EFFECTS OF PREGNANCY ON THE METABOLIC AND HAEMATOLOGICAL PROFILES OF FRIESIAN X BUNAJI AND BUNAJI COWS

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DECLARATION

I declare that the work in this thesis titled 'Effects of Pregnancy on the Metabolic and Haematological Profiles of Friesian x Bunaji and Bunaji Cows' was performed by me in the Department of Theriogenology and Production under the supervision of Dr. I. U. Ate, Professor P. I. Rekwot and Dr. J. S. Rwuaan.

The information derived from the literature has been duly acknowledged in the text and list of references provided. No part of this thesis has been previously presented for another degree or diploma in any University.

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CERTIFICATION

This thesis titled "EFFECTS OF PREGNANCY ON THE METABOLIC AND HAEMATOLOGICAL PROFILES OF FRIESIAN X BUNAJI AND BUNAJI COWS" by Christiana Chinedum DAVID meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to my parents

Elder and Mrs Charles David Okoro (JP, OFG)

for their support

and

To my husband and baby

Dr. Chikera and Chimdalu

for their love

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I thank God Almighty for giving me the opportunity to start and finish this research.

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ABSTRACT

This study was conducted to determine the body condition, metabolic and haematologic profiles reflecting the metabolic energy status prior to artificial insemination, during the different trimesters of pregnancy and at postpartum period (PP), in Friesian x Bunaji (FR x BJ) and Bunaji (BJ) cows, placed on the same feeding regimen. Twenty eight cows comprising of 11 FR x BJ and 17 BJ were observed for normal cyclicity. Only 8 cows (4 FR x BJ and 4 BJ) that showed normal cyclicity were used for the study. They where synchronized using a single intramuscular injection of 25 mg of $PGF_{2\alpha}$ and inseminated using Friesian semen. Body condition score (BCS) was obtained every morning before feeding during pre–insemination, pregnancy and PP at 30 days interval. Blood samples were similarly obtained, processed for serum and assayed for metabolites, electrolytes and calcium. Also, blood samples in sodium salt of ethylenediaminetetraacetate containing bottles were used for the determination of haematological parameters. The body condition score of the BJ cows was significantly ($P < 0.05$) higher than that of the FR x BJ cows in the second trimester of pregnancy (3.79 ± 0.10 vs 3.29 ± 0.07). The pre–insemination (control) values (range) for metabolic parameters for both breeds were glucose (GL) (2.17 ± 0.44 – 2.29 ± 0.32 mMol/L), tryglycerides (TRG) (0.61 ± 0.07 – 0.74 ± 0.08 mMol/L), cholesterol (CHL) (4.85 ± 0.77 – 5.15 ± 0.58 mMol/L), urea (UR) (7.41 ± 0.97 – 10.46 ± 2.68 mMol/L), creatinine (CRT) (188.68 ± 15.32 – 135.13 ± 23.05 mMol/L), sodium (Na^+) (152.38 ± 7.43 – 152.75 ± 5.53 mMol/L), potassium (K^+) (4.30 ± 0.29 – 4.31 ± 0.29 mMol/L) chloride ($Cl^–$) (103.38 ± 5.16 – 106.25 ± 3.69 mMol/L), bicarbonate ($HCO_3^{2–}$) (17.23 ± 2.72 – 23.00 ± 2.49 mMol/L), total protein (TP) (55.19 ± 5.16 – 59.54 ± 6.05 g/l) and albumin (ALB) (27.69 ± 3.10 – 29.02 ± 5.27 g/l). There was increase in GL, TRG, CHL, $HCO_3^{2–}$, Ca^{2+}, TP, and ALB levels in the two breeds, with onset of pregnancy,

but CRT, UR, Na^+, K^+, and Cl^- levels decreased with onset of pregnancy. There were no significant differences ($P > 0.05$) in the levels of GL, UR, CHL, Na^+, K^+, HCO_3^{2-}, Ca^{2+}, TP and ALB of the two breeds, throughout the reproductive stages. However, significant differences were obtained in other metabolic parameters at different reproductive stages. The control values for haematologic profiles for both breeds were Packed cell Volume (PCV) (37.38 ± 3.24 - 27.20 ± 5.34), Haemoglobin Concentration (Hb. Conc.) (13.10 ± 0.90 - 27.23 ± 5.27) and White blood cell count (WBC) (11.19 ± 2.01 - 14.67 ± 2.24). There was an increase in white blood cell count with onset of pregnancy in the FR x BJ, but a continuous drop throughout pregnancy, in the Bunaji cows. The present research has provided a baseline data on especially metabolic parameters of FR x BJ and BJ cows prior to insemination, during the different trimesters of pregnancy and at postpartum period, valuable in future clinical and experimental applications.

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LIST OF ABBREVIATIONS

ABBREVIATIONS	MEANING
AI	Artificial Insemination
ALB	Albumin
ANOVA	Analysis of Variance
BCS	Body Condition Score
BJ	Bunaji
CHL	Cholesterol
Cl^-	Chloride ion
CRT	Creatinine
EDTA	Ethylenediaminetetraacetic acid
FR x BJ	Friesian x Bunaji
GL	Glucose
HB	Haemoglobin
HCO_3^{2-}	Bicarbonate ion
K^+	Potassium ion
Na^+	Sodium ion
NAPRI	National Animal Production Research Institute
PP	Postpartum Period
RBC	Red Blood Cell
SEM	Standard Error of Mean
SPSS	Statistical Package for Social Science
TP	Total Protein
TRG	Tryglycerides
UR	Urea

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

The productivity of indigenous tropical cattle is generally low with late maturity, long calving intervals and low milk yield (Isaac and Olutogun, 2007). In an attempt to improve the productivity of the local cattle, there has been a need to introduce into the tropics, exotic cattle breeds such as the Friesian, an exotic cattle breed which has become very popular because of its high milk production and attendant low butterfat (Maule, 1990). The Friesian has been used in crossbreeding programmes, mostly with the Bunaji, a local breed that is relatively more tolerant to endemic tropical diseases such as streptotrichosis. The resultant cross, Friesian x Bunaji, has proven to be more disease tolerant than the pure Friesian and produces an average higher milk yield than the pure Bunaji (Adeneye, 1989; Ojango and Pollot, 2001).

Reproduction is the most important factor in determining profitability in a cow-calf enterprise (Edmonson *et al*., 1989). Thus, increased milk yield which is the main objective of trying to improve dairy cattle production in Nigeria is sequel to the reproductive status of the cow. However, several factors and the interactions of these factors, such as nutrition and body condition status affect fertility in dairy cows (Lucy, 2001).

Decline in fertility and reproductive efficiency in Friesian cows has of recent been one of the most important problems of the dairy industry (Lucy, 2001; Pryce *et al*., 2004). Increased magnitude and duration of postpartum negative energy balance, arising from

intense genetic selection for enhanced milk production, has been described as one of the main reasons responsible for this situation (de Vries and Veerkamp, 2000). Therefore, monitoring energy balance on a routine basis could facilitate improvement of fertility and reproduction (de Vries and Veerkamp, 2000).

Body condition score can be used for calculation of total body energy content and cumulative effective energy balance, which have also been proposed for energy balance studies (Banos *et al*., 2006). Furthermore, various blood metabolites such as serum glucose, triglycerides and cholesterol have been reported to be strongly correlated with energy balance (Reist *et al*., 2002; Clark *et al*., 2005).

1.2 STATEMENT OF RESEARCH PROBLEM

Cows should be in good health during and after pregnancy so as to produce viable calves. The identification of changes in the metabolism of such cows at various stages of reproduction, the determination of abnormal metabolic states, and the prediction of some metabolic disorders, such as ketosis and fatty liver could provide some advantages to producers (Andersson, 1988).

Metabolic and haematological profiles have been used to predict prepartum and postpartum metabolic problems, and for the diagnosis of metabolic diseases (Balıkcı *et al.* 2007). There is increasing evidence that nutrition and changes in metabolic state influence the reproductive performance through variations in blood metabolites (Schams *et al.,* 1999; Prunier and Quesnel, 2000).

The Friesian x Bunaji has been increasingly employed in dairy production in Nigeria, due to their ability to produce higher milk while resisting most tropical diseases (Mbap and Ngere, 1989). However, there is a dearth of information on the metabolic and haematologic profiles as well as body condition scores of the pregnant and lactating Friesian x Bunaji cows. Thus, there is unavailability of such information to aid clinicians in the appropriate diagnosis of diseases in pregnant and lactating Friesian x Bunaji cows. Reports of the biochemical profile of pregnant and lactating ewes (Krajnicakova *et al*., 1993; Karapehlivan *et al*., 2007; Piccione *et al*., 2009) and does (Krajnicakova *et al*., 2003; Iriadam, 2007) are available in the literature. Also, while much work has been done to elucidate the effect of different forms of nutrients on the blood metabolites and haematology of pregnant ruminants (Simpson *et al.,* 1998; Moallem *et al.,* 2004; Yang *et al.,* 2009), interest has not been directed to the effect of pregnancy on the same metabolites and haematological values in cows placed on the same nutrient. Therefore, this study was geared towards providing information on the effects of pregnancy on the metabolic and haematological profiles of pregnant Friesian x Bunaji and Bunaji cows. The potential value of these sensitive indices for accurately monitoring changes in energy intake in relation to animal needs, and the paucity of this information relevant to the pregnant and lactating Friesian x Bunaji and Bunaji cows prompted this study.

1.3 JUSTIFICATION

Profiles of blood metabolites have been used widely to identify problem herds and to indicate dietary causes of disease or low production. Metabolic profiles are useful when combined with haematologic values, diet evaluation, health tests and careful management monitoring of a herd (Khan *et al*., 1990).

The transition period (which overlaps the periparturient period) defined as 3 week prepartum to 3 week postpartum, is critical for a cow's health. It is marked by metabolic, hormonal, and immunological changes that have an impact on the incidence of infectious and metabolic diseases (Loiselle *et al*., 2008). This affects subsequent performance and productivity. Therefore, the availability of a blood metabolic profile in the Friesian x Bunaji and Bunaji cows during the transition period will help clinicians in drawing inferences of the incidence of metabolic diseases in these breeds.

In addition to the blood metabolites and haematological profile that indicate the metabolic status of dairy cows, the body reserves of cows, expressed as body condition score is used to monitor the amount and mobilisation of body adipose tissue. It has been reported that health and fertility parameters are related to the body condition score at pregnancy, calving and during early lactation (Broster and Broster, 1998). Body condition affects both cow and calf performance. Poor body condition is associated with reduced income per cow, increased postpartum interval, weak calves at birth, low quality and quantity of colostrum, reduced milk production, increased dystocia, and lower weaning weights (Royal *et al.*, 2000; Buckley *et al*., 2003). Hence, there is need to constantly monitor the body condition scores of cows and relate the findings to their metabolic status.

1.4 AIM

The general aim of the present study was to determine and compare the body condition, metabolic and haematological profiles of Friesian x Bunaji and Bunaji cows in different trimesters of pregnancy and the postpartum period, so as to describe their energy and metabolic status.

1.5 OBJECTIVES

The specific objectives of the study were to:

a) evaluate and compare the body condition scores of Friesian x Bunaji and Bunaji cows prior to insemination, during the different trimesters of pregnancy and the postpartum period.
b) determine and compare the blood metabolic and haematological profiles of the Friesian x Bunaji and Bunaji cows prior to insemination, during the different trimesters of pregnancy and the postpartum period.

1.6 STATEMENT OF RESEARCH HYPOTHESES

a) H_o: There is no significant difference in the body condition scores, metabolic and haematologic profiles of the Friesian x Bunaji and Bunaji cows prior to insemination, during pregnancy and postpartum period.
b) $H_{o:}$ The body condition scores, metabolic and haematologic profiles of the Friesian x Bunaji and Bunaji cows during each reproductive stage do not differ.

a) H_a: There is significant difference in the body condition scores, metabolic and haematologic profiles of the Friesian x Bunaji and Bunaji cows prior to insemination, during pregnancy and postpartum period.

b) $H_{a:}$ The body condition scores, metabolic and haematologic profiles of the Friesian x Bunaji and Bunaji cows during each reproductive stage differ.

CHAPTER 2

LITERATURE REVIEW

2.1 DAIRY PRODUCTION IN NIGERIA

In Nigeria, cattle provide more than 90 % of the total annual domestic milk output (Walshe *et al*., 1991) with the Bunaji or 'White Fulani' breed recognized as the principal producer (Adeneye, 1989). Unfortunately, the domestic output of about 407,000 metric tonnes of milk (Olaloku, 1999) from an estimated 14 million cattle (R.I.M., 1992) can hardly satisfy the dairy demands of an ever increasing population of Nigerians (Ibeawuchi *et al*., 2000).

Although natural selection over the years has provided the Bunaji with a high level of heat tolerance, tropical diseases resistance and the ability to survive long periods of feed and water shortage (Malau-Aduli *et al*., 1996), yet, poor nutrition (Adegbola, 2002) and low reproductive performance such as delay in puberty and prolonged calving interval (Rekwot *et al*., 1998) are major factors affecting milk production of the breed. To improve the milk yield potentials and reproductive efficiency of the indigenous Bunaji breed, Nigerian government, many decades ago, imported several Friesian bulls for crossbreeding with Bunaji heifers (Mbap and Ngere, 1989). The resultant Friesian x Bunaji, a cross between the tropical disease resistant Bunaji and the genetically high reproducing and milk yielding Friesian, was adopted for improved dairy production in Nigeria (Adeneye, 1989). A crossbreeding programme by means of artificial insemination, involving the local Bunaji cow and Friesian bulls imported from the United Kingdom, commenced at the National Animal Production Research Institute, Shika, Nigeria, in 1964 with the aim of improving the milk yield of the local Bunaji

(Malau-Aduli *et al*., 1996). Since then, there has been increasing interest in the use of pure Friesian or their imported semen to upgrade the indigenous cow. As the temperate dairy breeds cannot fully express their genetic potential for high milk yield and reproductive efficiency in the tropical environment, and with the low genetic potential of indigenous breeds, crossbreeding provides a platform for improved performance (Wiener, 1994). Crossbreeding brings about rapid genetic changes by incorporating genes from parents, removing inbreeding depressions and capitalizing on gene interactions that cause heterosis (Haille-Mariam *et al*., 2008).

