Effect of breed, parity and lactation stage on some blood metabolites in dairy cows in Sudan

By

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بسم الله الرحمن الرحيم

قال تعالى:

(وإن لكُم فِي الأنعامِ لَعِبرةً نسقِيكُم مما فِي بطُونِهِ مِـن بين فَرثٍ ودمٍ لبناً خالِصاً سآئغاً لِلشارِبين)

صدق الله العظيم

النحل الآية 66

Dedication

To my family

Father, Mother, Brothers and sisters

To my dear friends and colleagues.

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Abstract

The present study was conducted to investigate the effect of breed, parity order, lactation stage and their interactions on blood glucose, phosphorus and calcium levels. Thirty six lactating cows (18 crossbred and 18 pure Holstein Friesian) belonging to two farms in Khartoum State were selected. The cows in each group were divided into four groups according to parity order (first and fourth) and stage of lactation (early and mid lactation). Blood sampling was performed post milking and immediately analyzed.

The results revealed that all tested metabolites fell within the normal range. The breed however was found to affect significantly ($p< 0.5$) the plasma phosphorus but neither glucose nor calcium. Glucose and phosphorus levels were higher in Holstein Friesian(47.58 ± 2.01 mg/dl, 5.56 ± 0.27 mg/dl) compared to that for crossbred(46.54 mg/dl ± 2.01mg/dl, 4.58 ± 0.27mg/dl) , while plasma calcium level was higher in cross bred (8.57 ± 0.33 mg/dl) compared to that for Holstein Friesian (7.78 ± 0.33 mg/dl) .

The lactation stage showed a non significant effect on the levels of the studied metabolites. Serum glucose and plasma calcium levels were higher in mid lactation while plasma phosphorus level was higher in early lactation ($p > 0.05$). Parity order also didn't affect the level of the metabolites. The data analysis revealed that the interaction between breed and parity order didn't significantly affect ($p > 0.05$) serum glucose and plasma calcium levels, while plasma phosphorus level was affected ($p< 0.05$). The interaction between breed and lactation stage didn't affect the investigated metabolites. On the other hand, the interaction between parity order and lactation stage exerted a non significant effect on all investigated metabolites.

الخلاصه

أجريت هذه الدراسة لمعرفة تاثير السلاله وعـدد الـولادات ومرحلـة الإدرار و التداخل بينهما على نسب الجلكوز والفسفور والكالسيوم. أختيرت 36 مـن الابقـار المنتجـه للالبان (18أبقار مهجنه، 18 أبقار الهوليستاين فريزيان) من مزرعتين فى ولايه الخرطوم. تم تقسيم الابقار فى كل من السلالتين الى اربع مجموعات وفقا الـى عـدد الـولادات (الأولـى والرابعة) و مرحلة الإدرار (الأولى و الوسطى).أخذت العينات بعد الحلـب مباشـره و تـم تحليلها.

أسفرت النتائج التي بان كل من العناصر التى تم تحليلها فى معدلها الطبيعى بينمـا وجد ان السلاله لها تاثير معنوى على الفسفور($p<0.5$) لكن ليس لها اثر علـى كـل مـن الجلكوز و الكالسيوم. وكانت نسبة الفسفور والجلكوز أعلى فى أبقار الهوليستاين فريزيـان (47.58 ± 2.01 mg/dl, 5.56 ± 0.27 mg/dl) مقارنه مـع الأبقـار المهجنـة 46.54) (mg/dl ± 2.01mg/dl, 4.58 ± 0.27mg/dl) . بينما كانت نسبة الكالسيوم في البلازمـا أعلى في الأبقار المهجنة (8.57 ± 0.33 mg/dl) مقارنة مع أبقار الهوليـستاين فريزيـان (7.78 ± 0.33 mg/dl) .

