dry**	**Cold Dry**	**Hot Dry**	**Cold Dry**	**Hot Dry**	
Parameters							**LOS**
Milk yield (kg/d)	7.86±0.41[b]	6.52±0.37[c]	7.54±0.41[b]	6.62±0.37[c]	8.68±0.41[a]	7.63±0.37[b]	**
Milk Fat (%)	3.98±0.07[b]	4.10±0.06[a]	3.93±0.06[b]	4.21±0.06[a]	3.79±0.07[b]	3.96±0.06[b]	**
Milk Protein (%)	3.56±0.16	3.75±0.15	3.65±0.16	3.66±0.15	3.83±0.16	3.64±0.15	NS

[ab]Means with different superscript differs significantly (P<0.05) across the row, HF-Holstein Friesian, LOS- level of significance

Table 4.12:Correlation between Milk Yield and Environmental Conditions

	DMY	Milk Fat	Milk Protein	Temperature
RHM	-0.24*****	0.12	-0.10	0.48******
THIM	-0.23******	0.18	-0.03	0.97******
Temperature	-0.19******	0.17	0.003	0.99******
RHA	-0.26******	0.15	-0.07	0.54**
THIA	-0.24******	0.19**	-0.02	0.97******

DMY-daily milk yield, RHM- relative humidity for morning, RHA- relative humidity afternoon, THIM-temperature humidity morning, THIA- temperature humidity index afternoon

Table 4.13: Correlation between Serum Biochemical Parameters and Milk Yield

	Glucose	Cholesterol	ALT	AST
Phosphorus	0.52*	0.51*	0.43*	0.55**
T3	-0.51*	-0.69**	-0.17	-0.54**
T4	0.94**	0.88**	0.85**	0.96**
DMY	-0.23*	-0.28*	-0.26*	-0.28*

T3- triiodothyronine, T4-thyroxine, AST-aspartate; aminotransferase, ALT- alanine aminotransferase, DMY-daily milk yield.

4.5 Prediction Equation of Milk Yield in Dairy Cows Using Invasive and Non-Invasive Markers.

4.5.1 Multiple regression and prediction equation for milk yield from invasive parameterin dairy cows

The prediction equation of milk yield shown in (Table 4.14) showed that all the invasive markers combined together best explained daily milk yield (R^2= 0.52) in Brown Swiss dairy cows compare to Simmental and Holstein Friesian which were weakly predicted (R^2= 0.18). This means that combination of invasive markers (glucose, cholesterol, alt, ast, phosphorus, T_3 and T_4) given in equation (ii) in Brown Swiss explained daily milk yield better than equations (iii) in Simmental and (iv) in Holstein Friesian breeds. Although the regression analysis between Brown Swiss, Simmental and Holstein Friesian using invasive markers was not significant (P>0.05). When the breeds were pooled, the efficiency of prediction was (R^2= 0.50) equation (i). The estimated prediction equation / regression model are presented below:

PL=83.09-2.37Glu-0.47Cho-0.12ALT+0.01AST-6.78Phos-11.51T_3+0.33T_4.....(i)

BS = 18.49-4.40Glu-0.08Cho+0.13ALT-0.19AST+4.89Phos-0.33T_3+0.21T_4.....(ii)

SIM = 3.95-0.03Glu+0.02Chol+0.09ALT-0.07AST+4.78Phos-5.44T_3-0.06T_4.....(iii)

HF = 3.95-0.03Glu+0.02Chol+0.09ALT-0.07AST+4.78Phos-5.44T_3-0.06T4.....(iv)

Table 4.14: Multiple Regression and Prediction Equation for Milk Yield from Invasive Markers in Dairy Cows

Parameter	Coefficients											
	Constant	**Glucose**	**Cholesterol**	**ALT**	**AST**	**Phosphorus**	**T_3**	**T_4**	**R^2**	**N**	**P**	**LOS**
Pooled	Y=83.09±32.22	-2.37±2.87	-0.47±0.22	-0.12±0.13	0.01±0.14	-6.78±3.19	-11.51±4.78	0.33±0.11	0.50	60	0.196	NS
BS	Y=18.49±16.88	-4.40±4.47	-0.08±0.05	0.13±0.40	-0.19±0.20	4.89±5.27	-0.33±3.58	0.21±0.23	0.52	60	1.60	NS
Sim	Y=3.95±24.89	-0.03±3.37	0.02±0.11	0.09±0.44	0.07±0.25	4.78±5.38	-5.44±15.27	-0.06±0.27	0.18	60	0.893	NS
HF	Y=3.95±24.89	-0.03±3.37	0.02±0.05	0.09±0.44	0.07±0.25	4.78±5.38	-5.44±15.27	-0.06±0.27	0.18	60	0.893	NS

ALT = Alanine Amino Transferase, AST = Aspertate Amino Transferase, T_3 = Triiodothyronine, T_4 = Thyroxine, BS= Brown Swiss, Sim=Simmental, HF=Holstein Friesian

4.5.2 Multiple regression and prediction equation for milk yield from non-invasive markersin dairy cows.

Table 4.15 shows the prediction equation using non-invasive markers. The analysis showed that all the non-invasive markers (rectal temperature, respiratory, skin temperature and ear temperature) combined together explains only (R^2= 0.05) of milk yield when the breeds were pooled. However, combination of RT, ST, and RR explained (R^2= 0.05) milk yield in Brown Swiss. Prediction was weaker (R^2= 0.03) when only RT and RR were used as predictors in Simmental and (R^2= 0.02) when ST and ET were used as predictors in Holstein Friesian. This means that combination of all non-invasive markers given in equation (v) and combination of RT, RR and ST in equation (vi) explained milk yield better than when only two markers were used. Although, the regression analysis for non-invasise markers was significant ($P<0.05$) for milk yield in Brown Swiss, Simmental, Holstein Friesian and when breeds were pooled. The estimated prediction equations / regression models are presented below;

PL = 13.04-0.35RT+0.59ST-0.18±ET-0.12RR.....(v)

BS = 12.69-0.41RT+0.49ST-0.12RR.....(vi)

Sim = 18.37-0.11RT-0.11RR.....(vii)

HF = 10.75+0.42ST-0.53ET.....(viii)

Table4.15:Multiple Regression and Prediction Equation for Milk Yield from Non-Invasive parameterin Dairy Cows

Parameter		Coefficients							
	Constant	RT	ST	RR	ET	R^2	N	P	LOS
Pooled	Y=13.04±4.33	-0.35±0.18	0.59±0.25	-0.12±0.04	-0.18±0.27	0.052	330	0.001	**
Brown Swiss	Y=12.69±4.29	-0.41±0.16	0.49±0.20	-0.12±0.04	-	0.05	330	0.001	**
Simmental	Y=18.37±3.61	-0.11±0.98	-	-0.11±0.04	-	0.03	330	0.003	**
Holstein Friesian	Y=10.75±4.23	-	0.42±0.25	-	-0.53±0.24	0.02	330	0.082	NS

RT= rectal temperature, ST=skin temperature, RR=respiratory rate, ET=ear temperature