2.2 FACTORS AFFECTING DAIRY CATTLE REPRODUCTIVE PERFORMANCE

The ultimate goal of the dairy industry is to operate an economically efficient production system which depends upon high reproductive efficiency of the cow (Royal *et al*., 2000). A variety of environmental, genetic, health and nutritional factors were reported to influence fertility in dairy cattle (Duguma *et al*., 2012).

2.2.1 Genetic Factors

Genetic improvement programmes have done a great job selecting animals with superior genes for milk production. Selection with emphasis on milk production is generally recognized as the single most important objective of breeding programme in dairy cattle worldwide (Bruno, 2010). Records of the productive ability and reproductive characteristics of dairy cows are essentials for future improvement programmes. The best performance records of the cow can be obtained by accurate estimate of economic traits in the dairy cow, such as birth weight, age at puberty, age at first fertile service, age at first calving, gestation length, postpartum heat period, days

open, service per conception, lactation length, lactation yield and calving interval (Sarder *et al*., 2007).

Over the past decades, milk yield of cows have increased markedly. However, one negative impact of this improvement is reduced fertility and higher incidence of reproductive problems, which in turn, contribute to reduced fertility (Butler, 2000). Lucy (2001) attributed this decrease in fertility not only to increased milk production, but also to adverse events surrounding the periparturient period, such as the metabolic effects of lactation on reproduction.

The heritability of breed-based reproductive traits (e.g. days open or service per conception) is very low (Royal *et al*., 2002). This is probably due to the complexity of the reproductive process which depends upon the interaction of hundreds of different gene products and thus makes it difficult to improve genetic merit for these traits through selection. It is possible that genetic potential for reproduction has actually declined in dairy cows. Specific alleles that contribute to high milk production, and therefore, are positively selected, may inhibit reproductive performance directly (Silvia, 2003).

Reproductive efficiency has an important role in breeding efficiency and milk production of cows (Dabdoub, 2009). Genetic progress is dependent on having enough heifers for replacing old cows (Bahmani *et al*., 2011). The reproductive efficiency of a dairy cow can be evaluated based on calf birth weight. Calf birth weight is useful in selection criterion for increased production and for reproductive efficiency (Olawumi and Salako, 2010). According to Jain *et al*. (1996), birth weight of an animal is a

phenotypic expression of its genotype and could be used as an indicator of superior germplasm and thus aid in the selection of high production animals just after birth. In dairy cattle, the measure of repeatability estimate may be utilized to assess the real producing ability of individual cows in a population (Olawumi and Salako, 2010). Since, genetic improvement of any trait depend in part on the heritability of that trait, and since the precision of estimating heritability is increased by reducing the environmental variation, so that adjusting the performance record for the known environmental effects should make selection more effective. Therefore, the optimal reproductive trait depends upon the interaction of genetic, nutritional, physiological and environmental factors (Hammond, *et al*., 2010).

2.2.2 **Sire and Dam**

The reproductive performance of dairy cattle is dependent on the age, weight, hip height, pelvic area and parity of the dam (Colburn *et al*., 1997; Lucy, 2001; Steinbock *et al*., 2003; Sarder *et al*., 2007). The study of Gaafar *et al*. (2011) showed that the incidence of dystocia in Friesian cows decreased significantly with age progression of the dam. Roughsedge and Dwyer (2006) reported that first-calf heifers accounted for the majority of calving difficulties and associated calf losses. The high rates of poor reproductive performance among first-calf heifers and young cows are mostly due to their smaller size at first parturition than at subsequent calvings (Gaafar *et al*., 2011). Raphaka and Dzama (2009) reported the effects of age of dam on birth weight and weaning weight. According to the authors, there was an increase in birth weight from 3 year to 6 year old dams and a peak was reached between the dam ages of 6 and 9 years, whereafter it tended to decrease with an advancing age.

Pre-calving cow weight is a significant factor influencing cattle reproductive performance (Jenkins and Ferrell, 1994). According to Colburn *et al*. (1997), dams that were heavy (43.0 Kg) at birth experienced more calving difficulty than those that were less heavy (40.1 Kg).

Gaines *et al*. (1993) reported that pelvic area measurements at any time other than calving were not associated with dystocia. They observed that heifers with the smallest pelvic areas experienced significantly more dystocia. Virtually any level of dystocia causes a delay in the cow's ability to return to estrus, thereby contributing to a higher incidence of open and later calving cows during the next calving season (Gaines *et al.,* 1993). Jenkins and Ferrell (1994) reported that cows with hip height of 52.4 inches had a calving difficulty of 15.7 % while those with hip height of 54.3 inches had calving difficulty of 23.9 %.

Hammond *et al*. (2010) reported that sire had a significant effect on reproductive efficiency of Friesian cows, and these effects were shown on days open, calving interval, number of service per conception and age at first calving, indicating that sire selection may be used as a useful tool for the genetic improvement of these reproductive traits. Reurink *et al.* (1990) stated that insemination with semen from sub-fertile bulls is one of the factors related with impaired fertility in dairy cows.

2.2.3 **Body Condition Score**

The body condition score system is a subjective method to assess the body fat reserves of farm animals, particularly over the bony prominences like back and pelvic region (Alapati *et al*., 2010). It provides an easy and reliable method to estimate the nutritional

status and the efficiency of a feeding system for dairy cows (Chamberlain and Wilkinson, 1996). Variations in body condition scores in pregnant and lactating dairy cows are mirrored by changes of subcutaneous fat thickness, diameter of *longissimus dorsi* muscle as well as metabolic and endocrine status (Aeberhard *et al.,* 2001). In addition to the blood metabolites and haematological profiles that indicate the metabolic status of dairy cows, the body reserves of cows, expressed as body condition scores is also used to monitor the amount and mobilisation of body adipose tissue (Reist *et al.,* 2001). It has been reported that health and fertility parameters are related to the body condition score at pregnancy, calving and during early lactation (Broster and Broster, 1998). Body condition affects both cow and calf performance. Poor body condition is associated with reduced income per cow, increased postpartum interval, weak calves at birth, low quality and quantity of colostrum, reduced milk production, increased dystocia, and lower weaning weights (Royal *et al.*, 2000; Buckley *et al*., 2003).

Body condition scores have been related to milk yield and reproductive performance in cows (Ducker and Morant, 1984; Ducker *et al.,* 1985; Richards *et al.,* 1986). Conception rates declines with excessive losses of body condition score postpartum both in pasture based system and more intensive management system (Morton, 2003). Santos *et al*. (2009) reported that severe body weight and body condition score losses due to inadequate nutrition are associated with anoestrus in dairy cows. Despite the effect of energy balance on health and reproduction, until recently, most dairy cattle breeding programmes selected aggressively for increased milk production (Miglior *et al*., 2005), without much consideration for traits other than production. This has resulted in a cow that readily mobilises condition to support lactation (Roche *et al*., 2006), only regaining lost condition when energy surplus to milk production, maintenance, and

pregnancy is consumed. This relationship between body condition scores and milk production is consistent with fitted functions presented by Roche *et al*. (2006), which depicted body weight and body condition score profiles as mirror images of the lactation profile.

Many studies have identified a positive effect of either calving body condition score (Grainger and McGowan, 1982; Waltner *et al*., 1993) or amount of body condition score lost postpartum (Waltner *et al*., 1993; Ruegg and Milton, 1995) on milk production. Grainger and McGowan (1982) reported an increase in milk fat production for each body condition score unit increase between 2.0 to 3.5. This response is consistent with the results of Waltner *et al*. (1993), who reported a 322 kg increase in milk yield by increasing calving body condition score from 2.0 to 3.0. Furthermore, the majority of researches undertaken in grazing systems have reported a positive association between calving body condition score and milk production (Stockdale, 2004; 2005; Roche *et al*., 2005).

Following parturition, dry matter intake needs to increase 4- to 6-fold in order to meet the high nutrient demands of milk production (Bertics *et al*., 1992). However, the high yielding cow post calving is not able to increase dry matter intake as fast as the increased nutrient demands required for lactation; the cow copes with this nutrient shortage by mobilising body reserves of fat and protein (Keady *et al*., 2001). Energy partitioning in early lactation in one study resulted in mobilisation of 42 kg body weight, 31 kg fat and 5 kg protein (Tamminga *et al*., 1997). Cows mobilise an average of 0.7 kg body weight, 0.56 kg fat and 0.04 kg protein per day; however, the largest part of this mobilisation takes place in the first week of parturition with 37 % body

weight, 12 % total fat and 58 % total protein being mobilised (Tamminga *et al*., 1997). During this period of declining negative energy balance, luteinizing hormone pulses are suppressed and dominant follicles that develop have a decreased chance of producing sufficient oestradiol to induce a pre-ovulatory gonadotrophin surge. Delayed resumption of ovulation was related to the degree of negative energy balance (Kruip *et al*., 1998). Thus, the nutritional management of the dairy cow in the transition period; 3 weeks before calving to 3 weeks after calving has significant carry-over effects on future reproductive efficiency of high yielding dairy cows.

A number of scoring systems have been advocated (Lowman *et al*., 1976; Pullan, 1978; Wellington, 1981; van Niekerk and Louw, 1982; Buxton, 1982; Nicholson and Butterworth, 1985; Alapati *et al*., 2010). In general, evaluation of body condition score involves palpating the pelvis and lumbar processes, feeling for the sharpness and covering of the bones. In Australia, an 8-grade system for scoring dairy cows was developed by Earle (1976) and a similar 10-point system developed in New Zealand (Grainger and McGowan, 1982). Both the New Zealand and Australian scoring systems used photographs of individual cattle to define condition scores and have accompanied the photographs with a limited text description of the areas to be scored. Body condition scoring of dairy cows in the United States of America is generally performed according to a 1 to 5 scale (Wildman *et al.,* 1982). This method, like those used in the United Kingdom, involves palpating cows to assess the amount of tissue under the skin. These systems therefore require animals to be under restraint while scoring is performed. In many production systems, especially those with large herd sizes, the opportunity for this type of evaluation is limited. In Nigeria, body scoring techniques as described by Pullan (1978) basically use visual inspection, a preferred method when large numbers of freely

moving cattle are involved. The animals are scored on a scale from 0 to 5, summarized as follows:

1. Score 0 indicating emaciated animals with no apparent subcutaneous fats and a sharp spinous process of the lumber region;
2. Score 1 indicating animals whose spinous processes are sharp, but less than that of those in score 0; with some subcutaneous fats on these processes and on the points of the hips;
3. Score 2 representing animals whose individual spinous process are still fairly sharp to touch and the ribs can be seen individually;
4. Score 3 indicating animals whose spinous process can be felt but have a rounded feel and the ribs cannot be seen individually;
5. Score 4 indicating animals whose individual spinous processes can only be felt with firm pressure and point of the hips are covered with fat and are rounded.
6. Score 5 indicating animals whose spinous processes cannot be felt even with firm pressure and the animals have a blocky appearance (Pullan, 1978).

Body condition scoring performed in this way is a rapid and easy method of assessing the condition of cattle and is relatively unaffected by body size (Grainger and McGowan, 1982).

2.2.4 **Nutrition**

Dietary regimes should support milk production at an economic level whilst maintaining animal health and reproductive performance (Peters, 1996). The importance of nutrition as a factor limiting reproductive performance in ruminant livestock is well recognised (Robinson, 1990). Supplementary feeding is highly required for optimum milk production (Preston, 1989). Hamudikuwanda (1999) supplemented indigenous

cows with poultry litter, which is relatively cheap and readily available and reported daily milk offtakes of 1.5 kg and 1.8 kg for the non-supplemented and poultry litter groups, respectively. In another study, cows that received concentrate supplement had their first follicles 2 days before and ovulated 18 days earlier than cows that only grazed (Eduvie, 1985). Pregnancy rates of cows supplemented with concentrates (59.3 %) was significantly higher than cows not supplemented (32.8 %) (Voh *et al*., 1984).

2.2.4.1 Energy

The first and most important factor that affects energy status in dairy cows is feed availability (Grant and Albright, 1995). Vasconcelos *et al*. (2003) reported that energy balance and dry matter intake affect plasma concentration of progesterone which may interfere with follicle development and maintenance of pregnancy. Villa-Godoy *et al*. (1988) reported that variation in energy balance in postpartum dairy cows was influenced most strongly by dry matter intake and less by milk yield. When dairy cows experience a period of negative energy balance, blood level of non-esterified fatty acids increase as a consequence of body energy reserve mobilisation (adipose tissue), at the same time, glucose and insulin levels become low (Bruno, 2010). These metabolic changes may compromise animal health, ovarian function and fertility (Bruno, 2010). Kendrick *et al*. (1999) reported that changes in metabolism during the period of negetive energy balance could adversely affect prenatal follicles. Fertility of dairy cattle is therefore affected by negetive energy balance through inhibition of ovulation in early lactation, reduction of oestrus expression and impairment of conception and embryo survival during the breeding period (Santo *et al*., 2004).

2.2.4.2 Protein

Lactating dairy cattle require large quantities of metabolizable amino acids for synthesis of milk protein (Chakoma, 1995).). It is typical for lactating rations to contain crude protein between 16 and 18 % of its total dry matter (Manspeaker *et al*., 1988). Diets with limited crude protein can compromise microbial growth and rumen fermentation, which often reflects in declines in feed intake and milk production (Chakoma, 1995). On the other hand, feeding protein in excess to what is needed by the cows has been implicated in increased ammonia and urea concentrations in blood and milk, which have been used as markers for reduced fertility (Butler, 1998). The suggested decline in fertility of cattle fed excess of protein is caused by alterations in uterine physiology with a decline in uterine pH during the early luteal phase of the estrous cycle (Butler *et al.*, 2003). A more acidic uterine environment is less conducive with maintenance of pregnancy in cattle (Ocon and Hansen, 2003). This effect seems to be restricted to the early stages of embryo development (Rhoads *et al*., 2006).