مرحلة الإدرار ليس لها تأثيرا معنويا في نسب مكونات الدم التـى تمـت دراسـتها. وكانت نسبة الجلكوز في معدل الدم والكالسيوم في البلازما أعلى في مرحلة الإدرار الوسطي بينما كانت نسبة الفسفور في البلازما أعلى في المرحلة الأولى للإدرار($p > 0.05$). كمـا ان عدد الولادات ليس له تأثيرا في نسب مكونات الدم . وأوضح تحليل البيانات بان التداخل بـين كل من السلاله و عدد الولادات لم ليس له تاثيرا معنويا ($p < 0.05$) على نسبه الجلكوز فى معدل الدم و نسبه الكالسيوم فى البلازما بينما كان التأثير معنويـاعلى نـسبة الفـسفور فـي البلازما($p< 0.05$) . إن التداخل بين السلاله ومرحلة الإدرار ليس له تأثيرا علـى مكونـات الدم التي تم فحصها. ومن ناحية أخرى اوضح التداخل بين عدد الـولادات ومرحلـة الإدرار بعدم تاثيره معنويا على مكونات الدم التي تم فحصها.

Chapter ONE

Introduction

Dairy cattle productivity is the major concern to the dairy industry world wide. The productivity of tropical breeds of dairy cattle when compared with those reared in temperate climate is extreme, and has resulted in a tendency to seek improvement by the introduction of exotic dairy breeds. The diversity of climate, topography and conditions of husbandry in the tropics is so great such that the tropical environment imposes a number of stressors that curtail the full production potential of exotic breeds.

Metabolic profile testing (MPT) has generally been used as a part of a multi disciplinary approach for dairy herds in temperate climates (Whitaker *et al*, 1999). This approach highlights important potential constraints on productivity including predication of metabolic diseases even before the appearance of their clinical signs. Moreover, the MPT serves as a tool in evaluation of feeding strategies (adequacy of diet in terms of energy, protein and minerals), husbandry, health practices, herd fertility and general productivity of the dairy herd.

The analysis of some blood parameters at the proper time in proper groups of cattle may earlier identify productivity constraints and serve as a useful tool towards improved productivity (Stevenson, 2001).

Automated laboratory testing and ease of sampling have made the metabolic profile test popular and routinely implemented. In practice the metabolic profile test is considered as the most useful diagnostic aid in different herd situations, where other diagnostic techniques have failed to uncover the herd problems. Moreover the metabolic profile test is useful as a tool to support diagnostic hypothesis with farmers which will seldom lead directly to a solution. However blood metabolites analysis

can reveal some useful information when properly interpreted in Junction with animal and ration evaluation (Brent and Todd, 2003).

The metabolic profile test technique has recently been adopted in tropical and sub tropical countries. In the Sudan the technique was implemented in academic and research institutions to test blood metabolites including macro and micro minerals, glucose, total protein urea, globulin, triglycerides, cholesterol and albumen. A number of factors such as breed, stage of lactation, feed type, feeding frequency, season, sex, age and milk yield were reported to affect the concentration of these metabolites in cross bred dairy cattle (Osman, 1998 and Suleiman, 2005).

The present study was undertaken to test some hematological profiles (glucose, calcium, and phosphorus) in pure Holstein Friesian and cross bred cows as affected by breed, parity order and stage of lactation and their interactions.

CHAPTER Two
LITERATURE REVIEW

2.1 Metabolic profile test:

2.1.1 Definition

Kappel (1984) defined metabolic profile as series of specific analytical tests run in combination and used as a diagnostic aid.

The technique is based on an assessment of blood chemistry comparing results from a sample of cows in a herd with those of the normal population, so that metabolic problems in the herd can be detected (Payne et al, 1973). Also Brent and Todd (2003) concluded that metabolic profile test is most useful as a diagnostic aid in difficult herd situations where other, more direct, diagnostic techniques have failed to uncover the problem and it will seldom lead directly to the solution. However, blood metabolites analysis can reveal some useful information when properly interpreted in conjunction with animal and ration evaluation.

2.1.2 Uses of Metabolic profile test:

The aim of the metabolic profile test is to highlight abnormal changes in metabolites and which are harmful in terms of production, economic or health status. This could be achieved by identifying and investigating developing situations before they have had the chance to cause measurable changes in productivity and so preventive action can be taken to stop or minimize deleterious effects (Lee et al, 1978). This statement was insured by Whitaker et al (1999) who reported that the use of metabolic profile testing proved valuable in drawing attention to important potential constrains on productivity in dairy cows in tropical and subtropical environments.