4.5.3 Regression between milk yield, invasive and non-invasive parameters in dairy cows

Table 4.16 shows the regression between milk yield, invasive and non-invasive parameters of dairy cows. Serum glucose explained milk yield only up to (R^2=0.03). However, serum phosphorus, T_3 and T_4 explained milk yield by (R^2=0.019), (R^2=0.019) and (R^2=0.058) respectively. When milk yield was regressed against non-invasive traits, rectal temperature and respiratory rate explained milk yield by (R^2=0.014) and (R^2=0.03) respectively. This means that invasive and non-invasive markers were singularly weak and mostly insignificant (P>0.05) in influencing milk yield. Although RT and RR were significant (P<0.05) in influencing milk yield. The estimated regression models are presented below;

MY = 10.14-0.67Glu.....(ix)

MY = 9.36-0.01Chol.....(x)

MY = 8.99-0.04ALT.....(xi)

MY = 7.74+0.01AST.....(xii)

MY = 5.15+1.41Phos .....(xiii)

MY = 5.38+3.05T_3 .....(xiv)

MY = 7.59+0.02T_4 .....(xv)

MY = 14.86-0.02RT .....(xvi)

MY = 9.60-0.06ST .....(xvii)

MY = 15.34-0.13RR .....(xviii)

MY = 13.32-0.17ET .....(xix)

Table 4.16: Regression between Milk Yield, Invasive and Non-invasive parameters

Parameter	Constant	Coefficient	R^2	N	P	LOS
Milk Yield						
Glucose	10.14±1.43	-0.67±0.45	0.0365	60	0.144	NS
Cholesterol	9.36±1.94	-0.012±0.01	0.0077	60	0.506	NS
Alanine aminotransferase (ALT)	8.99±1.38	-0.04±0.05	0.017	60	0.503	NS
Aspertate aminotransferase (AST)	7.74±0.77	0.01±0.02	0.0043	60	0.619	NS
Phosphorus	5.15±2.72	1.4±1.29	0.0199	60	0.285	NS
Triiodothyronine (T_3)	5.38±1.46	3.05±1.60	0.0588	60	0.062	NS
Thyroxine (T_4)	7.59±0.58	0.02±0.01	0.0197	60	0.285	NS
Milk Yield						
Rectal temperature	14.86±3.37	-0.20±0.09	0.0146	330	0.028	*
Skin temperature	9.60±4.23	-0.06±0.12	0.0008	330	0.607	NS
Respiratory rate	15.34±2.47	-0.13±0.03	0.0304	330	0.0015	**
Ear temperature	13.32±3.96	-0.17±0.11	0.0067	330	0.1325	NS

CHAPTER FIVE

5.0 DISCUSSION

5.1 Environmental Conditions of Study Area during the Cold-Dry and Hot-Dry Seasons.

Mean morning and afternoonambient temperature (Ta), relative humidity (RH) and calculated temperature humidity index (THI) during the experimental period shown in (Table 4.1). The cold-dry period was characterized by lack of heat stress conditions; meanmorning and afternoon Ta and RH of the study area were(25.2 and 27.0^{O}C) and (34.7 and 35.3%,) respectively. Average calculated THI morning and evening was (70.3 and 72.4). In contrast to the cold-dry season, the hot-dry season was characterized by heatstress conditions; mean morning and afternoon Ta andRH were (40.6^{O}C and 41.5^{O}C)and (46.3 and 47.3%), respectively. Average calculated THI morning and evening was (91.3 and 92.2). TheTemperature-Humidity Index iswidely used in hot areas all over the worldto assess the impact of heat stress on dairycows. THImight describe more precisely the effect ofthe environment on the cow's ability to dissipate heat (West, 1999). Cows decrease milk production when THIexceeds the critical comfort level of 72 (Johnson, 1980b).High ambient temperature have a strong negative effect on milk yield and quality (Gregory, 2010), the upper critical temperature for Holsteins is 25 to 26^{O}C(Berman*et al*.,1985).When relative humidity is high, evaporative cooling is compromised, and even these heat stress-related behaviors may not effectively prevent a rise in body temperature (West, 2003).According to Johnson (1985) and Du Preez *et al*. (1990), milk production is not affected by heat stress when mean THI valuesare between 35 and 72. In the current study, average Ta and THI during hot-dry season by far exceededthe 25^{O}C and 72 critical points, respectively, Marai and Habeeb, (1994) indicated that between 0-30^{O}C, latent heat evacuation is only controlled by altering the breathing rate. Ta and THI values during the hot-dry season for thisstudy indicated

that cows were exposed tosevere heat stress during the hot-dry season. Thisis in agreement with Johnson *et al*. (1963) and Vesna *et al*. (2011)highly significant THI 71-81 decrease milk yield and reduce intake of feed and water. In the current study, THI in the cold-dry season (72) is considered as cool and indicated absence of heat stress. THI of 72 and below is considered as cool, 78-88 as moderate heat stress (Amstrong, 1994). Whereas and THI during the hot-dry season (92) indicates exposure of dairy cows to severe heat stress in agreement with (Igono *et al*.,1992) and (West, 2003) who reported that at THI of 84 or more, death occurs. However death of animals was not observed in current study, this means the animals were adapted to this environment by their ability to dissipate heat (West, 1999).However, milk production and feed intake begin to declinewhen THI reaches 72 and continue to decline sharply at a THI value of 76 or greater (Johnson, 1980).

5.2 Thermoregulatory Response of Dairy Cows

5.2.1 Effect of season on thermoregulatory response of dairy cows.

The results of this study showed that season had a significanteffect on thermoregulatory parameters, high environmental temperature during hot-dry season influence increase in the thermoregulatory parameters; rectal temperature respiratory rate and skin temperature indicating increased heat load on the dairy animals and inability to normally dissipate the accumulated heat, this consequently leads to rising body temperature. Significant increase in RT was recorded during heat stress in buffaloes (Alok *et al*.,2014).Significant increase in RT was seen in heat exposed buffalo calves as compared to cool comfortable conditions (Korde *et al*., 2007). Therefore the observed increase in RT in all the breeds of dairy cows as season changes from cold-dry to hot-dry indicates that heat dissipation was lower than heat gain and therefore, thermal balance could not be maintained and cooling mechanism become insufficient.

There was a significant rise in respiratory rate in dairy animals during the hot-dry season. This was probably adopted to increase evaporative cooling. (Tuner *et al.,* 1992) recorded 16breath/min. less in a cooled group than in un-cooled groups of dairy cows. Alok *et al*. (2014) observed higher RR in buffaloes during thermal stress. Benerjee and Ashutosh (2011b) observed significant rise in respiratory frequencies in Tharparkar and karan fries heifers at higher environmental temperatures. The cardiovascular and respiratory adjustments in accordance to increasing stress actsas the first line of defence in maintaining thermoregulation to prevent drastic metabolic alterations.