High-producing lactating dairy cows are more efficient in utilizing protein sources when diets are moderate in crude protein and balanced for metabolizable protein and limiting amino acids (Noftsger and St. Pierre, 2003). It is not justified to feed diets with protein concentrations that will increase urea nitrogen and harm fertility (Noftsger and St. Pierre, 2003).

2.2.4.3 Vitamins and Minerals

Requirements of vitamins and minerals need to be met to ensure optimal reproductive performance (Samardzija *et al*., 2011). Deficiencies of calcium, selenium, copper, iodine, and vitamins A and E can result in an increased incidence of retained placentas.

Vitamin A deficient animals are more susceptible to bacterial, viral, and parasitic infections than animals with sufficient vitamin A (Chew, 1987). A deficiency of vitamin A can cause abortions and increased incidence of retained placenta in cows, and increased morbidity and mortality in calves. The National Research Council (2001) recommends 75,000 IU of vitamin A/day for lactating cows and 80,000 IU/day for dry cows. Chew and Johnson (1985) reported that increasing the intake of vitamin A from 53,000 (66 % of current recommendation) to 173,000 IU/day in dairy cows starting at 30 days prepartum, decreased somatic cell counts in milk during early lactation. In contrast, Oldham *et al*. (1991) found that increasing vitamin A from 50,000 to 170,000 IU/day did not affect somatic cell count or mammary gland health in cows. Compared to cows receiving no supplemental vitamin A, cows supplemented with 120,000 IU of vitamin A/day for 4 weeks prepartum had reduced incidence of retained placenta (Eicher *et al*., 1994).

Beta-carotene is the major precursor of vitamin A that occurs naturally in feedstuffs. Fresh green forage is an excellent source of β-carotene, while cereal grains and grain by-products are extremely low in β-carotene (Michal *et al*., 1994). In addition to providing vitamin A, β-carotene can also serve as an antioxidant while vitamin A is not an important antioxidant (Michal *et al*., 1994). Chew (1993) reported that β-carotene supplementation at 300 mg/day to dry cows improved mammary gland health and reduces the incidence of retained placenta and metritis.

Low vitamin E status in cows reduces the immune response, leading to increase in the incidence of reproductive diseases, especially mastitis (Spears, 2011). Weiss *et al*. (1997) indicated that a plasma alpha tocopherol (Vitamin E) concentration of

approximately 3 μg/ml is needed to maximize health and immune response in dairy cows. The functions of vitamin E and selenium are related and responses in dairy cow's health to supplementation of either nutrient can depend on the cow's status of the other nutrient (Weiss *et al*., 1997). Supplementing vitamin E during the dry period has reduced the incidence of mastitis in cows fed diets low or marginal in selenium (Weiss *et al*., 1997). Vitamin E supplementation at high level lowered the risk of stillborn calves or calf deaths within 24 hours of birth (Bouwstra *et al*., 2010). Fresh green forages are good sources of vitamin E while concentrates and stored forages (hays, haylages, and silages) are low in vitamin E (National Research Council, 2001).

The selenium requirement of dairy cattle is approximately 0.3 mg/kg dry matter (National Research Council, 2001). Selenium deficiency in dairy cows reduces the ability of blood (Hogan *et al*., 1990) and milk (Grasso *et al*., 1990) neutrophils to destroy bacteria. Blood concentrations of selenium in dairy herds have been related to mammary gland health (Weiss *et al*., 1990). Low blood selenium concentrations were related to greater prevalence of intramammary infection (Erskine *et al*., 1987). In the study of Weiss *et al.* (1990), herds with high serum selenium concentrations had reduced rates of mastitis and lower bulk tank somatic cell counts.

Calcium deficiency in young calves prevents normal bone growth and retards their general growth and development (Taylor, 2007). Liesegang *et al.* (2007) reported the metabolic effects of dietary calcium concentration in the prepartum period in lactating cows. Cases of slow uterine involution, increased incidence of dystocia, retained placenta, and prolapsed uterus have been reported in cows with milk fever (Goff, 2008). Phosphorus absorption and metabolism are influenced by many variables including

dietary concentrations of calcium and magnesium (Taylor, 2007). Phosphorus results in a lower conception rate, a decrease in ovarian activity, irregular estrus cycles, anestrus and an increased incidence of cystic ovaries, reduced fertility, feed intake and milk production (Wu *et al*., 2000; Wu and Satter, 2000; Knowlton and Herbein, 2002). Vitamin D is necessary for normal calcium and phosphorus metabolism and, thus, affects reproduction indirectly (Taylor, 2007).Various forms of vitamin D and their derivatives have been examined in relation to calcium mobilisation and/or prevention of hypocalcemia (Okura *et al*., 2004).

Deficiency of copper is associated with reduced immunity, early embryonic death, reduced ovarian activity, delayed or reduced estrus activity, decreased conception rate, increased incidence of retained placenta, and increased difficulty in calving (Spears and Weiss, 2008). Scaletti *et al*. (2003) evaluated the effect of dietary copper on responses of heifers to an intramammary *Escherichia coli* challenge at 34 days of lactation and realised that heifers supplemented with copper had lower *Escherichia coli* numbers and somatic cell counts in milk, lower clinical scores, and lower peak rectal temperatures than control heifers. The availability of copper is reduced by excesses of calcium, sulfur, iron, zinc, and molybdenum in the diet or water (Spears, 2011).

Lactating dairy cows require 45 to 65 mg zinc/kg of dry matter (National Research Council, 2001). Increasing dietary zinc from 40 to 65 mg/kg dry matter reduced somatic cell counts in dairy cows (Cope *et al*., 2009). Severe zinc deficiency impairs immune responses, reduces disease resistance and delays sexual maturity (Spears and Weiss, 2008).

Manganese deficiency is rare in ruminants. Cows deficient in manganese are likely to have poor development of the follicles, delayed ovulation, abortion, more silent heats, and lower conception rates (Spears, 2011). Cobalt deficiency is associated with an increased incidence of silent heats, a delayed onset of puberty, nonfunctional ovaries, and abortion (Spears, 2011).

2.2.4.4 Fats

Feeding fat to dairy cattle increases the risks associated with pregnancy, although responses have not been consistent (Santos *et al*., 2009). When fat feeding improved production and increased body weight, primiparous cows experienced reduced pregnancy risks at first artificial insemination (Sklan *et al*., 1994). In grazing cows, supplementation with 0.35 kg of fatty acids increased the risk of pregnancy after the first postpartum artificial insemination (McNamara *et al*., 2003). Feeding calcium salts of long chain fatty acids of palm oil did not improve fertility of dairy cows (Scott *et al*., 1995).

The benefits of feeding fat may originate from specific fatty acid (Staples and Thatcher, 2005). Thus, it has been evaluated whether feeding fatty acids differing in the degree of saturation might influence fertility of cows (Juchem, 2007). The essential fatty acids of the n-6 and n-3 families are available in much smaller supply due to microbial biohydrogenation of fatty acids in the rumen, suggesting that their supplementation may benefit reproduction (Santos *et al*., 2009). When cows were fed 0.75 kg of fat from flaxseed, a source rich in n-3, or sunflower seed, a source rich in n-6, pregnancy tended to be greater for cows fed n-3 (Santos *et al*., 2009). According to Mattos *et al*. (2004), fatty acids with n-3 suppresses uterine secretion of PGF2α and thus has the potential to

improve embryonic survival in cattle. Collectively, these data suggest that feeding fat to dairy cows generally improves fertility and responses are observed when the energy density of the ration increased with fat feeding. Also, these data suggest that fertility responses to fat feeding is altered according to the type of fatty acids supplemented in the diet.

2.2.5 **Season of the Year**

The incidence of oestrus and success rate of artificial insemination was high in cold dry seasons in Friesian cows (Pongpiachan *et al*., 2003). This was because reproduction in the purebred Friesian cows was affected by high temperature. Zebu and crossbred cows tended to be in peak reproductive activity during the rainy season when grazing conditions and nutrient availability were optimal (Mukasa-Mugerwa, 1989). Colburn *et al.* (1997) reported high incidence of calving difficulty in winter and attributed it to severe spring temperatures.

In Nigeria, Lamorde and Franti (1973) noted that most calvings occurred during the wet season. Osori (1976) reported that there are seasonal variations in the number and size of graffian follicles, ovarian size and weight, with ovarian activity peaking between July and August. Based on the effect of season on reproductive parameters, Voh *et al*. (1987) proposed a breeding plan for cattle in Northern Nigeria: start breeding in mid August to end of September (45 days), with calving from mid May to end of June (45 days), and this can result in a 365 days calving programme.

2.2.6 **Reproductive Diseases**

Epidemiological studies have clearly demonstrated strong relationships between postparturient diseases and subsequent reproductive performance in dairy cattle (Haile *et al*., 2010). The postpartum period coincides with the peak of milk production, uterine involution, resumption of ovarian activity, conception and greater risk of infections (Farin and Slenning, 2001). Bovine brucellosis is the most notable cause of infertility in female cattle, which impacts the greatest economic damages (Hafu and Gashaw, 2009). Cattle brucellosis causes high rate of abortion, calf mortality, reduced milk yield and uterine infections (Radostitis *et al*., 2000; Hafu and Gashaw, 2009). A survey of bovine brucellosis and campylobacteriosis in Kaduna State showed an infection rate of 6.6 % and 7 %, respectively (Bawa *et al*., 1986). A case of severe vulver dermatophilosis in a Bunaji cow in a settled herd, leading to infertility, was reported in Northern Nigeria by Ate *et al*. (2007). Veneral campylobacteriosis in Nigeria was first reported in a herd of South Devon cattle imported into Kano state (Nuru, 1974). An incidence of 2.1 % of genital tract malformations of reproductive organs of Nigerian indigenous cows has been reported (Abdullahi and Chaudhri, 1996).

Cows diagnosed with clinical hypocalcemia were 3.2 times more likely to experience retained placenta than cows that did not have clinical hypocalcemia (Curtis *et al*., 1983). Whiteford and Sheldon (2005) found that hypocalcemia was associated with occurrence of uterine disease in lactating dairy cows. Markusfeld (1985) reported that 80 % of cows with ketonuria developed metritis.

Cows with retained placenta have increased risk of developing metritis compared with cows not experiencing retained placenta (Santos *et al*., 2009). Both metritis and retained

placenta double the risk of cows remaining with uterine inflammation at the time of first postpartum insemination (Rutigliano *et al*., 2008). A study on 5 dairy farms observed that retained placenta was diagnosed in 13.1 % and 9.2 % of multiparous and primiparous cows, respectively (Goshen and Shpigel, 2006). In the same study, metritis affected 18.6 % and 30 % of the multiparous and primiparous cows, respectively. Both retained placenta and metritis can have devastating effects on reproductive efficiency in lactating dairy cows, with reduced conception rates and extended intervals to pregnancy (Goshen and Shpigel, 2006). Subclinical endometritis, a disease characterized by increased proportion of neutrophils in uterine cytology without the presence of clinical signs of inflammation of the uterus, has major deleterious effects on conception rates of lactating dairy cows at first postpartum insemination (Urton *et al*., 2005).

2.2.7 **Lactation**

Breeding for increased milk production in dairy cattle has negative side effects on fertility traits (Roxstrom *et al*., 2001). The negative energy balance recorded during early lactation have negative effects on the health and fertility of dairy cows. The study of Nebel and McGilliard (1993) have reported that reproductive performance is compromised, primarily through delayed ovarian activity and reduced conception rates, by the demands of high milk yield. Muller *et al*. (2000) reported that days to first heat, days to conception and number of inseminations per conception increased with higher milk yields in primiparous Holstein cows.

Selection for milk yield has increased blood concentrations of somatotropin and prolactin, stimulators of lactation, and decreased insulin, a hormone that is antagonistic to lactation and may be important for normal follicular development (Nebel and

McGilliard, 1993). These changes in hormone concentrations promote higher milk yield but may be potentially detrimental to other physiological functions, such as reproduction, if the management is not adequate to meet the metabolic demands of lactation (Nebel and McGilliard, 1993).

Daily managerial decisions to obtain efficient reproductive performance have considerable impact on the effect of milk yield on reproductive performance in dairy cows. A study by Farin *et al*. (1994) using 1.2 million Holstein and 50, 000 Jersey cows indicated that as the milk yield in dairy herds increased with increasing herd size, days to first heat and days to conception (days open) decreased in both Holstein and Jersey herds. Number of services per conception increased only in Holstein herds. This information illustrates the effects that managers, inseminators and other factors have on reproductive performance as well as milk yield.

2.3 METABOLIC PROFILE

It is known that in high-yielding dairy ruminants, as well as in other mammals, major changes occur during pregnancy and lactation in the metabolism of several tissues and organs, such as placenta, udder, liver, adipose tissue, muscle tissue and gastrointestinal tract (Ingvartsen and Andersen, 2000). These changes are controlled and coordinated by the body system in order to distribute the nutrients throughout the maternal body and finally to supply glucose and amino acids to the growing foetus, or acetate, propionate and butyrate to the mammary gland, during its growth and function (Ingvartsen and Andersen, 2000). The regulatory mechanism of homeorhesis is essential for the successful completion of pregnancy and lactation (Bauman and Currie, 1980). Alterations in homeorhesis can result in decreased reproductive and productive

efficiency, as well as detrimental effects on the animal's health such as fatty liver, pregnancy toxaemia in sheep, and ketosis during lactation in cows (Ingvartsen and Boisclair, 2001). Metabolic changes during pregnancy and lactation have been extensively studied in high yielding cows over the last few decades (Bauman and Currie, 1980; McNamara, 1995; Vernon and Pond, 1997; Chilliard *et al*. 1998; Ingvartsen and Andersen, 2000; Ingvartsen and Boisclair, 2001).