Khanamas and Broarbent (1990) used metabolic profile test as routine analysis for prediction of metabolic disease before the appearance of their clinical signs, so that metabolic profile can act as a monitor for detecting any abnormality which may affect herd health, production and reproduction performance. This statement comply with Stevenson (2001) who stated that the analysis of some blood parameters in proper time or in a proper group of cattle may earlier identify health problems and may highlight the weak points on farm management. Hence the test can be considered as a useful tool for farmers to demonstrate and improve health and productivity of their animals. Moreover Payne (1970) proposed four applications for blood metabolic profiles which are monitoring the metabolic health of the herd, helping to diagnose metabolic problems, aiding to diagnose production diseases and selection of individuals that possess superior metabolism.

Concerning metabolic profile tests used to evaluate a feeding system in dairy farms, Kida (2003) stated that the value which deviated from reference value for metabolic profile test components could assess milk production and feeding. In addition it can be considered as practical tool for auxiliary feeding evaluation. Similarly Dobbs (2005) concluded that metabolic profile and other monitoring of the blood in peripartum period is being used as an aid to the management of dairy cattle nutrition as well as management of their fertility and productivity. Kida (2002) Postulated that the metabolic profile is a useful tool for assessing feeding management and perparturient disease of dairy cattle.

Metabolic profile test is a good and quick test for testing the adequacy of diet in term of energy, protein and mineral (Paletier et al, 1985). Measurement of blood parameters can be useful to estimate energy and protein (Bertoni et al, 1994). Similar results were reported by (Paletier et al, 1985) who stated that blood parameters are good indicator

of nitrogen and energy nutritional status of dairy cows. Payne (1973) confirmed this and reported that metabolic profile test is good and quick test for testing the adequacy of diet in terms of energy, protein and minerals.

2.2 Blood metabolites:

2.2.1 Blood glucose:

2.2.1.1 Blood glucose level:

Glucose is a major source of energy for maintaining life and for different reproductive processes. Ruminant maintains low level of blood glucose compared to monogastrics (Bergman, 1963). Blood glucose is derived principally from gluconeogenesis of amino acid propionate and lactic acid and to a lesser extent butyric acid (Hietman et al, 1973). The liver and to lesser extent the kidneys are the only endogenous source of blood glucose.

Kappel et al (1984) reported an average of plasma glucose for Holstein cow as 62 ± 8 mg/dl and they assured it as a normal range, however, Blood et al (1986) reported a mean glucose concentration of 50 mg/dl as the normal. Dew (1975) defined the normal mean glucose concentration in British dairy herd as ranging from 36.5 to 53.5 mg/dl.

Glucose and amino acids are the major fuel supply of the developing fetus in ruminants. Moreover glucose and amino acids are also needed by mammary glands for lactose and milk protein synthesis (Herdet, 2000).

2.2.1.2 Effect of nutrition on blood glucose level:

Blood glucose level is significantly affected by the type of diet and level of feeding (Bensadoun, 1962). In an experiment used to study the effect of dietary forage to grain ratios on blood constituents. The result indicated that increasing the percentage of forage in the diet decrease blood glucose concentration. Dhiman et al (1991) also found that glucose level was low at the beginning of lacation and increase as dry matter intake increased.

Anderson et al (2004) reported that the concentration of plasma glucose of Holstein dairy cows in early lactation is (3.43 mmol/L) when fed high energy diet. They also concluded that cows in early lactation will benefit from receiving high energy density diet and thereby avoid a low metabolic imbalance when mobilizing body tissue in support of milk production. This was confirmed by the findings of Dhiman et al (1991) who found a strong relationship between the energy value of fed rations and the content of glucose in cow's blood.

Feeding cows a high grain diet change the rumen fermentation pattern, resulting in greater amount of propionate production, which a long with increase flow of starch to the intestine may give rise to higher blood glucose levels than when a high forage diet is fed (Jenny and Polan, 1975). Cezary et al (2005) on the other hand provided contradicting data that the feeding system had no significant effect on the content of glucose, triglycerides and total cholesterol in the cows serum.