Season had significant effect ST, as a result of animal's exposure to heat stressduring the hot-dry season, increased in skin temperature was observed. This result agrees with those of Fahmy (1994) and Marai *et al*. (1997), who reported that heat stress increased both skin and rectal temperatures in goat which have a decreasing effect on the productivity. One of the thermoregulatory mechanisms available to animals to conserved body heat is vasoconstriction. Bianca and Kunz (1978) stated that vasoconstriction leads to reduction in heat delivery to the skin so that when ambient temperature is below core temperature, a low skin surface temperature would reduce the outer temperature gradient and consequently slow the sensible heat dissipation from animal's body. The observed elevation in ST in the dairy animals can be attributed to the fact exposure to heat stress alter blood flow and redistribution of blood flow and increase blood flow to the surfaces (Marai and Habeeb, 2010). This could indicate that all the dairy breeds were stressed under the hot-dry season of sahel-savannah condition of Adamawa State, Nigeria. The result of this study showed that season had no significant effect on ear temperature (ET).Ear temperature is one of the most important dissipation pathways. The observed variations in thermoregulatory parameters RT, RR, ST and ET between cold-dry and hot-dry season could be

due to stress-induce hyperthermia (Piccione *et al*., 2007). The significant increase in each of the thermoregulatory parameters was similar to result of Habeeb *et al*. (1997) and Marai *et al*. (2001).

5.2.2 Interaction between season and breed on thermoregulatory response of dairy cows

The results of this study showed that season and breed had a significant interaction effect on some of the thermoregulatory parameters; rectal temperature and skin temperature increased significantlyin all the breeds of dairy cows during the hot-dry season indicating increased heat load on the dairy animals and inability to normally dissipate the accumulated heat, this consequently leads to rising body temperature. The study results showed thatseason and breed hadno significant interaction on respiratory rate and ear temperature. Significant increase in rectal temperature was recorded during heat stress in buffaloes (Alok *et al*.,2014). Holstein Friesian and Brown Swiss had slightly higher RT than Simmental, this is in line with (Correa-Calderon *et al*.,2004), who reported higher RT in heat-stressed Brown Swiss and Holstein cows under a cooling system as a result of high THI of 73-85.RT is a sensitive indicator of thermal balance and may be used to assess thenegative effects of hot environments ongrowth, lactation and reproduction of dairycows (Johnson, 1980aand West, 1999). It has been shown that a rise of $1^{O}C$ or less in rectal temperature isenough to reduce intake and production indairy cows (Johnson *et al*., 1963). Johnson *et al*. (1963) reported that milk yield declines when bodytemperature exceeds $38.9^{O}C$, and, for each$0.55^{O}C$ increase in RT, milk yield and intake of total digestible nutriment decline by1.8 and 1.4 kg, respectively.

Skin temperature (ST) for all the breeds had a significant increase during the hot-dry season and Holstein Friesian cows had slightly higher ST compare to Brown Swiss and Simmentalcows as result of animals' exposure to heat stress. This result agrees with those ofPiccione *et al*., (2007),

Fahmy (1994) and Marai *et al*. (1997), who reported that heat stress increased skin temperatures (ST) in goat.Animals body temperature expressed as skin temperature increased when the body fails to maintain its heat balance (Marai *et al*.,2007). This means that Brown Swiss and Simmental breeds maintained heat balance better than Holstein Friesian.The mammalian skin is an important pathway for heat exchange between body surface and the environment. ST is the result of the adjustment of the skin blood flow that ends with regulation of heat between body core and skin (Habeeb *et al*., 1992). Resistance of animal coat to environmental heat flow is of greater importance in tolerance to heat stress, coat color also affect heat exchange, compare to black color, brown reduces the inward flow of heat Finch (1986). Brown Swiss and Simmental cows might have benefited from their lighter coat color compare to the Holstein Frisian. Brown Swiss had higher rate of cutaneous evaporation than Holstein cows resulting in a lower skin temperature (Armstrong and Hillman, 1998). Correa-Calderon *et al.* (2004) reported higher RR in heat-stressed Brown Swiss and Holstein cows under a cooling system as a result of high THI of 73-85.The behaviour of the dairy cows changed during the hot-dry season, Lower frequencies of rumination and urination during the hot-dry season were also observed, which possibly indicates changes in the reticular-rumen environment, changing the fermentation pattern and water conservation mechanism respectively (Benejee and Ashutosh, 2011a). They were more restless, nervous and showed reduced salivation accompanied with panting. Srikandakumar *et al*. (1992) found a reduction in RR of Holstein cows subjected to spray and fan cooling system compare to those under shade. Dairy animals uses breathing rate, reduced feed intake, ear temperature, reduced urination and salivation as devices to maintain homeostasis, however, respiration and ear temperature are most important dissipation pathways (Akinsola, 2012).

5.2.3 Circadian rhythm

The body temperature of homoiotherms is not always constant, but displays day-night and rhythm changes, normally higher in the day time and lower during the night. The information on circadian rhythm is hypothesized to be related to the difference between heat production and dissipation (Webb, 1997). Hillman *et al*. (1985) indicated that the rhythm of body temperature was related to endocrine system and more in particular thyroid hormones. Based on the current study results, the changes of ambient temperature, relative humidity and Temperature Humidity Index during the morning and afternoon periods for both seasons agrees with (Adenkola *et al*., 2009) who reported low THI in the morning and late evening hour, when it was high in the afternoon alongside windy and dusty conditions.

The thermoregulatory parameters RT, RR, ST and ET in dairy cows shown in (Table 4) were not significantly affected by circadian rhythm, the values recorded in the morning period were similar to those of the afternoon period. It has been reported that circadian rhythm of body temperature is affected by physiological status (Bishop *et al*., 2000).

The changes in the thermoregulatory parameters RT, RR, ST and ET in dairy cows shown in (Table 5) shows that thermoregulatory parameters varies with season and circadian rhythm. Mornings and cold-dry season values were lower than afternoon and hot-dry season values. Food intake, physical activity, light; dark cycle and light intensity all play a part (Whittow, 1986). Higher values were observed for RT, RR, ST and ET in the afternoon time of the day, the diurnal variations agrees with observation made by (Piccione *et al*., 2007) who observed that such fluctuations was driven by biological clock in the hypothalamus. Akinsola, (2012) observed similar diurnal variations in rabbit.

5.3 Biochemical Response of Dairy Cows

5.3.1 Effect of breed on biochemical response of dairy cows

The results of the present study shows that breed had different effects on biochemical parameters of the Dairy Animals. There was an increased plasma glucose concentration of the breeds; being in the order of Brown Swiss, Holstein Friesian and Simmental. Glucose is a major component of Cow's milk and a coordinator of the endocrine mechanism controlling homeorhesis (Lucy *et al*., 2014). Whereas the breeds showed increase activities of the enzymes ALT and AST,they did not show any effect on serum concentrationsof cholesterol, phosphorus, T_3 and T_4. The significantly high enzymes level in all the breeds was probably a mechanism for better adaptation. Nazifi *et al*. (1999) suggested that the increase activities of these enzymes are to increased heat adaptation.Simmental and Brown Swiss breed showed increased activities in the enzymes AST than Holstein Friesian cows, this could mean that Brown Swiss and Simmental were better adapted to the Sahel Savannah zones of Nigeria. However, Brown Swiss had higher concentration of glucose, ALT activities and lower T_3 and T_4 serum concentration compared to Simmental and Holstein Friesian. Johnson *et al*. (1988) also showed that the decline in thyroid hormones (T_3 and T_4) in response to heat stress is an attempt to reduce metabolic heat production in the cow. This could be a mechanism for better adaptation to the environment by Brown Swiss. T_3 concentration was higher in Holstein Friesian compare to Simmental and Holstein Friesian. T_3 is an important hormone for animal's adaptation to hot environment (Correa-Calderon *et al*., 2004). It has been shown that factors that have inhibitory effects on thyroid function could reduce production efficiency (Robinson *et al*., 1996; Huszenicza *et al*., 2000). Spicer *et al*.(2001) reported a direct stimulatory effect of T_3 and T_4 on thecal cells during steroidogenesis in cattle.