Profiles of blood metabolites have been used widely to identify problem herds and to indicate dietary causes of disease or low production (Lee *et al*., 1978). Metabolic profiles are useful when combined with haematologic values, diet evaluation, health tests and careful management monitoring of a herd (Khan *et al*., 1990).

The majority of dairy cows enter a period of negative tissue energy balance in the early postpartum period which may last for up to 15 weeks (Pushpakumaraa *et al.,* 2003). Tissue energy balance is a function of energy intake, body maintenance and milk energy yield. This negative energy balance has been attributed to the increased mobilisation of tissue energy reserves for milk synthesis. For example, Jelinek *et al.* (1996) pointed out that during lactation, the mammary gland secretory cells utilize 80 % of the circulating metabolites in the blood for milk synthesis. Also, during lactation and advanced pregnancy in ruminants, the insulin concentration in the blood and responsiveness of glucose reserves to insulin are decreased (Regnault *et al.,* 2004). In this case, fat mobilisation is enhanced to obtain free fatty acids from adipose tissue as an alternative energy resource during tissue negative energy balance (Regnault *et al.,* 2004).

Although selection for yield has involved an increase in appetite, high producing cows in early lactation are still unable to consume sufficient feed to fulfill their needs (Kellems, 2012). This shortfall in energy is met by mobilisation of lipids from adipose tissue and from skeletal muscle protein which is achieved by both endocrine and metabolic adaptations (Bauman and Currie, 1980). Growth hormone secretion increases and insulin secretion decreases after calving, thus increasing lipolysis and resulting in raised levels of non-esterified fatty acids. Liver metabolism is altered and in severe cases, hepatic lipidosis occurs, accompanied by the formation of ketone bodies (Kruip *et al.,* 1998). The body reserves available for mobilisation during early lactation depend on the nutritional management during the transition period. However, cows that had very high body condition score during transition subsequently had a lower feed intake and a higher incidence of metabolic diseases (Huyler *et al.,* 1999).

The energy requirements for maintenance and pregnancy of dairy cattle increase during the last month prepartum but during this time feed intake may be reduced (Ingvartsen *et al.,* 1999). Therefore, the energy density of the diet should be increased prior to calving (Grummer, 1999). The increase in energy density may also stimulate papilla growth and increase the absorption of volatile fatty acids from the rumen, adapt the microbial population to higher energy dense diets, increase blood insulin and decrease fatty acid mobilisation from adipose tissue (Minor *et al.,* 1996). However, high energy density diets have in some instances led to a significant reduction in total energy intake (National Research Council, 2001) due to increased feed inappetance.

The protein requirement for growth of the conceptus also increases as pregnancy advances (Bell *et al*., 1995). Based on the estimates of dry matter intake, 12 % crude

protein should be sufficient for maintenance and gestation in transition cows but feeding protein above the National Research Council (National Research Council, 2001) recommendation during the final 3-6 weeks prepartum decreased the risk of retained placenta and uncomplicated ketosis (Curtis *et al.,* 1985), increased milk yield (Park *et al.,* 2002) and improved fertility (Van Saun *et al.,* 1993). However, most studies have shown that milk yield is not influenced by prepartum protein status (Doepel *et al.,* 2002).

There is increasing evidence that the changes in blood metabolites and metabolic hormones which accompany the post-partum period of negative energy balance can act as signals to the reproductive system, to reduce fertility (Wathes *et al.,* 2001). This may be an adaptive mechanism to protect the cows from the additional metabolic load of pregnancy. The extent and duration of the negative energy balance at this time is an important determinant of when the cow will return to normal ovarian cyclicity after calving (Beam and Butler, 1999).

2.3.1 **Glucose**

2.3.1.1 *Glucose Metabolism in the Rumen*

The carbohydrate components in ruminant diets are mainly hexose polymers such as cellulose, starch, fructans, and pentose polymers such as xylan (Martin, 1994). However, plasma glucose in dairy cows is derived almost exclusively by gluconeogenesis (Busato *et al*., 2002). The most important pathway of ruminal glucose catabolism is by glycolysis resulting in the degradation of glucose to pyruvate which generates adenosinetriphosphate, reduced equivalents, and precursor metabolites for a multitude of essential cellular processes (Baldwin, 1965). The pyruvate may be utilized

by at least, two alternative pathways. One involves the microbial release of lactate into the medium followed by the conversion of the free lactate to the volatile fatty acids (Baldwin, 1965). The other process simply involves a direct microbial conversion of pyruvate to acetate, carbondioxide, and a reduced product such as hydrogen gas, succinate, propionate, or butyrate (Baldwin *et al*., 1963). Ahmad *et al*. (2004) reported a mean serum glucose value of 50.72 ± 1.12 g/dL (equivalent to 2.82 mMol/L) in normal cyclic cows.

2.3.1.2 *Significance of Serum Glucose in Pregnancy and Lactation*

Dairy cows experience many metabolic and hormonal challenges in the periparturient period, including rapid growth of the foetus, calving and initiation of lactation (Stevenson, 2001). This is further confounded by the genetic drive to produce vast quantities of milk. During the catabolic phase of early lactation, a dairy cow's energy requirements can increase by up to 300 % (O'Boyle, 2008). The principal demand is for glucose and, at the onset of lactation, virtually all the glucose is directed towards the mammary gland for milk synthesis (Drackley, 2005). Glucose is the precursor of lactose, which dictates milk volume via osmotic regulation (O'Boyle, 2008). Serum concentration of glucose is considered to be an indicator of the energy balance of a cow (Oikonomou *et al*., 2008). Low serum glucose levels in dairy cows can be the consequence of nutritional restrictions, impaired liver function or high glucose needs (Busato *et al.,* 2002).

Dairy cows at the onset of lactation need more energy due to milk synthesis than those during pregnancy and the dry period (Block *et al*., 2001). Glucose requirements for lactose production in dairy cows are highest in the early postpartum period (Busato *et*

al., 2002). During this period, secretory cells of the mammary gland utilize 80 % of the circulating metabolites including glucose for milk synthesis, so that the synthesis and composition of the milk depends on the speedy infiltration of glucose into the mammary gland cells (Karapehlivan *et al.*, 2007).

Glucose is the major precursor for lactose synthesis (Komatsu *et al.,* 2005). Therefore, the supply of glucose to the mammary gland is an important factor in the control of milk yield (Komatsu *et al.,* 2005). To supply glucose to the mammary gland, insulin-independent glucose uptake decreases in tissues, except for the mammary gland (McDowell *et al*., 1987). This increased glucose flow to the mammary gland is the cause of a fall in serum glucose at the onset of lactation, causing a negative energy balance in the lactating dam (McDowell *et al*., 1987). This can be prevented by stepping up the dry matter intake or concentrate feed during pregnancy and lactation in dairy cows (McDowell *et al*., 1987). Antunovic *et al*. (2002) and Karapehlivan *et al*. (2007) reported increased serum glucose concentration at onset of lactation in dairy ruminants, attributable to good concentrate feeding, leading to increase in milk production during lactation (El-Sherif and Assad, 2001; Regnault *et al*., 2004).

2.3.2 **Triglycerides**

2.3.2.1 *Synthesis and Metabolism of Triglycerides*

Triglycerides are used in animals to either generate energy or to be deposited as adipose tissue (Silva and Dangolla, 2002). Many cell types and organs have the ability to synthesise triglycerides, but in animals the liver and intestines are most active, although most of the body stores of this lipid are in adipose tissue (Williams and Stanko, 1999). Ruminal microflora hydrolyze triglycerides to unsaturated fatty acids and glycerol

(Williams and Stanko, 1999). Fats of plant or animal origin contain the unsaturated fatty acids such as palmitoleic, oleic, linoleic, and α-linolenic acids, all of which are metabolized in the rumen (Williams and Stanko, 1999). A high proportion of the fatty acids are then partially or completely hydrogenated and much of the glycerol is fermented to propionic acid (Noble, 1978). Of the three major volatile fatty acids derived from ruminal fermentation, propionic acid, in the form of propionate, is the only volatile fatty acid that contributes directly to glucose synthesis during gluconeogenesis in the Krebs cycle (O'Boyle, 2008). Detailed reviews regarding lipid metabolism (Jenkins, 1993), absorption and transport (Bauchart, 1993), and metabolism (Grummer, 1993) are available. The normal concentrations vary tremendously among animals (Williams and Stanko, 1999).

2.3.2.2 *Significance of Serum Triglyceride in Pregnancy and Lactation*

Lactose and glycerol play a relatively minor role in the dairy cow experiencing a state of positive energy balance (O'Boyle, 2008). However, the extraordinary demand for milk production renders the early lactating dairy cow in a state of early negative balance, as dietary intake is inadequate to meet energy demands (O'Boyle, 2008). Consequently, the role of other gluconeogenic substrates increases and body reserves are then utilized (O'Boyle, 2008). Adipose tissue is the main energy reserve utilized during negative energy balance. Adipose tissue in the cow is oriented towards mobilisation of non-esterified fatty acids in early lactation rather than lipid deposition (McNamara, 1995), and when the non-esterified fatty acids mobilised from the adipose tissues are present in large quantity, they are converted into triglycerides (Bertics *et al.,* 1992). These circulating blood triglycerides contribute significantly to milk fat synthesis (Nazifi *et al*., 2002).

Non-esterified fatty acids, which are converted to triglycerides, are also mobilised from adipose tissue to provide a source of energy for the dam after parturition (Pullen *et al*., 1989). During late pregnancy in ruminants, blood serum lipid profile is characterized by increased concentration of triglycerides (Schlumbohm *et al*. 1997). Similarly, Balıkcı *et al.* (2007) recorded a gradual increase in serum triglyceride levels, with advancement of pregnancy in ewes. This increase during late pregnancy is hormone dependent; it is due to insulin, which plays a direct role in adipose tissue metabolism during pregnancy and its responsiveness is significantly reduced in ruminants during late pregnancy (Schlumbohm *et al.*, 1997). The diminished responsiveness of target tissues towards insulin, together with an increased mobilisation of fatty acids from adipose tissue, result in increased triglyceride level, thus creating alternative source of energy for the dam and for foetal growth (Schlumbohm *et al*., 1997).

2.3.3 **Cholesterol**

2.3.3.1 *Synthesis and Metabolism of Cholesterol*

Cholesterol is a lipid obtained from dietary sources and also synthesized in the liver (Jackson *et al*., 2010). Hepatic cholesterol synthesis is high when animals are fed a cholesterol-free diet or when there is an increased demand for cholesterol, and it declines dramatically when cholesterol is fed (Horton *et al*., 1998). Hepatic synthesis of cholesterol utilizes acetyl CoA as a precursor which undergoes transformation under the influence of the enzyme hydroxyl methylglutaryl-CoA reductase, to cholesterol (Jackson *et al*., 2010). Because significant elevation in cholesterol levels is a risk factor for coronary heart disease, clinicians aim to reduce cholesterol levels by prescribing hydroxyl methylglutaryl-CoA reductase inhibitors, also referred to as statins, to inhibit

the action of the enzyme (Jackson *et al.*, 2010). Cholesterol is metabolized in the gluconeogenic pathway for alternative energy source (Schlumbohm *et al.*, 1997).

Cholesterol is important in the synthesis and maintenance of cell membranes and synthesis of steroidal hormones (Kaneko *et al.*, 1997). Variation in blood cholesterol content has been observed during oestrus and pregnancy, as precursor of the steroid hormones (Iriadam, 2007). Ahmad *et al.* (2004) reported that serum cholesterol concentration in cyclic cows is 199.12 ± 9.38 mg/dL (equivalent to 5.15 mMol/L).

2.3.3.2 *Significance of Cholesterol in Pregnancy and Lactation*

Because the majority of serum cholesterol is derived from hepatic biosynthesis, blood cholesterol level has been used as an indirect indicator of liver function in pregnant and lactating cows (Amanlou *et al.*, 2008). Fat accumulation in the hepatocytes at parturition can impair normal liver function in cows that were overfed during the dry period (Drackley *et al.*, 2005; Dann *et al.*, 2006). The impaired liver function would be expected to alter the patterns in hepatic protein synthesis and lipoprotein export, and specifically, reduce blood cholesterol concentrations. Cholesterol is indeed a key component of the circulating lipoproteins (Mayes, 2000).

In dairy cows with negative energy balance during the transition period, cholesterol and triglycerides are utilized in the gluconeogenic pathway to generate energy for the dam (Schlumbohm *et al.*, 1997). Thus, cholesterol is also important as an alternative source of fuel when glucose is depleted at late pregnancy and early lactation (Schlumbohm *et al.*, 1997).

2.3.4 **Urea**

2.3.4.1 *Synthesis and Metabolism of Urea*

Urea is the most common nitrogen-containing end product of protein catabolism (Wright, 1995), mainly resulting from the detoxification of the ammonia derived from amino acids deamination (Meijer *et al.,* 1990). Ruminants recycle substantial amount of nitrogen as urea by transfer from blood to the lumen of the gut rather than excretion in urine (Huntington, 1989). Bacteria in the lumen of the gut in turn use urea nitrogen for their metabolic needs; ruminants then reabsorb portions of the nitrogen in the form of amino acids, nucleic acids or ammonia (Huntington, 1989).

Although blood urea concentrations are positively related to crude protein intake when diets containing sufficient energy are fed (Rowlands, 1980), report has shown that high urea concentrations can also be induced by low energy diets (Diab and Hillers, 1996). An elevated blood urea nitrogen level may also be caused by excessive protein intake or protein catabolism (Bruce and Nugent, 2005). A decreased level may be seen in malnutrition or impaired nutrient absorption (Hanuš *et al*., 2008).

2.3.4.2 *Significance of Urea in Pregnancy and Lactation*

Reports have ascribed increase in plasma urea with advancement of pregnancy in dairy animals to the cortisol-stimulated catabolism of proteins in the body (Silanikove, 2000). However, when much of the dietary protein is taken up for synthesis of milk proteins resulting in minimal catabolism, urea production in the body will drop and plasma urea concentration will decrease (El-Sherif and Assad, 2001).