2.2.1.3 Effect of stage of lactation on blood glucose level:

There appeared to be an interaction between feed quality and stage of lactation in their effects on blood glucose concentration (Mc Clure, 1977). Similarly Suleiman (2005) found that the stage of lactation had significantly affected the blood level of glucose in Sudanese cross bred dairy cattle.

Blood glucose was lower for cows in early lactation (Paletier et al, 1985). A similar trend was observed by Brent and Todd (2003) who study the effect of stage of lactation in blood glucose level in Ontario dairy herds. The authors found that blood glucose level in early lactation was 2.6 - 3.5 mmol/L while the blood glucose level in mid lactation was 3.0-4.0 m mole/L. The studies of Suleiman (2005) provided evidence that blood glucose level in early lactation was lower than the level in mid lactation in Sudanese cross bred dairy cattle.

In Sudanese cross bred dairy cattle, Osman (1998) noted that plasma glucose level of dairy cows was slightly affected by the stage of lactation which tend to increase with advancing lactation. Also Spicer et al (2002) reported that plasma glucose concentration increase between first and fifth weeks of lactation (56 – 60 mg/dl), while after fifth weeks of lactation, plasma glucose concentration didn't change. Similar trends were observed by Koprowski and Tuker (1973). In contrast Dunshea et al (2000) found no significant variation in blood glucose during different stage of lactation. Similar results were claimed by Wolonczyk and Rutkowia (1986) who stated that the age and stage of lactation didn't affect blood glucose level in Black- White dairy cattle.

2.2.1.4 Effect age, breed and parity on blood glucose level:

Many factors such as breed, parity, stage of lactation, feed type, feeding frequency and season can influence some blood constituents such as calcium phosphorus and glucose (Brent and Todd, 2003). Roussel et al (1982) claimed matching finding where they concluded that serum glucose was influenced by age of Jersey cows.

Variation due to herd origin cause variation on concentration of metabolites in Jugular blood of dairy cattle (Lee et al, 1978), However, Suleiman (2005) reported that the breed had no effect on blood glucose

level in cross bred Sudanese dairy cattle of different grades. On the other hand Sahlu et al, (1993) postulated contradicting evidence that the breed and diet had no effect on glucose level.

The parity order have clear effect on blood glucose level in dairy cattle as Shin-ichi-kume et al (2003) observed that the plasma glucose of Holstein primiparous cows was higher than that of multiparous cows. That finding was supported by Kappel et al (1984) who reported that first lactation heifers had higher blood glucose level than cows in their second or later lactation. Also Dhiman et al (1991) found multiparous cows had lower blood glucose concentration than primparous cows at beginning of the lactation.

2.2.2 Blood calcium:

2.2.2.1 Blood calcium level:

Calcium is the most abundant mineral element in the animal's body; the 1% of calcium and 20% of phosphorus not present in the skeletal tissues are widely distributed in the fluid and soft tissues of the body where they serve a range of essential functions (Underwood, 1981).

About 45-50% of the total calcium in plasma exists in ionized, soluble form which must be maintained at a relatively constant value of 1-1.25 mM to elaborate systems to maintain calcium homeostasis.

In most species calcium is maintained closely to about 10 mg/dl by the regulatory action of pararthyroid, calcitonin and active metabolite of vitamin D3 (1, 25 dihyroxy cholecalciferol).

Normal plasma calcium was found to be about 9.04 ± 0.5 mg/100ml in cattle as determined by Payne et al (1970), however Jesse et al (2005) stated that blood calcium in adult cows is maintained at 8.5-

10 mg/dl, similarly there is 10 mg of calcium in each 100 ml of blood (Bredon and Dugmore , 2005).

In the study carried out to evaluate normal level of blood calcium in dairy cattle, Goff,(1999) reported that more than half of all mature dairy cows have total blood calcium concentration below 7.5 mg/dl(< 1.8 mmol/L) after calving without any evidence of clinical signs. Thus Larsen (2001) stated that the level of plasma cattle is 1.95 ±0.62 mmol/l 12 hours after calving.

2.2.2.2 Calcium tissue distribution:

Calcium is the most abundant mineral in the body, about 99% of which is stored in the skeleton as a constituent of bone and teeth and the remaining 1% is widely distributed in soft tissues and body fluids (Schuette and Linkswiler, 1984), with the largest concentration in blood plasma (Mc Dowell et al, 1993).