5.3.2 Effect of season on biochemical response of dairy cows

The present study showed that season had a significant effect on serum biochemical parameters of dairy cows. Heat stress had an increasing effect on the serum biochemical parameters. The results also showed that the concentration of serum $T_4$and T_3in thehot-dry season was significantly lower than that of the cold-dry season. This adaptive response may be associated with decrease in feed intake and metabolic heat production during the hot-dry season. It has been reported that in hot environment, heat acclimation and physiological adjustment by the thermoregulatory centre induces a decrease in endogenous heat production influenced mainly by thyroid hormone (Barnabucci *et al*., 2010). However, the difference in concentration of serum triiodothyronine (T_3) in both seasons was not significant. T_3 level may be affected by food intake than thermal stress (McGuire *et al*., 1991). This could be the case in the present study because similar feed was offered to the animals during cold-dry and hot-dry season. In the dog, serum T_3 and T_4 levels were lowest in summer and their concentrations were at the highest levels in winter (Tuckova *et al*., 1995), this agrees with the current finding on thyroid hormones. Also, it is been recorded that during summer, the exposure of animals to high environmental temperatures depressed the functional activity of the thyroid hormones(Nazifa *et al*. 1999). Based on this study results on T_4 and T_3, cold environment may be a stimulus to increase the thyrotrophic hormones output there by resulting in a higher concentration of thyroid hormones in the serum. The reason for decline in the thyroid hormones (T_4 and T_3) during the hot-dry season could an adaptive response to the prevailing heat stress during this period. Johnson *et al*. (1988) also showed that the decline in thyroid hormones (T_4 and T_3) in response to heat stress is an attempt to reduce metabolic heat production in the cow.

The serum glucose and cholesterol concentration of the three breeds of dairy cows in this study were significantly affected by season. Their concentrations were low in cold-dry than hot-dry seasons. The observed increase in plasma glucose concentration during the hot-dry season may be related to reduction in feed intake and insulin concentration, (Habeeb, 1987) and the action of glucocorticoids (Nessim, 2004). Some authors reported that in cattle and neonatal calves, season had no effect on serum glucose (Kweon *et al.,* 1986, Chand and Georgie, 1989). On the other hand, Eldon *et al*. (1988) showed higher serum glucose concentration of cows in winter. The decrease in serum cholesterol concentration during the cold dry season is similar with the observation of Sinha *et al*. (1981) which showed that the concentration of cholesterol in cattle was higher during the summer. In contrast to the current findings, Kataria *et al*. (1993) and Soveri *et al*. (1992) reported that serum cholesterol concentration were higher during the winter (cold) in Marwa goats and reindeer calves respectively.

AST and ALT activities were significantly higher in hot-dry season compared to cold-dry season. The significantly high enzymes level during hot-dry was probably due to stimulation of gluconeogenesis by glucocorticoids to meet the increasing energy demands for production during heat stress.Nazifi *et al*. (1999) suggested that the increase in activities of the enzymes is to increased heat adaptation; this is in agreement with the current findings. The increase in the concentration of these enzymes in plasma is might be due to the leakage of these enzymes from the liver cytosol into the blood stream, which reflects liver damage and disruption of normal liver function. Marai *et al*. (1997) reported similar result showing significant increase in the enzymes AST and ALT in summer compared to winter in Friesian calves.The current study showed that serum inorganic phosphorus concentration increased in hot-dry compared to cold-dry season, this agrees with the result of Fatemi, (1989) who reported that mean serum inorganic phosphorus

concentration decreased significantly during the winter in dairy animal. In contrast, Seifi *et al*. (1997) reported that serum inorganic phosphorus concentration was highest in winter and lowest in spring and autumn. The lower level of phosphorus during the cold-dry season in the current study may be associated with lower rate of synthesis of vitamin D_3 which increases extracellular levels of calcium and phosphorus. Serum vitamin D_3 concentration in sheep has been associated with seasonal variations in solar radiation, (Smith and Wright, 1981). Yokus and Cakir (2006) reported that for dairy cows reared under tropical conditions, phosphorus varied only with seasonal but not physiological changes due to thermal stress.

5.3.3 Interaction between season and breed on biochemical response of dairy cows

The present study showed that season and breed had a significant interaction on some serum biochemical parameters in Holstein Friesian, Brown Swiss and Simmental breed of dairy cows during the cold and hot dry season. In this study, the concentration of serum T_4in hot dry season was significantly lower than that of the cold-dry season. This could be as a result of adaptive response associated with decrease in food intake and metabolic heat production during the hot dry season.The prolonged hot weather in hot dry season had a significant effect on T_4suppression in all the breed of dairy cows. It might be suggested that the low serum thyroid hormone levels in hot dry season for all the breed in our study was a consequence of an adaptive decrease in production and secretion rate of $T_4$and altered T_4 to T_3 conversion in extrathyroidal tissues. In the brain and the peripheral tissues (liver, mammary epithelium and others) the most inactive T_4 may undergo extra thyroidal enzymatic activation by 5-deiodinase producing much more potent T_3 (Huszenicza *et al*., 2000). Therefore, the depression of thyroid secretion rate in all the exotic Dairy Cows in present study might be linked to heat stress. Thermal exposure acts directly on the hypothalamic pituitary axis and causes a reduction in TSH secretion (Tal andSulman, 1973).

For dairy cows in early lactation, the state of hypothyroidism is present and it is the cause of the secretion of thyroid hormones in the milk (Pezzi *et al.,* 2003). During summer, Holstein cows under cooling system showed higher levels of T_3 in serum than shaded groups of cows (Deresz 1987). Yagil *et al*. (1978) and Nazifi*et al*. (1999) reported higher levels of serum T_3 and T_4 in summer in comparison to winter in camels. The serum concentration of T_4 was lower and higher in Brown Swiss compare to Holstein Friesian and Simmental during the cold-dry and hot-dry season respectively. It is therefore likely that species difference could have an effect in changes in these hormones in cold and hot seasons. Adaptation to environment could play a bigger effect than the season. Correa-Calderon *et al.* (2005) reported that Brown Swiss cows appeared to be affected less adversely by hot weather compare to Holsteins. Johnson *et al*. (1988) also showed that decline in thyroid hormones (T_3 and T_4) in response to heat stress is an attempt to reduce metabolic heat production in the cow.Cold environment may be a stimulus to increase the thyrotrophic hormones output there by resulting in a higher concentration of thyroid hormones in the serum. Johnson and Vanjonack (1976), Correa-Calderon *et al*. (2004) reported that Holstein cows showed greater sensitivity to heat stress than Brown Swiss. Brown Swiss had higher rate of cutaneous evaporation than Holstein cows resulting in a lower skin temperature Armstrong and Hillman (1998). Concentrations of T_3 in Simmental was within the normal rangeof 0.91±0.28 for dairy cows while that of Brown Swiss and Holstein Friesian was above the normal range during cold-dry season a reverse case was observed during the hot-dry season. T_4in all breed of dairy cows was outside the normal range of 3.20±0.90 for healthy dairy cows(McGuire *et al*., 1991).