Since urea is a product of dietary protein catabolism, plasma urea concentration is a reflection of protein intake (King *et al*., 1993). As dietary concentration of the protein increases, muscle tissue loss decreases and, blood urea concentration is increased (King *et al*., 1993). El-Sherif and Assad (2001) reported plasma urea to start increasing in pregnant ewes from 10^{th} week of pregnancy, reaching a maximum level at parturition. Rodriguez *et al*. (1996) stated the glomerular filtration and urea clearance in ewes to be reduced during late pregnancy and lactation. Shetaewi and Daghash (1994) found the level of serum urea during pregnancy to slightly exceed that of early lactation but it was highest at 55 days of lactation.

2.3.5 **Albumin**

2.3.5.1 *Synthesis and Metabolism of Albumin*

Albumin is a protein synthesised by the liver and found in abundance in the bloodstream. Its level can be abnormal in a variety of diseases and malnutrition conditions. Albumin is the most abundant protein in plasma and transports many small molecules, including bilirubin, calcium, progesterone, and certain drugs (Eichner and Sommermeyer, 2002). It is also of prime importance in maintaining the oncotic pressure of the blood, since its concentration in blood is much greater than it is in extracellular fluid (Golab *et al*., 2011). Because albumin is synthesized by the liver, decreased serum albumin may result from liver disease. It can also result from kidney disease, which allows albumin to escape into the urine, or be caused by malnutrition or a low-protein diet (Golab *et al*., 2011).

The ratio of albumin to globulin in the blood may be abnormal in certain blood and immune system conditions as well as some liver and kidney diseases. A low

albumin/globulin ratio may be due to overproduction of gamma globulin, as seen in monoclonal/polyclonal gammopathy, multiple myeloma or autoimmune diseases (Jacobson *et al*., 2003) or low levels of albumin due to low production, as in cirrhosis, or excessive loss, as in nephrotic syndrome or protein-losing enteropathy (Crowley and Coltman, 1990). A high ratio suggests disorders involving low gamma-globulin production, such as agammaglobulinemia (Geha *et al*., 1973).

2.3.5.2 *Significance of Albumin in Pregnancy and Lactation*

It has been established that protein requirement increases during late pregnancy and lactation as maternal serum protein concentrations decrease with advancement in pregnancy, due to an increased foetal growth, especially the utilization of amino acids from the maternal circulation for protein synthesis in the foetal muscles (Jainudeen and Hafez, 1994) and for the development of maternal mammary glands (Everts, 1998). Reist *et al*. (2001) and Busato *et al*. (2002) recorded that plasma albumin concentrations increased significantly during the studied lactation period in dairy cows. They attributed this to be an expression of higher protein synthesis postpartum as reported by Bell (1995).

2.3.6 **Total Protein**

2.3.6.1 *Protein Metabolism*

The liver is a major site for the synthesis of blood proteins, and altered or impaired liver function may change protein levels in circulating blood (DeSancho and Pastores, 2012). Proteins provide the amino acids needed for maintenance of vital functions, reproduction, growth and lactation. Non-ruminant animals need pre-formed amino acids in their diets, but ruminants can utilize many other nitrogen sources due to ruminal

microbial activities resulting in the synthesis of amino acids and protein from non-protein nitrogen sources (Doepel *et al*., 2002).

2.3.6.2 *Significance of Total Protein in Pregnancy and Lactation*

Maternal serum protein is highly indicated as a source of amino acids for protein synthesis in the foetal muscles (Antunovic *et al*., 2002), and this may cause a decrease in blood protein concentration during the late stages of gestation in ruminants (Antunovic *et al*., 2002). The foetus synthesizes all its proteins from the amino acids derived from the dam, and growth of the foetus increases exponentially reaching a maximum level, especially in muscles, during late pregnancy (Jainudeen and Hafez, 1994).

During late pregnancy and early lactation in dairy cows, changes take place in the maternal muscular tissues where proteolysis is increased, and the mobilised amino acids contribute as substrates in the gluconeogenesis and milk protein synthesis (Ingvartsen, 2006). However, proteolysis is only substantially increased in the first 1-2 weeks postpartum (Plaizier *et al*., 2000; Doepel *et al*., 2002).

During the periparturient phase, a dramatic shift occurs from a non-lactating to a lactating state (Drackley, 1999). As a result, protein and energy demands rise markedly to sustain maternal immunity (Drackley *et al*., 2005). Decreased ability of the immune system to respond to infectious challenges is responsible for the high incidence of environmental mastitis around calving, as well as the high incidence of metritis (Kimura *et al*., 2002). Inadequate supply of metabolizable protein has been associated with impaired function of the immune system (Houdijk *et al*., 2001). Evidence linking

retained placenta to a malfunction of the immune system (Kimura *et al*., 2002) suggests that protein nutrition also might impact the incidence of retained placenta.

As parturition approaches, blood progesterone concentration decreases while oestrogen concentration remains high (Grummer, 1995). The high circulating oestrogen is an important factor that contributes to decreased dry matter intake at that period (Grummer, 1993). During the last 3 weeks of pregnancy, nutrient demands by the foetal calf and placenta are at their peak, yet dry matter intake may be decreased by 10 to 30 % compared with intake during the early dry period (Bell, 1995). Elevated nutrient requirements and inadequate dry matter intake associated with periparturient phase, causes massive body fat mobilization, thus, large fat accumulation occurs in the liver (Van Saun, 2012). Excess hepatic lipidosis may interfere with hepatic gluconeogenesis and ureagenesis (Rukkwamsuk *et al*., 1999). Meanwhile, the immune cells are greatly activated to accommodate the above changes in peripheral and mammary metabolism (Goff and Horst, 1997).

Amino acids, alongside glucose and lactate are the primary foetal metabolic fuels (Battaglia and Meschia, 1978). Complete oxidation of glucose and lactose can only account for 60-70 % of the total foetal caloric requirement (Reynolds *et al*., 1986; Bell *et al*., 1987). This suggests that amino acids account for 32-40 % of the total conceptus energy requirement in addition to providing the necessary substrate to support substantial protein synthesis activity (Faichney and White, 1987; Bell *et al*., 1989). In periods of maternal undernutrition, foetal glucose and acetate uptake concentrations and utilization decline, but foetal amino acid uptake is essentially unaffected by maternal nutrient status, suggesting a greater role for amino acids in foetal energy production,

especially during periods of maternal undernutrition, resulting in additional protein utilization burden on the dam (Van Saun and Sniffen, 1996).

2.3.7 **Creatinine**

2.3.7.1 *Synthesis and Metabolism of Creatinine*

Creatinine is a protein waste product generated by muscle metabolism and eliminated by the kidneys (Burton *et al*., 2003). Creatinine is a useful indicator of kidney function, and its levels are significantly elevated when kidney function is impaired (El-Sherif and Assad, 2001). Because creatinine is released at a constant rate (depending on muscle mass), its serum level is a good indicator of glomerular filtration rate and renal function (Swedko *et al*., 2003). Daily excretion of creatinine can be increased by 10 % to 30 % as a result of dietary intake (Chizzotti *et al*., 2008).

2.3.7.2 *Significance of Creatinine in Pregnancy and Lactation*

When lactating animals are fed dietary amino acid concentrations below the requirement, maternal tissue protein is catabolized in an attempt to supply the nutrients to maintain milk production leading to a rise in plasma creatinine concentration (Yang *et al*., 2009). A major source of mobilised amino acids is protein breakdown in skeletal muscles, although skin, uterine involution and myometral protein degradation may have some contribution (Bell and Ehrhardt, 2000).

2.3.8 **Calcium**

2.3.8.1 *Synthesis and Metabolism of Calcium*

Diets and bones are the primary sources of calcium in mammals and birds (Horst *et al*., 1994). The enhancement of intestinal calcium absorptive and bone calcium resorptive

processes are under the influence of the calcium-regulating hormones, which are parathyroid hormone, secreted by the parathyroid glands, and 1, 25-dihydroxyvitamin D, produced in the kidney (Horst *et al*., 1994). Calcium can be absorbed from the lumen of the intestine by passive diffusion between the intestinal epithelial cells (paracellular transport) and by active transport across the epithelial cells (Kovacs, 2012). Paracellular transport is strictly related to the concentration of calcium ions in the lumen of the gut (Kovacs, 2012).

Calcium is required for the normal functioning of a wide variety of tissues and physiologic processes. It is indicated in bone formation, muscle contraction, nerve transmission, blood clotting, and as a second messenger regulating the actions of many hormones (Horst *et al*., 2003). Hypercalcemia may be caused by hyperparathyroidism or excessive vitamin D intake, while hypocalcemia is essentially caused by metabolic alkalosis in the cow induced by high potassium diets. (Goff, 2008).

2.3.8.2 *Significance of Calcium Concentration in Pregnancy and Lactation*

The passage of calcium across the placenta is unidirectional; back transfer of this element is very limited (Piccione *et al*., 2012). The mobilisation of calcium from maternal bone and the increased absorption from the gastrointestinal tract are required to re-establish homeostasis (Liesegang, 2008). Blood calcium in the adult cow is maintained between 2.1 and 2.5 mmol/L (Goff, 2008). Maintaining blood calcium is a key management goal in any modern dairy farm. This is mainly because calcium plays crucial roles in smooth muscle contraction (Partiseti *et al*., 1994). Through muscular malfunction, the peripartal hypocalcaemia predisposes the cow to dystocia, retained placenta, metritis, displaced abomasum and milk fever (Moore *et al*., 2000). Subclinical

hypocalcemia is believed to be a contributing factor in displaced abomasum and ketosis by decreasing smooth muscle function, which is critical for normal function of the digestive tract (Goff and Horst, 1997). Hypocalcemia also leads to increased secretion of cortisol, which is believed to be a factor in increased incidence of retained placenta (Goff, 1999).

Calcium is also significant in pregnancy as it is necessary for normal immune responsiveness (Horst *et al*., 1997; Kimura *et al*., 2006). According to Kimura *et al*. (2006), decreased calcium stores may depress the cell calcium ion release in response to the immunity activating signals. Such a blunted response in calcium ion release to the immunity activation may lead to immunosuppression (Kimura *et al*., 2006). Thus, any feeding strategy that can improve calcium status of the periparturient cows will subsequently contribute to improving the immunity (Kimura *et al*., 2006).

Amanlou *et al*. (2008) demonstrated that prepartal dietary inclusion of wheat grain increased prepartum feed intake and elevated serum calcium concentration, thus, attenuated hypocalcaemia and increasing milk fat yield. Although the treatment did not affect milk protein, changes in body condition score, and serum cholesterol level, the authors concluded that prepartal dietary use of wheat grain proved effective in the simultaneous improvement of calcium and energy states, and thereby, in easing the metabolic transition from gestation into lactation in dairy cows.

During lactation, there is increased absorption of calcium ions from the intestines (Kovacs, 2012) and osteolytic resorption from the maternal bones (Horst *et al.,* 1994). Metabolic acidosis caused by a negative dietary cation-anion difference favours

mobilisation of calcium from bone, whereas high dietary potassium concentration suppresses this process (Horst *et al*., 1997). This increased calcium demand during lactation can result in a precipitous drop in blood calcium concentrations at calving, leading to milk fever (Goff, 2008).

Metabolic alkalosis prevents the response of the cow to parathyroid hormone (Phillippo *et al*., 1994). The conformation of the parathyroid hormone receptor is altered during metabolic alkalosis, rendering the tissues less sensitive to parathyroid hormone (Goff, 2008). Failure of the kidneys to respond to parathyroid hormone reduces renal reabsorption of calcium from the glomerular filtrate and enhanced intestinal absorption of dietary calcium which will normally restore blood calcium to normal, fails to be instituted (Goff, 2008). The physiological factors, such as metabolic alkalosis and hypomagnesemia, that cause milk fever, have been reviewed by Goff (2008).

2.4 HAEMATOLOGICAL PROFILES AND REPRODUCTION

Haematological parameters are of value in the evaluation of physical and health status of animals (Hawky and Dennet, 1989). They are influenced by pregnancy and lactation (Al-Shami, 2007, Mohammed *et al*., 2007). The study of haematology of prepartum and postpartum cows is a useful approach in understanding the metabolic state of the animals (Ate, 2005). Rekwot *et al*. (1997) stated that haematological data under field conditions may be good indication of the nutritive status of Nigerian indigenous cattle. Saror and Cole (1975) stated that cows under better management system of institutional and research herds had higher haematological values due to better management and nutritional factors. Thus, there is a need to ascertain the haematological values during the different reproductive stages for effective diagnosis of metabolic disorders which

may affect the reproductive efficiency, leading to heavy economic loss in the dairy industry.

Hematological parameters of cattle are used for screening examination or to establish initial diagnosis, therefore they are often taken into account in diagnosis (Cole *et al*., 1997). According to Deptuła and Dorynek (1993), red blood cell count and white blood cell count as well as hemoglobin concentration do not show great seasonal differences. Other examinations (Horst *et al*., 1997) indicate that during the last period of pregnancy such parameters as hematocrit value (packed cell volume) and erythrocyte count decrease physiologically while increasing after delivery. Kuleta *et al*. (1993) proved other relationships finding a downward tendency of hematological parameter values in cows during the period of 2 weeks before to 2 months after delivery. These variations, however, do not hinder the clinical evaluation of test results since they were within normal ranges (Cole *et al*., 1997).

The normal value for packed cell volume, haemoglobin concentration and white blood cell count in bovine has been stated as 26-46 %, 8-14 g/dL and 4-12 x 10^3/µL (Coles, 1967). However, the author emphasized that in most geographic areas, normal values of haematological values and blood metabolites must be determined as the values are affected by altitude, temperature and humidity. In Nigeria, Saror and Coles (1973) reported that the white Fulani cattle (Bunaji breed) under intensive management had higher red blood cell count, haemoglobin concentration and packed cell volume than those managed extensively.