The serum glucose and cholesterol concentration of the three breeds of dairy cows in this study were lower in cold-dry than hot-dry seasons. Low glucose level during the cold-dry could be as a result of increased insulin in circulation and glucose utilization by the lactating mammary gland.

Glucose is a blood parameter defining energy metabolism in late pregnancy and lactating cows (Djokovic *et al.,* 2010) indicating that the sudden activity of mammary gland and increase lactose synthesis will leads high demand of glucose by the lactating cows.

Serum glucose concentration for Brown Swiss and Holstein Friesian dairy cows in this study significantly increased during the hot-dry season, this is in line with (Nazifi *et al*., 1999) who reported that glucoseconcentrations was higher during the summer months than the winter period. However, Simmental breed showed a slight decrease in serum concentration of glucose during the hot-dry season. However, reduction in plasma glucose concentration could be attributed to acceleration of respiration in heat stressed animals, (Schroter *et al.,* 1987) which cause high utilization of glucose by respiratory muscle (Kamal *et al*., 1962, Shaffer *et al*., 1981).

Some authors reported that in cattle and neonatal calves, season had no effect on serum glucose (Kweon *et al.,* 1986, Chand and Georgie, 1989). On the other hand, Eldon *et al*. (1988) showed higher serum glucose concentration of cows in winter.Djokovic *et al.* (2010) reported significantly low glucose level in the blood of puerperal healthy cows compare to pregnant cows.

Current observation on cholesterolis similar with that of Sinha *et al*. (1981) which showed that the concentration of cholesterol in cattle was higher during the summer. In contrast to the current findings, Soveri *et al*. (1992) and Kataria *et al*. (1993) reported that serum cholesterol concentration were higher during the winter (cold) in Marwa goats and reindeer calves respectively. Significantly lower total cholesterol concentration was observed in the blood of ketotic cows than the values obtained from healthy cows before and after lactation (Djokovic *et al.,* 2010). During early lactation, cows are in the state of metabolic stress, in order to satisfy the increase energy demand of mammary gland and adjustment of neuro-endocrine system of dairy cows to new metabolic needs of the body (Bauma *et al.,* 1980).

The results of this study showed that serum AST and ALT activities were significantly higher in hot dry season compared to cold dry season for all the breeds. Marai *et al*. (1997) reported similar result showing significant increase in the enzymes AST and ALT in summer compared to winter in Friesian calves.The enzymes AST and ALT have a key role in gluconeogenesis hence are essential for stress adaptation (Kaneco *et al*., 2008). The significantly high enzymes level during thermal stress was probably due to stimulation of gluconeogenesis by glucocorticoids to meet the increasing energy demands for production during heat stress. Marai *et al*. (1997) reported similar result showing significant increase in the enzymes AST and ALT in summer compared to winter in Friesian calves. Higher AST activities in summer was also reported by Georgie *et al*. (1973) and Shaffer *et al*. (1981) in cattle, Kataria and Bhatia (1991) in camel, Kataria *et al*. (1993) in camel and Marwari goats. The increase in blood concentration of AST is considered one of the indicators of hepatic steatosis (fatty liver) in postpartum period (Cebra *et al*., 1997).

The current study showed that serum inorganic phosphorus concentration for all the exotic breeds increased in hot-dry compared to cold-dry season, this agrees with the result of Fatemi, (1989) who reported that mean serum inorganic phosphorus concentration decreased significantly during winter period in dairy animals. The concentration of inorganic phosphate in the winter months were higher than that of summer month (Nazifi *et al*.,1999). The lower level of phosphorus in the current study may be associated with lower rate of synthesis of vitamin D_3 which increases extracellular levels of calcium and phosphorus. Serum vitamin D_3 concentration in sheep has been associated with seasonal variations in solar radiation, (Smith and Wright, 1981). Yokus and Cakir (2006) reported that for dairy cows reared under tropical conditions, phosphorus varied only with seasonal but not physiological changes due to thermal stress. The

result of Nazifi *et al*. (1999) also showed significant difference in the concentration of serum glucose, inorganic phosphate, triiodothyronine (T_3), thyronine (T_4), and in the activities of ALT and AST under heat stress and cold stress conditions in dromedary camels, this validate the present results.

5.3.4 Milk yield and composition of dairy cows

The current results showed significant daily milk yield and percentage fat for the three breeds groups, compared to Brown Swiss and Simmental breeds, Holstein Friesian had the highestdaily milk yield and lowest milk fat percentage and higher protein percentage. Brown Swiss also had higher daily milk yield compare to Simmental breeds. Correa-Calderon *et al*. (2005) reported that milk production was significant ($P<0.05$) 6.9kg/d in Holstein cows under evaporative cooling system koral kool and 8.1kg/d higher ($P<0.05$) under spray and fan cooling system than the group under ordinary shade. The reductions in voluntary intake and thesubsequent declines in milk production areconsistent responses to heat stress in lactating dairy cows Beede and Collier, (1986);Mohamed and Johnson (1982) The adverse effect on milk yieldwas most likely mediated through a reduction in DMI, which decreased by 1.73 kg or9.6%, and changes that occurred in bodytemperature and plasma hormone concentrations.

The percentages fat recorded for the breeds of dairy cows in this study was 4.07%, 4.04% and 3.88% for Simmental,Brown Swiss, and Holstein Friesian respectively. Similar values 4.049% and 4.04% were reported by USDA (2007) and Alade *et al*. (2013) in cows. However, the values recorded in this study is higher than 2.7% reported by Ozrenk and Selculk (2008) for Cows in Van-province, Turkey and 3.6 % for cows in Romania reported by Silvia *et al*. (2011). The values recorded in this study is lower than 4.77 % reported for Friesian-Sahiwa cross breed by

(Muhuyi *et al*., 2000), 4.60 % for Guernsey (USDA, 2007), and 4.94 % for Hungarian grey cattle (Kovacs *et al*., 1999). A depression in milk fat and protein percentages associated with heat stress environment was observed by (Rodrigeuz *et al*., 1985). On the other hand, Knapp and Grummer (1991) found no significant decrease in milk fat percentage for cows under heat.

The result of this study also showed that percentage protein in all the groups is insignificant, although Holstein Friesian had high protein percentage (3.74%) compare to Brown Swiss (3.66%) and Simmental (3.65%). Neitz and Robertson (1991) reported 3.3% in Guernsey in kwazulu-natal, 3.18 %, 3.38 %, 3.37 % and 3.06 % in Ayrshire, Brown Swiss, Guernsey and Holstein, respectively. Muhuyi *et al*. (2000) reported 3.03 % in Friesian-Sahiwal cross bred in Kenya, relatively higher values of 3.9 % in Jersey and 3.5 % in Ayrshire were reported by Neitz and Robertson (1991).Emery (1978) quoted that decrease in levels of food intake during lactation are usually associated with decrease in protein content.