The findings of Peterson *et al*. (1960) indicated an effect of gestation and lactation on haemoglobin levels. Ullrey *et al*. (1965) observed a decline in haematocrit value during pregnancy in sheep followed by an increase at parturition and a decline at the second week of lactation. Schultze (1960) showed a significant correlation between average erythrocyte count and milk producing ability, and thus concluded that cows that secrete large amount of milk may have a larger blood volume than less productive ones.

The influence of the stages of reproduction on selected parameters such as mineral metabolism levels has been investigated on 55 clinically healthy dairy cows of the Bohemian Spotted breed (Doubek *et al*., 1985). Late pregnancy and lactation are associated with decrease in packed cell volume and red blood cells counts (Ozegbe, 2001). Ate (2005) reported that haematological profiles of cows from herds in Kaduna State at prepartum and postpartum periods were not significantly different.

Examination of physiological indicators is a diagnostic method for early recognition of a number of metabolic disorders of subclinical nature in dairy cows (Stec *et al*., 2006). It is of great importance to diagnose as early as possible the latent diseases which may occur during the perinatal period spanning the range from the last 3 weeks of pregnancy to the first 3 weeks of lactation. Possible disorders occurring in this period influence further fertility and health of cows and calves (Tothova *et al*., 2008).

CHAPTER 3

MATERIALS AND METHODS

3.1 STUDY LOCATION

This study was conducted at the Dairy Research Programme, National Animal Production Research Institute (NAPRI), Ahmadu Bello University, Shika. Shika is located about 20 km along the Zaria - Sokoto road in Giwa Local Government Area of Kaduna State, Nigeria. It is geographically located between latitude 11^{o} 12'N and longitude 7^{o} 33'E at an altitude of 640 M above sea level (Akpa *et al*., 2002). It has three distinct climatic seasons; the cold dry season (November - February), the hot dry season (March - May) and the wet season (June - October) (Abeke *et al*., 2008). The total annual rainfall ranges from 617 to 1365 mm with and most of the rains fall between July and September (Oni *et al*., 1991). The mean annual minimum and maximum temperature in Shika are 17.7 ^{o}C and 31.5 ^{o}C, respectively, and the relative humidity is 28.8 % during the cold dry and 67.6 % during the wet season (Rekwot *et al*., 2000).

3.2 EXPERIMENTAL ANIMALS AND MANAGEMENT

Twenty eight cows of 5 to 6 years of age, comprising of 11 Friesian x Bunaji and 17 Bunaji were observed for normal cyclicity. Only 8 cows (4 Friesian x Bunaji and 4 Bunaji) that showed normal cyclicity were used for the study. They where synchronized using a single intramuscular injection of 25 mg of $PGF_{2\alpha}$ as the dinoprost tromethamine (Lutalyse®, Pharmacia and Upjohn Company LLC, Division of Pfizer Inc. New York). All synchronized cows were inseminated using Friesian semen. The cows were kept in a pen and released every morning into paddocks to graze. The cows grazed on woolly

finger grass (*Digitaria smutsii*), signal grass (*Brachiaria decumbens*), gamba grass (*Andropogon gayanus*), golden bristle grass (*Setaria sphacelata*) and thatch grass (*Hyparrhenia rufa*). Grazing was supplemented with whole cotton seed (39.66 %), maize (35.31 %) maize offal (22.02 %), bone meal (2.00 %) and salt (1.00 %), while water was provided *ad libitum*. The animals were preconditioned for two weeks before commencement of the experiment. During this period, they were screened for endoparasites by the analysis of their faecal and blood samples. The animals were clinically healthy before they were utilized for the study. The cows prior to insemination were used as the control to compare the effect of pregnancy at different trimesters, on the body condition score, metabolic and haematological profiles (self pairing).

3.3 EXPERIMENTAL SAMPLING

The study was carried out in 3 phases: prior to insemination, during the 3 trimesters of pregnancy and 2 months postpartum. Jugular venous blood samples were collected from dams using 20 ml syringe and 18 Gauge 1½ inch sterile needle. Blood samples were collected at 30 days interval in the last two months prior to insemination, and the mean value was obtained for the control. Thereafter, venous blood samples were collected at 30 days interval during the different trimesters (first, second and third) and during the first 2 months postpartum. The blood samples were used for the analyses of metabolic and haematological profiles. Body condition scores were similarly obtained.

3.3.1 Evaluation of Body Condition Scores

Body condition scores were obtained at 30 days interval, using the method of Pullan (1978), on a scale of 0 to 5. According to Pullan (1978), score 0 indicated emaciated animals with no apparent subcutaneous fats and a sharp spinous process of the lumber

region. Score 1 indicated animals whose spinous processes are sharp, but less than that of those in score 0. There were some subcutaneous fats on these processes and on the points of the hips. Score 2 indicated animals whose individual spinous process are still fairly sharp to touch and the ribs can be seen individually. Score 3 indicated animals whose spinous process can be felt but have a rounded feel and the ribs cannot be seen individually. Score 4 indicated animals whose individual spinous processes can only be felt with firm pressure and point of the hips are covered with fat and are rounded. Score 5 indicated animals whose spinous processes cannot be felt even with firm pressure and the animals have a blocky appearance.

3.3.2 **Evaluation of Metabolic and Haematological Profiles**

Jugular blood samples were obtained early in the morning at 30 days interval. Fifteen ml of blood was collected from each animal using the 20 ml syringe and 18 G needle. Ten ml of the blood was poured immediately into ice packed serum bottles (MEUS sri PIOVE DI-ITALY) for metabolic profile determination, while the remaining 5 ml was gently poured into sterile bottles containing 0.5 ml ethylenediaminetetraacetic acid (EDTA) anticoagulant for the determination of haematological profile. The blood samples were transferred to the Department of Chemical Pathology of Ahmadu Bello University Teaching Hospital, Shika.

The samples for the determination of blood metabolites were centrifuged at 2343 x g for 10 minutes and the serum samples obtained were aliquoted into test tubes and analyzed immediately. The samples were analysed for glucose, triglycerides, cholesterol, creatinine, blood urea nitrogen, total protein and albumin using the Express plus analyser (Bayer Diagnostic, Norwood, Massachusetts). Samples for serum calcium were

analysed using a calcium analyser (Randox laboratories Ltd. Antrim, UK) which employs the colorimetric method. Sodium and potassium ions were analysed using Corning 410 analyser (Sherwood, UK) which employs the flame photometric method while chloride and bicarbonate ions were analysed using the conventional titrimetric method. Packed cell volume was estimated by the microhaematocrit method and the haemoglobin concentration by the cyanmethaemoglobin method. White blood cell count was determined using a haemocytometer.

3.4 STATISTICAL ANALYSES

Data was expressed as mean ± SEM (standard error of mean) and presented in tables. The mean values of body condition scores, blood metabolites and electrolyte and haematological values in the different trimesters of pregnancy and at postpartum were subjected to one-way analysis of variance (ANOVA), followed by Turkey's post hoc test using Statistical Package for Social Science (SPSS) version 17 to determine significant differences. Also, student's t-test was used to compare between Friesian x Bunaji and Bunaji cows the mean values of the corresponding parameters under consideration. Values of $P < 0.05$ were considered significant.

CHAPTER 4

RESULTS

4.1 BODY CONDITION SCORE

The mean values of the body condition scores in the two breeds throughout the experiment are shown (Table 4.1). There was an initial drop in the value of body condition score of the Friesian x Bunaji, from 3.31 ± 0.16, prior to insemination, to 3.17 ± 0.11 at the end of the first trimester. Thereafter, the value increased steadily during pregnancy, before decreasing to 3.19 ± 0.16 at postpartum. In the Bunaji cows, there was an increase in body condition score during pregnancy, from 3.54 ± 0.19 at first trimester to 3.96 ± 0.11 at third trimester and a subsequent decrease in the value to 3.50 ± 0.19 at postpartum. The body condition score of the Bunaji cows at the second trimester was significantly ($P < 0.05$) higher than the value in the Friesian x Bunaji cows (3.79 ± 0.10 vs 3.29 ± 0.07).

In the Friesian x Bunaji, there was a significantly ($P < 0.05$) higher body condition score at the third trimester, than at the first trimester (3.75 ± 0.12 vs 3.17 ± 0.11). Also, in the Friesian-Bunaji Cross, there was a significant difference ($P < 0.05$) in body condition score at the second and third trimesters, with a higher value in the third trimester (3.29 ± 0.07 vs 3.75 ± 0.12). Similarly, values of the body condition scores of the third trimester in the Friesian x Bunaji was significantly ($P < 0.05$) higher than the value obtained at postpartum (3.75 ± 0.12 vs 3.19 ± 0.16). In the Bunaji cows, there was no significant difference ($P > 0.05$) in body condition scores across all the reproductive stages.

Table 4.1: Mean ± SEM body condition scores during the reproductive stages for Friesian x Bunaji and Bunaji cows

Breed	Reproductive Stages and Body Condition Score (Mean ± SEM)				
	Prior to Insemination	First Trimester	Second Trimester	Third Trimester	Postpartum
FR-BJ Cross	3.31 ± 0.16	3.17 ± 0.11[a]	3.29 ± 0.07[a*]	3.75 ± 0.12[b]	3.19 ± 0.16[a]
BJ	3.69 ± 0.16	3.54 ± 0.19	3.79 ± 0.10[*]	3.96 ± 0.11	3.50 ± 0.19

[*]means ± SEM within a column with asterisks are significantly different ($P < 0.05$)
[a, b]means ± SEM in rows with different letter superscripts are significantly different ($P < 0.05$)
SEM - Standard error of mean; FR x BJ – Friesian x Bunaji; BJ - Bunaji

4.2 METABOLIC PROFILE

4.2.1 Serum Metabolites

The mean values of serum glucose, triglycerides, cholesterol, urea and creatinine concentrations in the two breeds are shown (Table 4.2). The Bunaji cows had a significantly ($P < 0.05$) higher glucose concentration than the Friesian x Bunaji at postpartum (5.48 ± 0.54 mMol/L vs 4.91 ± 0.57 mMol/L). In each of the two breeds, the postpartum serum glucose concentration was significantly ($P < 0.05$) higher than the value at the third trimester.

The difference in the triglyceride concentration between the Friesian x Bunaji cows and Bunaji cows at postpartum was significant ($P < 0.05$), with a higher value in the Bunaji cows. In the Friesian x Bunaji, the value of triglyceride concentration prior to insemination was significantly ($P < 0.05$) lower than the value at the third trimester (0.74 ± 0.08 mMol/L vs 1.58 ± 0.26 mMol/L) and at postpartum (0.74 ± 0.08 mMol/L vs 1.86 ± 0.32 mMol/L). The postpartum triglyceride values was significantly ($P < 0.05$) higher than the first and second trimester values in the Friesian x Bunaji. In the Bunaji cows, the triglyceride value at postpartum was significantly ($P < 0.05$) higher than the triglyceride value prior to insemination and during pregnancy. Also, the triglyceride value at the third trimester was significantly ($P < 0.05$) higher than the values prior to insemination and during the first and second trimesters.

In both the Friesian x Bunaji and Bunaji cows, there was a significant ($P < 0.05$) difference in the value of cholesterol between the first and third trimesters, while the difference in all other reproductive stages was not significant ($P > 0.05$).

In the Bunaji cows, the decrease in urea level from prior to insemination to the first trimester was significant ($P < 0.05$). Also, in the Bunaji cows, urea level prior to insemination was significantly ($P < 0.05$) higher than the value obtained at postpartum (7.41 ± 0.97 mMol/L vs 4.13 ± 0.52 mMol/L) and the urea level at the first trimester was significantly ($P < 0.05$) lower than the value at the third trimester.

In the third trimester, creatinine value of the Bunaji was significantly ($P < 0.05$) higher than the value of the Friesian x Bunaji (113.00 ± 9.65 mMol/L vs 90.25 ± 4.63 mMol/L). In the Friesian x Bunaji, the creatinine value prior to insemination was significantly ($P < 0.05$) higher than the value at pregnancy and postpartum. In the Bunaji cows, the creatinine value at postpartum was significantly ($P < 0.05$) lower than the value prior to insemination (54.38 ± 10.13 mMol/L vs 135.13 ± 23.05 mMol/L) and the value at the third trimester (54.38 ± 10.13 mMol/L vs 113.00 ± 9.65 mMol/L).

Table 4.2: Mean SEM values of blood metabolites during the reproductive stages in Friesian x Bunaji and Bunaji cows

		Reproductive Stages and Level of Metabolites (Mean ± SEM)				
Parameter (mMol/L)	Breed	Prior to Insemination	First Semester	Second Trimester	Third Trimester	Postpartum
Glucose	FR x BJ	2.17±0.44	3.10±0.38	2.89±0.32	3.60±0.30[a]	4.91±0.57[b,*]
	BJ	2.29±0.32	3,50±0.39	2.83±0.33	3.83±0.49[a]	5.48±0.54[b,*]
Triglyceride	FR x BJ	0.74±0.08[a]	0.91±0.06[a, b]	0.93±0.06[a, b]	1.58±0.26[b, c]	1.86±0.32[c,*]
	BJ	0.61±0.07[a]	0.93±0.08[a]	1.02±0.06[a]	1.45±0.12[b]	2.69±0.16[c,*]
Cholesterol	FR x BJ	5.15±0.58	6.17±0.63[a]	5.29±0.46	4.12±0.46[b]	4.78±0.33
	BJ	4.85±0.77	7.08±0.73[a]	5.92±0.83	3.72±0.34[b]	4.95±0.41
Urea	FR x BJ	10.46±2.68	3.73±0.40	4.63±0.78	4.75±0.51	9.50±5.08
	BJ	7.41±0.97[a]	2.86±0.20[b, c]	4.92±0.64	6.08±0.67[a, d]	4.13±0.52[b, d]
Creatinine	FR x BJ	188.63±15.32[a]	97.08±12.07[b]	89.50±11.06[c]	90.25±4.63[d,*]	77.36±12.95[e]
	BJ	135.13±23.05[a]	94.00±9.76	96.25±5.78	113.00±9.65[a,*]	54.38±10.13[b]

[*]Means ± SEM in column for metabolite with asterisks are significantly different ($p < 0.05$)

[a,b,,c,d,e] Means ± SEM in rows for each metabolite with different letter superscripts are significantly different ($p < 0.05$)

SEM - Standard error of means; FR x BJ - Friesian x Bunaji; BJ - Bunaji

4.2.2 **Serum Electrolytes and Minerals**

The mean values of sodium, potassium, chloride, bicarbonate and calcium ions of the Friesian x Bunaji and Bunaji cows are shown (Table 4.3). In each of the two breeds, there was a continuous decrease in sodium ion from the period prior to insemination to the second trimester, followed by an increase in the value up to postpartum. The difference in the value of sodium ion of the Friesian x Bunaji and Bunaji cows at each reproductive stage was not significant ($P > 0.05$). In the Friesian x Bunaji, the value of sodium ion prior to insemination was significantly ($P < 0.05$) higher than the value during pregnancy, while the difference in the value prior to insemination and the value at postpartum was not significant ($P > 0.05$). In the Bunaji cows, the value of sodium ion prior to insemination was significantly ($P < 0.05$) higher than the value during the stages of pregnancy and at postpartum.