5.3.5 Milk yield and composition during cold-dry and hot-dry seasons

The present study shows that heat stress had a significant effect on milk production and percentage fat. Milk production was significantly higher (8.03 kg/d) during the cold-dry season compared to (6.93 kg/d) hot-dry season where as fat percentage values was higher (4.09%) during the hot-dry season compare to (3.91%) cold-dry.This result agrees with several studies that reported higher milk production under cool weather (Correa-Calderon *et al*., 2005; Igono *et al.*, 1987; Goodwin *et al*.,1997). Animals' capacity to dissipate heat by evaporation was reduced as a result of high ambient humidity and consequently high THI, this is in line with results reported by (Correa-Calderon*et al*., 2005; Flamenbaum *et al*., 1986).

The reduction in milk yield during hot season may be due to decreased nutrient intake and nutrient uptake by portal drained viscera of the cow. Blood flows shifted to peripheral tissues for cooling purposes may alter nutrient metabolism and contribute to lower milk yield during hot weather. A mean daily THI value of 72 is considered to be the critical point at which milk yield is reduced (Johnson, 1987) higher THI values 72.3 and 92.2 were observed in our study for cold and hot dry seasons respectively.

The highest percentage fat recorded in this study was 4.10%, 4.21% and 3.96% for Brown Swiss, Simmental and Holstein Friesian respectively during the hot dry season; similar values 4.049% and 4.04% were reported by USDA (2007) and Alade *et al*. (2013) in cows. Shearer and Beede (1990) suggested that heat stress is associated with changes in the milk production and composition, which may be due to reduction in feed intake. Correa-Calderon *et al.* (2005) reported significantly ($P<0.05$) high fat percentage (3.37%) in heat stressed Brown Swiss under cooling system as compare to those under shade (3.3%).Knapp and Grummer (1991) found no significant decrease in milk fat percentage for cows under heat stress.

Percentage protein was not significantly ($P>0.05$) different for both cold and hot dry seasons.Knapp and Grummer (1991) observed decrease in milk protein with increased maximum daily temperature. Emery (1978) quoted that decrease in levels of food intake during lactation are usually associated with decrease in protein content.Shearer and Beede (1990) suggested that heat stress is associated with changes in the milk production and composition, which may be due to reduction in feed intake. Correa-Calderon *et al*. (2005) did not find differences for protein percentages in milk of Holstein cows under spray and fan cooling system compared to group of animals under shade.

5.3.6 Interaction between Season and Breed on Milk Yield and Milk Composition of Dairy Cows

The present study shows that season and breed had a significant interaction on milk production and composition. Milk production was significantly higher during the cold-dry season for all the breeds compared to hot-dry season in the order of Holstein Friesian>Brown Swiss>Simmental where as fat percentage values were higher during the hot dry season. Compared to Brown Swiss and Simmental breeds, Holstein Friesian had the highest milk yield and lowest milk fat percentage incold-dry and hot-dry seasons. Brown Swiss did not show any milk production response to cooling systems Correa-Calderon *et al.* (2005), he reported higher milk production for cooled groups during the hottest weeks.There was a significant reduction in daily milk yield for all the breeds of dairy cows during the hot-dry season. The reduction in milk yield during hot-dry season may beas a result of blood flows shifting to peripheral tissues for cooling purposes which may alter nutrient metabolism and contribute to lower milk yield during hot weather. Also, decreased in nutrient intake and nutrient uptake by portal drained viscera of the cow could result in low daily milk yield. In line with this study results, Holstein and Brown Swiss have reduced milk production under heat stress condition, Correa-Calderon *et al.* (2005). Milk yield decreases of 10 to 40%have been reported for Holstein cows during the summer as compared to the winter(Du Preez *et al*., 1990). Moreover, heat stress is associated withchanges in milk composition, milk somaticcell counts (SCC) and mastitis frequencies(Du Preez *et al*., 1990; Nickerson, 1987;Rodriguez*et al*., 1985).

Berman *et al*. (1985) noticed that the upper limit of ambient temperature at which Holstein cattle may maintain a stable body temperature is 25-26^{O}C and that above 25^{O}C, practices should be instituted to minimize the rise in body temperature which eventually reduces milk production. A mean daily THI value of 72 is considered to be the critical point at which milk yield is reduced

(Johnson, 1987) which is lower than the value 92.2 observed in this study for hot-dry seasons.Holstein and Brown Swiss had reduced milk production under heat stress condition, Correa-Calderon *et al*. (2005).

Higher values for percentage fat recorded in this study were 4.10%, 4.21% and 3.96% for Brown Swiss, Simmental and Holstein Friesian respectively during the hot-dry season whereas lower values were recorded during the cold dry season. Similarly, Correa-Calderon *et al.* (2005) reported significantly ($P<0.05$) high fat percentage (3.37%) in heat stressed Brown Swiss under cooling system as compare to those under shade (3.3%).Similar values 4.05% and 4.04% were reported by USDA (2007) and Alade *et al*. (2013) in cows. On the contrary, Ozrenk and Selculk (2008) recorded lower values for fats; 2.7% for dairy Cows in Van-province, Turkey and 3.6 % for cows in Romania reported by Silvia *et al*. (2011). The values recorded in this study is lower than 4.77% reported for Friesian-Sahiwa cross breed by Muhuyi *et al*. (2000), 4.60 % for Guernsey (USDA, 2007), and 4.94 % for Hungarian grey cattle (Kovacs *et al*., 1999). Higher fat values recorded during the hot-dry season in this study may be as a result of reduced milk yield during this season. A depression in milk fat and protein percentages associated with heat stress environment was observed by Rodriguez *et al*. (1985). On the other hand, Knapp and Grummer (1991) found no significant decrease in milk fat percentage for cows under heat stress.

Percentage protein was not significantly ($P>0.05$) different in both seasons for all genotypes of the cows. Brown Swiss had the highest protein percentage value (3.83%) during the cold-dry season. Several authors reported similar values for protein in different breeds of cattle; Neitz and Robertson (1991) reported 3.3% in Guernsey in kwazulu-natal, 3.18 %, 3.38 %, 3.37 % and 3.06 % in Ayrshire, Brown Swiss, Guernsey and Holstein, respectively. Holstein averaged lower protein percentage under cooling system compared to groups under shade whereas protein

percentage in Brown Swiss was greater (P<0.05) for groups under shade than those under cooling system (Correa-Calderon *et al*., 2005), this validate the present study results.Knapp and Grummer (1991) observed decrease in milk protein with increased maximum daily temperature.The reduced milk production in hot season is accompanied by a decrease in milk protein contents related to a decrease in casein fraction (Bernabucci*et al*., 2002).

5.4 Correlation Analysis between Temperature, Serum Biochemical Parameters, Milk Yield and Composition in Dairy Cows

5.4.1 Correlations between milk yield, protein, fat and environmental conditions of dairy cows.

Daily milk yield was significant, low and negatively correlated with temperature (R=-19, P=0.0005) while moderate, significant and negative correlations was observed with temperature humidity index and relative humidity. The inverse relationship between milk yield and temperature, THI and RH may be due to decreased nutrient intake as a result of reduced feed intake there by resulting in reduced milk production.High ambient temperature has a strong negative effect on milk yield and quality (Gregory, 2010) Shearer and Beede (1990) suggested that heat stress is associated with changes in the milk composition and production, which may be due to reduction in feed intake. Cows decrease milk production when THIexceeds the critical comfort level of 72 (Johnson, 1980).According to Johnson (1985) and Du Preez et al. (1990), milk production is not affected by heat stress when mean THI valuesare between 35 and 72. Berman *et al*. (1985) reported that high temperature reduced milk production. Enhanced milk yield increases the overall thermal load on the cows, because of increased metabolic heat production (West, 1994).Johnson *et al*. (1963) reported that milk yield declines when bodytemperature exceeds 38.9^{O}C.

Fat had significant, low and positive relationship with temperature (R=0.17, P=0.0019) while low and positive relationship existed with temperature humidity index and relative humidity. The reduced milk yield as a result of high environmental conditions might have led to high fat percentage. Although, Knapp and Grummer (1991) found no significant decrease in milk fat percentage for cows under heat stress. Protein had low and negative correlations with all the environmental factors with the exception of temperature which was positively associated (R =0.003, P=0.9501). Temperature had significant, high and positive relationship with all the environmental factors. A depression in milk fat and protein percentages associated with heat stress environment was observed by Correa-Calderon *et al.* (2005).

5.4.2 Correlations between milk yield and serum biochemical variables of dairy cows.

Daily milk yield was significant and highly correlated with phosphorus (R=0.52) and T_4 (R =0.94) but significant, high and negatively correlated with T_3 (R=-0.51). This implies that increase in the concentration of phosphorus and T_4 will cause a corresponding increase in milk yield while decrease in the activities/concentration of T_3 will cause a corresponding decrease in milk yield. The reduction and increment(positive and negative correlation of T_3 and T_4 concentrations with milk yield) is probably a reflection of the decreased thyroid hormone secretion rateas well as to the large demand for these hormones by the mammary gland during milk production. At the beginning of galactopoiesis, there is an increase in the number of T_3 receptors in the mammary gland secretory cells during lactation (Wilson and Gorewit, 1980), and there is a greater activity of the organ-specific type-2 deiodinase enzyme, which generates T_3 intracellularly from T_4, and also of the type-3 deiodinase enzyme, which in turn deactivates the thyroid hormones (Pezzi *et al.*, 2003); there is an actual secretion of T_4 through the milk, which

may represent between 4 and 7% of the total T_4 required for the maintenance of metabolic functions (Akasha and Anderson, 1984).

Alterations in plasma T_4 levels associated with energy balance and metabolism reflect both the changes in TSH-regulated thyroid secretion rate (central regulation) (Riis and Madsen, 1985) and the balance of extra thyroidal enzymatic T_4 activation and inactivation (peripheral autoregulation) (Capuco *et al*., 2001; CassarMalek *et al*., 2001). In addition, the decrease in T_3 concentrations could yet be caused by the additional mammary gland secretion of maternal iodine during lactation. The inverse relationship between the changes in serum concentrations of T_3 and milk yield could be in part ascribed to the large demand for these hormones by the intensively lactating mammary gland and to their increased rate of metabolism in mammary tissue during the first months of lactation, when milk yield rapidly rise and reach peak values.

Enhanced milk yield increases the overall thermal load on the cows, because of increased metabolic heat production (West, 1994).T_3 had a significant negative correlation with AST and ALT whereas T_4 had a significant positive correlation with serum ALT and AST activities. This implies that increase in the concentration of T_4 will cause a corresponding increase in activities of ALT and AST, The serum T_4 had a significant ($P<0.05$) positive correlation with serum concentrations of glucose, AST, ALT and cholesterol whereas T_3 had a negative correlation with serum concentration of glucose, AST, ALT and cholesterol. The inverse relationship observed could be linked to the activity of some of these metabolites during production because they are required in different proportions there was a positive correlation between serum glucose, cholesterol and milk yield.

5.5 Prediction Equation of Milk Yield in Dairy Cows Using Invasive and Non-Invasive Markers.

5.5.1 Multiple regression and prediction equation for milk yield from invasive parameter in dairy cows

The combination of all the invasive markers (glucose, cholesterol, AST, ALT, phosphorus, T_3 and T_4) explained milk yield better in Brown Swiss dairy cows than Simmental and Holstein Friesian breeds. Equation (ii) seems to be the best predictor of milk yield (R^2 =0.52) when compare to equations (iii) (R^2 =0.18) and (iv) (R^2 =0.18). It is observed that invasive markers insignificantly explain milk yield. It is therefore shown from the result of the study that invasive markers are good predictors of milk yield in Brown Swiss dairy cows under Sahel savannah conditions of Nigeria compare to Simmental and Holstein Friasian dairy cows. The reason for this could be that Brown Swiss are more tolerant and adaptive to the climatic conditions prevailing in Sahel Savannah environmental conditions of Nigeria compare to Holstein Friesian and Simmental breeds.Similarly, Correa-Calderon *et al.* (2005) reported that Brown Swiss were better adapted to heat stress compare to Holstein. Johnson and Vanjonack (1976) and Correa-Calderon *et al*. (2004) reported that Holstein cows showed greater sensitivity to heat stress than Brown Swiss. Brown Swiss had higher rate of cutaneous evaporation than Holstein cows resulting in a lower skin temperature Armstrong and Hillman (1998).

5.5.2 Multiple regression and prediction equation for milk yield from no-invasive parameterin dairy cows.

The combination of all the non-invasive markers (RT, ST, RR and ET) in equation (v) explained milk yield better (R^2 =0.05) than when ST, ET and RT, RR were excluded in equations (vii) and (viii) respectively. Combination of RT, ST and RR in equation (vi) explained milk yield better (R^2 =0.05) when compare to equations (vii) and (viii). Therefore, equations (v) and (vi) are better predictors than (R^2 =0.03) in equation (vii) and (R^2 =0.02) in equation (viii). It was observed in this study therefore that non-invasive markers are weak predictors. Although non-invasive markers significantly explain milk yield in all the breed of dairy animals. Since our data was

collected in the Sahelian region characterized with high temperature and THI as compared to the temperate country where the weather supports optimal production for dairy cattle, non-invasive parameters does not explain milk yield. Also, there may be a time lag between environmental events (Collier *et al.,* 1981; Spiers *et al.,* 2004; West *et al.,* 2003). Spiers *et al*. (2004) observed a decreased dry matter intake (DMI) within 24 hours of heat stress and a decline in milk yield after 48 hours of heat stress. Moreover, Collier *et al.* (1981) reported that high ambient temperature 24 and 48 hours prior to milking were associated with decreased milk yield. However, the most obvious parameter for decreased milk yield is the total time with high THI under previous days (West *et al.,* 2003). Studies have shown that mean air temperature and THI two days earlier have the greatest impact on DMI and milk yield and they both declined linearly with increases in air temperature or THI (West *et al.,* 2003).