In both the Friesian x Bunaji and Bunaji cows, there was a decrease in the value of potassium ion from the period prior to insemination to the first trimester. Thereafter, there was an increase in the value throughout pregnancy, followed by a decrease in the value at postpartum. The difference in value of potassium ion of the Friesian x Bunaji and Bunaji cows at each reproductive stage was not significant ($P > 0.05$). Comparison across the reproductive stages revealed that there was no significant difference ($P > 0.05$) in the value of potassium ion in the Friesian x Bunaji. However, in the Bunaji cows, the value of potassium ion at postpartum was significantly ($P < 0.05$) lower than the value obtained prior to insemination (2.51 ± 0.24 mMol/L vs 4.31 ± 0.29 mMol/L) and during pregnancy. Also, in the Bunaji cows, the potassium ion at the first trimester was significantly ($P < 0.05$) lower than the value at the third trimester (4.15 ± 0.23 mMol/L vs 5.52 ± 0.47 mMol/L).

The value of chloride ion in the Bunaji cows at the third trimester was significantly ($P < 0.05$) higher than the value in the Friesian x Bunaji (104.92 ± 1.71 mMol/L vs 100.17 ± 1.42 mMol/L). In each of the two breeds, there was no significant ($P > 0.05$) difference in the values of chloride ion across the different reproductive stages.

The value of bicarbonate ion in both the Friesian x Bunaji and Bunaji cows increased at the onset of pregnancy. This was followed by a fall in value at the second trimester, with a final postpartum value of 23.50 ± 2.68 mMol/L and 25.13 ± 1.86 mMol/L in the Friesian x Bunaji and Bunaji cows, respectively. There was no significant difference ($P > 0.05$) in the value of bicarbonate ion of the Friesian x Bunaji and Bunaji cows during each reproductive stage. In the Friesian x Bunaji, bicarbonate ion at the first trimester was significantly ($P < 0.05$) higher than the value obtained prior to insemination (25.42 ± 0.92 mMol/L vs 17.23 ± 2.72 mMol/L).

In each of the two breeds, there was an increase in the blood calcium level from prior to insemination to the second trimester. Thereafter, there was a drop in the value by the third trimester in both the Friesian x Bunaji and Bunaji cows. The difference in calcium ion of the Friesian x Bunaji and Bunaji cows at each reproductive stage was not significant ($P > 0.05$). Comparison across the reproductive stages revealed that in the Friesian x Bunaji, calcium level in the second trimester was significantly ($P < 0.05$) higher than the value at postpartum (3.40 ± 0.32 mMol/L vs 2.04 ± 0.17 mMol/L). In the Bunaji cows, there was no significant difference ($P > 0.05$) in the blood calcium level across all the reproductive stages.

Table 4.3: Mean SEM values of serum electrolytes and minerals during the reproductive stages in Friesian x Bunaji and Bunaji cows

		Reproductive stage and Level of metabolites (Mean ± SEM)				
Parameter (mMol/L)	Breed	Prior to Insemination	First Semester	Second Trimester	Third Trimester	Postpartum
Sodium	FR x BJ	152.38±7.43[a]	137.17±1.77[b]	122.17±4.93[b]	123.75±2.68[b]	134.50±2.80
	BJ	152.75±5.53[a]	134.17±1.62[b]	117.67±5.36[b]	124.83±2.65[b]	135.00±2.36[b]
Potassium	FR x BJ	4.3±0.029	3.85±0.18	4.22±0.25	6.64±0.61	3.23±0.33
	BJ	4.31±0.29[a, b]	4.15±0.23[a]	4.77±0.36[a, b]	5.52±0.47[b]	2.51±0.24[c]
Chloride	FR x BJ	103.38±5.16	101.17±2.26	102.33±2.58	100.17±1.42[*]	107.13±1.92
	BJ	106.25±3.69	100.83±2.12	103.58±2.14	104.92±1.71[*]	107.13±1.04
Bicarbonate	FR x BJ	17.23±2.72[a]	25.42±0.92[b]	22.92±1.35	21.10±2.10	23.50±2.68
	BJ	23.00±2.49	24.83±1.59	20.58±1.35	22.33±1.39	25.13±1.86
Calcium	FR x BJ	2.47±0.14	2.83±0.12	3.40±0.11[a]	2.72±0.30	2.04±0.17[b]
	BJ	2.38±0.34	3.14±0.26	3.77±0.34	2.98±0.36	3.20±0.76

*Means ± SEM within column for electrolyte with asterisks are significantly different ($p < 0.05$)

[a,b,c] Means ± SEM in rows for each electrolyte and mineral with different letter superscripts are significantly different ($p < 0.05$)

SEM - Standard error of means; FR x BJ - Friesian x Bunaji; BJ - Bunaji

4.2.3 Total Proteins and Serum Albumins

The mean values of total protein and serum albumin in the two breeds are shown (Table 4.4). In the Friesian x Bunaji, there was no significant difference ($P > 0.05$) in the value of total protein across the reproductive stages. Conversely, in the Bunaji cows, the total protein was significantly ($P < 0.05$) lower at postpartum, compared to the values prior to insemination and during pregnancy.

The difference in value of albumin between the Friesian x Bunaji and Bunaji cows during each reproductive stage was not significant ($P > 0.05$). The serum albumin concentration at third trimester was significantly ($P < 0.05$) higher than the value at postpartum in both the Friesian x Bunaji (36.67 ± 7.61 g/l vs 18.50 ± 2.20 g/l) and Bunaji (28.25 ± 4.32 g/l vs 17.25 ± 2.30 g/l) cows.

Table 4.4: Mean ± SEM values of total protein and serum albumins during the reproductive stages in Friesian x Bunaji and Bunaji cows

		Reproductive Stages and TP/ALB levels (Mean ± SEM)				
Parameter (g/l)	Breed	Prior to Insemination	First Trimester	Second Trimester	Third Trimester	Postpartum
Total Protein	FR x BJ	59.54 ± 6.05	58.33 ± 5.75	62.42 ± 2.12	56.33 ± 3.70	45.00 ± 4.45
	BJ	55.19 ± 5.16[a]	58.92 ± 6.06[a]	63.92 ± 3.69[a]	56.83 ± 4.20[a]	34.75 ± 2.74[b]
Albumins	FR x BJ	29.02 ± 6.48	31.50 ± 4.84	37.00 ± 1.97	36.67 ± 7.61[a]	18.50 ± 2.20[b]
	BJ	27.69 ± 3.10	29.00 ± 4.29	35.92 ± 2.50	28.25 ± 4.32[a]	17.25 ± 2.30[b]

Means ± SEM of TP/ALB within column of each reproductive stage are not significantly different ($P > 0.05$)
[a, b]Means ± SEM in rows with different letter superscripts are significantly different ($P < 0.05$)
SEM - Standard error of means; FR x BJ – Friesian x Bunaji; BJ - Bunaji; TP: Total protein; ALB: Albumin

4.3 HAEMATOLOGICAL PROFILE

The mean haematological profiles of the Friesian x Bunaji and Bunaji cows during the reprodutive stages are shown (Table 4.5). Packed cell volume of the Friesian x Bunaji at postpartum was significantly ($P < 0.05$) lower thant the value in the Bunaji cows. In the Friesian x Bunaji, packed cell volume prior to insemination was significantly ($P < 0.05$) higher than the value at the second trimester and at postpartum. In the Bunaji cows, packed cell volume prior to insemination was significantly ($P < 0.05$) lower than the value at the second trimester. There was a significant difference ($P < 0.05$) in haemoglobin concentration of the two breeds prior to insemination, with a higher value in the Bunaji cows (27.23 ± 5.27 g/dL vs 13.10 ± 0.90 g/dL). In the Friesian x Bunaji, haemoglobin concentration prior to insemination was significantly ($P < 0.05$) higher than the value at the second trimester and at postpartum. In the Bunaji cows, haemoglobin concentration prior to insemination was significantly ($P < 0.05$) higher than the value at the second (27.23 ± 5.27 g/dL vs 10.38 ± 0.35 g/dL) and third trimesters (27.23 ± 5.27 g/dL vs 11.82 ± 0.57 g/dL). Also, in the Bunaji cows, haemoglobin concentration in the first trimester was significantly ($P < 0.05$) higher than the second trimester, while the value in the second trimester was significantly ($P < 0.05$) lower than the value in the third trimester.

In Friesian x Bunaji, white blood cell count at the first trimester was significantly ($P < 0.05$) higher than the values at the second and third trimesters and at postpartum. In the Bunaji cows, white blood cell count prior to insemination was significantly ($P < 0.05$) higher than the values at the second and third trimesters and at postpartum.

Table 4.5: Mean ± SEM values of haematological profile during the reproductive stages in Friesian x Bunaji and Bunaji cows

Parameter	Breed	Reproductive Stages and Haematological Profile (Mean ± SEM)				
		Prior to Insemination	First Trimester	Second Trimester	Third Trimester	Postpartum
Packed cell Volume (%)	FR x BJ	37.38 ± 3.24^{a}	32.53 ± 1.04	29.75 ± 0.92^{b}	32.00 ± 1.26	$27.50 \pm 3.03^{b,*}$
	BJ	27.20 ± 5.34^{a}	34.75 ± 1.67	31.25 ± 1.02^{b}	35.50 ± 1.72	$36.13 \pm 1.75^{*}$
Haemoglobin Concentration (g/dL)	FR x BJ	$13.10 \pm 0.90^{a,*}$	11.14 ± 0.46	9.90 ± 0.30^{b}	10.65 ± 0.42	9.51 ± 1.13^{b}
	BJ	$27.23 \pm 5.27^{a,*}$	$12.06 \pm 0.68^{a,c}$	10.38 ± 0.35^{b}	11.82 ± 0.57^{c}	12.13 ± 0.67
White blood cell count ($10^3/\mu L$)	FR x BJ	11.19 ± 2.01	13.37 ± 0.69^{a}	$8.93 \pm 0.68^{b,d}$	$9.30 \pm 0.80^{c,d}$	8.85 ± 1.42^{d}
	BJ	14.67 ± 2.24^{a}	11.42 ± 0.85	$8.10 \pm 0.82^{b,d}$	$8.23 \pm 0.72^{c,d}$	7.64 ± 0.89^{d}

*Means ± SEM within a metabolite column with asterisks are significantly different ($P < 0.05$)

a, b, c, dMeans ± SEM in rows with different letter superscript are significantly different ($P < 0.05$)

SEM = Standard error of means; FR x BJ = Friesian x Bunaji; BJ = Bunaji

CHAPTER 5

DISCUSSION

The body condition scores of both breeds remained constantly above 3.0 which is an adequate condition to maintain good health (Burton *et al*., 2003). Skidmore *et al*. (2001) reported that this body condition score is an optimum body condition at calving which will allow the cow to meet any forthcoming dietary inadequacies without incurring excessive rates of postpartum mobilization in support of a potential production. The increase in body condition score in the Friesian x Bunaji and Bunaji cows during pregnancy is consistent with the report of Friggens (2003) who stated that dairy cows undergo a normal cycle of body energy storage and mobilization, with increased body fat storage during mid-gestation. The non-significance of the increased body condition score in the Bunaji cows during pregnancy is not very clear in this study. The significant decrease in the body condition score in the Friesian x Bunaji following parturition, unlike in the local Bunaji, has been attributed to the genetic merit for milk production in the cross breed by Veerkamp *et al*. (1994) and Buckley *et al*. (2000). The authors reported that cows of higher genetic merit for milk production have greater body condition score loss in early lactation than those of lower genetic merit. The significantly higher value of body condition score in the Bunaji cows in the second trimester, compared to the Friesian x Bunaji could be attributed to breed disposition, as the Bunaji cows had a higher value of body condition score throughout the reproductive stages, although the difference was significant in the second trimester.

The significant increase in values of serum glucose concentration during the third trimester and postpartum periods (when lactation must have set in) in the two breeds

agrees with the report by Djoković *et al*. (2010) in dairy cows and by Karapehlivan *et al*. (2007) in ewes. Conversely, Busato *et al.* (2002) recorded a decrease in plasma glucose concentrations in the first 12-16 days of lactation in dairy cows. Although it has been established that the increased glucose flow to the mammary gland leads to a fall in serum glucose at the onset of lactation, causing a negative energy balance in the lactating dam (Reist *et al*., 2002; Overton and Waldron, 2004), this has been greatly prevented by adequate dry mater intake especially during the third trimester and early lactation (National Research Council, 2001). The concentrate feeding employed for animals in the present study was probably adequate to prevent a negative energy balance during pregnancy and lactation, as indicated by their serum glucose level. The significantly lower values of serum glucose concentration in the Friesian x Bunaji than Bunaji cows at postpartum indicated that the Friesian x Bunaji is more prone to negative energy balance than the Bunaji cows, as it produces more milk and thus takes up more serum glucose than the Bunaji cows. Thus, there is a need to place the Friesian x Bunaji breeds on more concentrate than the pure Bunaji cows especially during pregnancy and the postpartum period.

The higher mean values of blood glucose level recorded in this study may have provided more co-factors and induced the expression and activity of the enzymes involved in milk fat synthesis (Koumatsu *et al*., 2005). This agrees with the report of El-Sherif and Assad (2001) and Regnault *et al*. (2004) which concluded that increase in milk production during lactation is attributed to increased serum glucose concentration at late pregnancy and postpartum.

The increased serum triglycerides at early lactation in both the Friesian x Bunaji and Bunaji cows observed in the present study has been reported in ewes by Karapehlivan *et al.* (2007). The increase in serum triglyceride concentration during late pregnancy observed in this study is necessary to maintain positive energy balance. This increase is under the influence of growth hormone and insulin, which play direct role in adipose tissue metabolism during pregnancy (Ingvartsen, 2006). According to Grum *et al*. (2002), the diminished responsiveness of adipose tissue to insulin during late pregnancy and the increased responsiveness of adipose tissue to growth hormones during the same period, predispose the ruminant to increased mobilisation of fat deposited during gestation, resulting in increased blood concentration of triglycerides (as is the case in this study), which are used as alternative fuel source for the dam and for milk synthesis.

The decrease in serum cholesterol level at the third trimester and lactation periods in both breeds observed in the present study is similar to the report of Piccione *et al*. (2009) but contrary to the report of Karapehlivan *et al*. (2007). A significant decrease in serum cholesterol during late pregnancy and early lactation maybe indicative of an impaired liver (Grum *et al*., 2002), as the majority of serum cholesterol is derived from hepatic biosynthesis, and fat accumulation in the hepatocytes at parturition can impair normal liver function in cows (Dann *et al*., 2006).

The significant rise in the value of serum urea concentration at postpartum in both breeds agrees with Gonzalez and Rocha (1998) who reported that serum urea level was higher in lactating cows, compared to non-lactating cows. Similarly, Antunovic *et al.* (2002) reported that serum urea concentration was higher during lactation and the last trimester of pregnancy in sheep. Conversely, Brozostowski *et al.* (1996) observed an

increase in urea level during early pregnancy, while there was a decrease below the reference levels in late pregnancy. The drop in urea during gestation is probably associated with the use of urea for protein synthesis on the rumino-hepatic pathway as a compensation for the low protein uptake prior to pregnancy (Yokus *et al*., 2006), while the elevated value at postpartum is consistent with the significant drop in total protein at postpartum as urea level increases during protein catabolism (Bruce and Nugent, 2005).

The significant drop in serum creatinine level at postpartum in both the Friesian x Bunaji and Bunaji has been reported in Holstein x Friesian cows by Piccione *et al*. (2012). The significant drop in serum creatinine level in this study indicates the absence of maternal tissue (muscle) breakdown for milk production as suggested in small ruminants by Roubies *et al*. (2006). This also implies that although the maternal protein supply declined during this period, as indicated by a drop in total protein, the decline was not devastating to induce maternal tissue catabolism as alternative protein source. This was also supported by the adequate body condition score of the two breeds at postpartum.

The significant drop in serum calcium concentration in Friesian x Bunaji at third trimester indicated an increased uptake of calcium for foetal bone growth (Martz *et al*., 1990). The continuous decline of serum calcium at postpartum lactation in the Friesian x Bunaji is an adaptation required to meet the high demand for milk production associated with the breed, relative to the local Bunaji (Adeneye, 1989), since an especially large loss of calcium to milk occurs during lactation (Loiselle *et al*., 2008). Thus, as both breeds were exposed to the same dietary level of calcium, more calcium was mobilised for milk synthesis in the high milk producing Friesian x Bunaji.

The result of sodium, potassium and chloride ion concentrations in the Friesian x Bunaji cows in the present study agrees with the findings of Ate (2005) who reported that there was no significant difference in the third trimester of pregnancy and postpartum values of serum electrolytes in cattle herds in Zaria. Azab and Abdel-Maksoud (1999) reported a decrease in sodium and potassium ions at parturition in Baladi goats and speculated that the decrease was due to the cations being lost in the colostrum. The present result suggests that the homeostasis of the electrolytes at parturition was maintained in the Friesian x Bunaji despite their increased demand in colostrum production. Similar result was obtained for sodium and chloride ions in the Bunaji cows. The significant decrease in potassium ion postpartum in the Bunaji cows could be due to an increased uptake of the ion for colostrum, relative to the feed intake of the ion.

According to Kaneko *et al*. (1997), sodium ion concentrations would be assumed to follow urea concentrations with respect to the body recycling nitrogen. The present result is in agreement with the assumption of Kaneko *et al*. (1997) as the concentrations of both urea and sodium ions decreased with the onset of pregnancy and increased at postpartum in both breeds. The non-significant difference in mean values of bicarbonate ion prior to insemination and postpartum in the two breeds used in the present study contradicts the result of Ate *et al*. (2009a) who reported that the prepartum (third trimester) value of bicarbonate ions was significantly higher than the postpartum value in a settled cattle herd. The non-significance in the values of bicarbonate ion between the stage prior to insemination and at postpartum in the present study might be attributed to the adequacy of bicarbonate salts in the grazing forage of the animals.

The drop in serum total protein soon after parturition in both breeds suggests that while maternal protein supply via dry matter intake was adequate for foetal muscle growth during pregnancy, there was more demand for gluconeogenesis and milk protein synthesis to maintain milk production during lactation (Mohri *et al*., 2007), hence the increased uptake and subsequent drop in value at the peak of lactation. Fox *et al*. (1992) suggested that current models underestimate actual gestational protein requirement throughout late gestation. This potential discrepancy would result in depletion of protein reserves to support gestation rather than early lactation.

In the present study, the decreased level of serum albumin in both breeds as pregnancy advanced into the third trimester as well as at postpartum has earlier been reported by Brozostowski *et al*. (1996). Conversely, Busato *et al.* (2002) recorded that plasma albumin concentrations increased slightly, but significantly, during lactation period in dairy cows. The dairy cows used by Busato *et al.* (2002) were feed with rations of high protein content. It has been established that albumin requirement increases during late pregnancy and lactation as maternal serum protein concentrations decrease with advancement in pregnancy, due to an increased foetal growth, especially the utilization of amino acids from the maternal circulation for protein synthesis in the foetal muscles (Jainudeen and Hafez, 1994) and for the development of maternal mammary glands (Everts, 1998). Therefore, there is a need to improve on protein nutrient availability for the dam.

The decreased packed cell volume and haemoglobin concentration during lactation in the Friesian x Bunaji seen in this study is in agreement with the report of Esievo and Moore (1979), Sattar and Mirza (2009) and Mirzadeh *et al*. (2010). The reason for the

high value of haemoglobin concentration (27.23 g/dL) in the Bunaji cows prior to insemination, in the present study, is not fully understood as the value decreased to normal during pregnancy. Although the packed cell volume and haemoglobin concentration of the Friesian x Bunaji used in the present study remained within the reference range, continuous decline in the values during early lactation could point to postparturient haemoglobinuria which has been reported to cause decrease in packed cell volume and haemoglobin concentration during early lactation in high yielding cows (Jubb *et al*., 1990; Pirzada and Hussain, 1998). Thus, there is a need to constantly monitor the haematological profiles of the cross breeds or other improved breeds during early lactation to avoid the occurrence of postparturient haemoglobinurea.

Studies on white blood cell count during pregnancy have produced variable results (Ate, 2005). In this study, there was significant decrease in white blood cell as pregnancy progressed. This result disagrees with that of Ate *et al.* (2009b) who reported no significant difference between third trimester – pregnancy and postpartum values in dairy cows (Friesian x Bunaji). In ewes, white blood cell decreased with pregnancy progression (Pisek *et al*., 2008). According to Entrican *et al*. (2002), the development of an embryo is associated with increased stress in the dam's immune system resulting in increase in maternal white blood cell count. However, Nasar *et al*. (2002) revealed through histological examinations that the onset of immunotolerance in pregnant ruminants is concentrated in the uterine tissues with proportions of lymphocyte subsets in the peripheral blood being uninfluenced.

CHAPTER 6

SUMMARY, CONCLUSSION AND RECOMMENDATIONS

6.1 SUMMARY

The present study has provided information on the body condition scores, metabolic and haematologic profiles of the Friesian x Bunaji and Bunaji cows prior to insemination, during pregnancy and at postpartum.

Throughout the experiment, the body condition scores of both breeds remained constantly above 3.0. The body condition scores were 3.75 and 3.96 at third trimester of pregnancy in the Friesian x Bunaji and Bunaji cows, respectively.

The first trimester values (range) for metabolic parameters for both breeds were glucose (3.10 ± 0.38 – 3.50 ± 0.39 mMol/L), tryglycerides (0.91 ± 0.06 – 0.93 ± 0.08 mMol/L), cholesterol (6.17 ± 0.63 – 7.08 ± 0.73 mMol/L), urea (2.86 ± 0.20 - 3.73 ± 0.40 mMol/L), creatinine (94.00 ± 9.76 - 97.08 ± 12.07 mMol/L), sodium (134.67 ± 1.62 - 137.17 ± 1.77 mMol/L), potassium (3.85 ± 0.18 - 4.15 ± 0.23 mMol/L) chloride (100.83 ± 2.12 - 101.17 ± 2.26 mMol/L), bicarbonate (24.83 ± 1.59 - 25.42 ± 0.92 mMol/L), total protein (58.33 ± 5.75 - 58.92 ± 6.06 g/l) and albumin (29.00 ± 4.29 - 31.50 ± 4.84 g/l), while the second trimester values (range) were glucose (2.83 ± 0.33 - 2.89 ± 0.32 mMol/L), tryglycerides (0.93 ± 0.06 - 1.02 ± 0.06 mMol/L), cholesterol (5.29 ± 0.46 - 5.92 ± 0.83mMol/L), urea (4.63 ± 0.78 - 4.92 ± 0.64 mMol/L), creatinine (89.50 ± 11.06 - 96.25 ± 5.78 mMol/L), sodium (117.67 ± 5.36 - 122.17 ± 4.93 mMol/L), potassium (4.22 ± 0.25 - 4.77 ± 0.36 mMol/L) chloride (102.33 ± 2.58 - 103.58 ± 2.14 mMol/L), bicarbonate (20.58 ± 1.35 - 22.92 ± 1.35 mMol/L), total protein (62.42 ± 2.12

- 63.92 ± 3.69 g/l) and albumin (35.92 ± 2.50 - 37.00 ± 1.97 g/l). The postpartum serum glucose levels were 4.91 ± 0.57 mMol/L and 5.48 ± 0.54 mMol/L in the Friesian-Bunaji Cross and Bunaji cows, respectively. Serum creatinine level decreased during lactation in the two breeds. Significant decrease in serum cholesterol in the two breeds was found at advanced stage of pregnancy (third trimester). There was a decrease in serum calcium concentration of both breeds, at the third trimester. Total protein level dropped during the postpartum period. Finally, with the exception of high haemoglobin concentration prior to insemination in the Bunaji breed, the values of haematological parameters of the two breeds were within the reference values.

6.2 CONCLUSION

From the results of this study, the following conclusions may be drawn:

1. The body condition scores of both breeds were optimal for normal pregnancy progression, calving and the postpartum period.
2. Serum metabolites (glucose, triglycerides, cholesterol, electrolytes, urea, and creatinine) and electrolyte and minerals (sodium, potassium, calcium) of both Friesian x Bunaji and Bunaji cows were modified by pregnancy in varied ways. The significant drop in calcium levels at the third trimester suggested the higher demand for foetal growth and probably enhanced lactogenesis at this stage. Also, the significant drop in cholesterol levels at advanced stage of pregnancy in both breeds may suggest a tendency towards the development of fatty liver.
3. Total proteins and albumins were also significantly changed during the course of pregnancy and postpartum period.
4. Haematological parameters though modified during the course of pregnancy and the postpartum period the values were within normal range reported.

5. The significantly reduced haemoglobin concentration especially in the Friesian x Bunaji cows may suggest a vulnerability to the development of postpartum haemoglobinuria in this breed than the Bunaji and therefore the need to monitor this breed closely.
6. Since none of the cows showed clinical signs of metabolic disorders, the values of metabolites, electrolytes and minerals obtained in this study may be considered baseline values for clinical and field application for diagnostic purposes. Thus, the current findings regarding the metabolic profile prior to insemination, during the different trimesters of pregnancy and at postpartum have expanded the knowledge for the diagnosis and prognosis of reproductive and metabolic diseases in Friesian x Bunaji and Bunaji cows in the study area.

6.3 RECOMMENDATIONS

Based on the findings of the study, the following recommendations are made to improve dairy production in the National Animal Production Research Institute, Shika:

1. Cows should be placed on feeding regimen that will improve the protein percentage during the early postpartum periods, especially for the Bunaji cows.
2. There is a need to constantly monitor cholesterol level during the transition period as a continuous drop in cholesterol is indicative of a fatty liver and such animals may need immediate medical attention.
3. There is a need to increase dietary calcium for the Friesian x Bunaji, especially at late gestation because of their increased milk synthesis relative to the Bunaji breed. This will help prevent sub-clinical hypocalcaemia in the former breed.
4. There is a need to increase potassium supplement in the feed of the Bunaji breed.

5. There is a need to constantly monitor the packed cell volume and haemoglobin concentration of the Friesian x Bunaji to predict the possible occurrence of postparturient hemoglobinuria.
6. Further research regarding the investigation of metabolic diseases and nutrition programmes as regards the energy requirements of Friesian x Bunaji and Bunaji cows during pregnancy and lactation is essential.
7. More extensive studies are needed to investigate the metabolic profile of these breeds especially on a higher animal number and under field conditions.

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