5.5.3 Prediction equation between milk yield, invasive and non-invasive parameters

From the result, an increase or decrease in the concentration of a biochemical variables increase milk yield by; glucose (R^2=0.03), cholesterol (R^2=0.0077), ALT (R^2=0.071), AST (R^2=0.0043), phosphorus (R^2=0.0019), T_3 (R^2=0.058), T_4 (R^2=0.019), RT (R^2=0.014), ST (R^2=0.0008), RR (R^2=0.034) and ET (R^2=0.0067), these does not give good explanation for milk yield. Therefore Invasive and Non-Invasive markers were singularly weak in predicting milk yield. This could perhaps be explained that an increase or decrease in a single invasive and non-invasive markers is not enough to explain milk yield in dairy animals under cold-dry and hot-dry seasons of Sahel savannah zones of Nigeria. Although T_3, RT and RR values were significant in explaining milk yield. Lower than the 76% was reported by Rejeb *et al*., (2016) in dairy cows using biochemical parameters as predictors of milk yield.

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1Summary

A study was carried out to assess the effect of thermal indices and relationship with milk yield in exotic dairy cowsusing invasive and non invasive markers in Sebore farm, Adamawa State Nigeria.

The effect of invasive and non invasive parameters on milk yield were estimated using the GLM procedure of the Statistical Analysis Software (SAS, 2002) package. Means were separated within breed using Orthogonal pair wise difference method.

The cold-dry seasonmorning and afternoonambient temperature (Ta) and relative humidity (RH) were (25.2 and 27.0^{o}C) and (34.7 and 35.3%), respectively. THI morning and evening was (70.3 and 72.4). The hot-dry season morning and afternoon Ta andRH were (40.6^{o}C) and (41.5^{o}C)and (46.3 and 47.3%), respectively. Average THI morning and evening was 91.3 and 92.2. Cold-dry and hot-dry season had a significanteffect on all thermoregulatory parameters except ET, high environmental temperature and THI during hot-dry season influence increase inthermoregulatory parameters; (rectal temperature, respiratory rate, and skin temperature).

Season and Breed had a significant interaction effect on some of the thermoregulatory parameters; RT and ST increased significantlyin all the breeds of dairy cows, whereas RR and ET were statistically ($P>0.05$) similar. Season and circadian rhythm had a significant ($P<0.05$) effect on all the thermoregulatory parameters; RT, RR, ST and ET in dairy cows.

Breeds had significant ($P<0.05$) effect on serum glucose and activities of the enzymes ALT and AST,but they did not show any significant ($P>0.05$) effect on serum concentrationsof cholesterol, phosphorus, T_3 and T_4. However, all the biochemical parameters differed significantly ($P<0.05$) between the cold-dry and hot-dry seasons with the exception of T_3 which was statistically similar ($P>0.05$). Glucose, cholesterol, ALT, AST, inorganic phosphorus had significantly higher concentration/activity during hot dry season than the cold dry season while

T_4 concentration was significantly (P<0.05) high during the cold-dry (47.9±0.84) compare to hot-dry season (5.30±0.84), T_3 was statically similar (P>0.05) during the cold-dry and hot-dry season but decrease slightly in hot-dry season.There was significant (P<0.05) breed by season interaction in all parameters measured (glucose, cholesterol, inorganic phosphorus, ALT, AST and T_4) with the exception of T_3 which was statistically similar (P>0.05).

Milk yield and percentage fat were significant (P<0.05) for all the breeds. Holstein Friesian cows had the highest average daily milk yield (8.16 kg) followed by Brown Swiss (7.19 kg) and Simmental cows (7.08 kg). Percentage protein was statistically (P>0.05) similar.Milk yield was significantly (P<0.05) affected by season. Daily milk yield was significantly (P<0.05) higher during the cold-dry season (8.03±0.24 kg) than the hot-dry season (6.93±0.21 kg). Whereas daily fat yield was higher during the hot-dry season (4.09) compare to cold-dry season (3.91)

Daily milk yield was significant, low and negatively correlated with temperature (R=-19, P=0.0005) while moderate, significant and negative correlations was observed with THI and RH (R=-0.24, P=0.0005 and R=0.26, P=0.0061) Fat had significant, low and positive relationship with temperature (R=0.17, P=0.0019) while low and positive relationship existed with THI and RH (R=0.19, P=0.76 and R=0.12, P=0.059).

Daily milk yield was significant and highly correlated with phosphorus (R=0.52; p=0.02) and T4 (R=0.94; p=0.05) but significant, high and negatively correlated with T3 (R=-0.51).Cholesterol was significant, high and negatively correlated with DMY (R=-0.28). AST was significant, moderately and negatively correlated with DMY (R=-0.26). ALT was negatively correlated with DMY.

The regression analysis for prediction of milk yield showed that all the invasive markers combined together best explained daily milk yield (R^2= 0.52) in Brown Swiss dairy cows

compare to Simmental and Holstein Friesian which were weakly predicted (R^2= 0.18). The regression analysis for prediction equation using non-invasive markers, showed that all the non-invasive markers (rectal temperature, respiratory, skin temperature and ear temperature) combined together explains only (R^2= 0.05) of milk yield when the breeds were pooled. However, combination of RT, ST, and RR explained (R^2= 0.05) milk yield in Brown Swiss. Prediction was weaker (R^2= 0.03) when only RT and RR were used as predictors in Simmental and (R^2= 0.02) when ST and ET were used as predictors in Holstein Friesian. However, invasive and non-invasive markers were singularly weak and mostly insignificant (P>0.05) in influencing milk yield. Although T3, RT and RR among others were significant (P<0.05) in influencing milk yield.

6.2Conclusions

Breedand season had significant variations on the thermoregulatory, blood, milk yield and milk component parameters. This suggests thatthese factors should be taken into consideration in an index design for genetic adaptation of dairy cows in the Sahel Savannah zone of Nigeria.

There was an inverse relationship between milk yield, temperature, THI and RH. High ambient temperature and THI had a strong negative effect on milk yield and positive effect on percentage fat.

Accuracy of prediction in modeling daily milk yield was higher in invasive variables than the non-invasive variables. This suggests that the invasive variables could serve as a biomarker for predicting average milk yield under Sahel condition. Brown Swiss proved to be better adapted to Sahel Savannah zone of Nigeria compared to Simmental and Holstein Friesian dairy breeds.

6.3Recommendations

Superficial measurements of stress (e.g. increased pulse, respiration ratesand sweating) can be considered as an added tool on the spot assessment of heat stress in dairy cows reared under the Sahel conditions of Nigeria. More sophisticated measurements could include the determination of blood levels of metabolites, such as cortisol and adrenalin.

Invasive variables can be used to explain milk production and assess the acclamatory responses of dairy cows to thermal stress under Sahel Savannah zones of Nigeria, milk collection in the Sahel Savannah zones should be targeted towards the cold-dry season period for better output.

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