Plasma calcium is mainly found in three states; as free ion (60%), bound to protein (35%), or complex with organic acids such as citrate or with inorganic acids, such as phosphate (5-7%) (Arthur and Martin, 1973).

2.2.2.3 Calcium functions:

Calcium is required for the normal functioning of a wide varity of tissues and physiological processes. Calcium is needed for bone and teeth formation, blood clotting, muscle contraction and synthesis of milk which represent 12% in whole milk (Schroder, 2004). Further more it is required for nerve transmission (Horst, 1986).

Calcium is involved in activating certain enzymes, control neuromuscular excitability and maintaining normal heart beat. In addition to that it serves as second messenger regulating the action of many hormones (Underwood, 1989).

Extra cellular calcium is required for transmition of nervous tissue impulses and excitation of skeletal and cardiac muscle contraction. Recent work has demonstrated that calcium regulate the level of phosphorylation of a number of indigenous protein in the nervous system.

2.2.2.4 Calcium Requirements:

Adequate calcium and phosphorus nutrition depend only on sufficient total dietary supplies, but also on the chemical forms in which they occur in the diet and on vitamin D status of the diet or the animal. In addition to that ca: p ratio also can be important, thus for a cow producing 3.5% milk fat, requires 1.17 g/kg calcium and 1.05 g/kg phosphorus (Underwood, 1981). NRC (2001) recommended that feeding diet of 0.6% calcium is sufficient for lactating cows.

2.2.2.5 Calcium absorption:

Dietary calcium is absorbed largely from the duodenum and jejunum of most animals, which occurs both by active and passive transport (Church and Pond, 1988), however recently it was found that the small intestine appear to be the major site for calcium absorption.

The enhancement of intestinal calcium absorption and bone calcium resorptive process are under the influence of calcium regulating hormones, parathyroid hormone, which is secreted by parathyroid gland and 1.25 dihydroxy vitamin D which is produced in the kidney (Deluca, 1984). The same author mentioned that low calcium intake increase parathyroid hormone secretion and result in greater conversion of 25-dihydroxy cholecalciferol to 1, 25- (OH)2 vitamin D3 which enhance dietary calcium absorption.

Parathyroid hormone, calcitonin derived from vitamin D and it's active metabolites 1.25-(OH)2 cholecalciferal and minimal degree of gonodal hormones, as well as selected glucocorticoids, all of which affect absorption of calcium, phosphorus and magnesium from the digestive system and removal from the skeleton and their excretion through urination.

During the past decade, it has been discovered that vitamin D3 is converted into 25-hydroxycholecalciferol in the liver and this compound is converted in the kidney to1, 25- dihydroxycholcaliceferol. These metabolites particularly 1,25dihydroxycholcaliceferol rather than vitamin D itself have powerful effect on calcium metabolism, stimulation absorption of calcium from the gastrointestinal tract and resorption of calcium from bone (Sanson, 2006).

Many factors affect the efficiency of calcium absorption such as dietary calcium concentration, lactose as well as other amino acids, and oxalic acid (Church and Pond, 1988). Absorption efficiency is well known to fall with age and at least a part of this decline, perhaps most of it, is due to decrease in vitamin D stores (Robert, 1989). Similarly Horst, et al (1990) observed that intestinal calcium absorption efficiency decrease with age in the bovine as well as intestinal 1.25(OH) 2. D3 receptor number which decline with age in both rats and cows.

2.2.2.6 Calcium excretion:

Calcium is excreted primarily via feces; therefore the fecal and urinary excretion route plays little part in calcium homeostasis. Fecal out put include both an unabsorbed fraction and endogenous fraction, while urinary out put of calcium is generally considerably less than that of fecal out put in most species (Church and Pond, 1988). About half of plasma calcium, mainly ionized calcium is filtered in the kidney, but

more than 99% is reabsorbed under normal condition (Consolazio et al, 1964).

It has been established that the fecal endogenous loss of calcium is independent of the amount of calcium ingested or adsorbed, but directly proportional to the live weight of the animal (Kenneth, 1990).

2.2.2.7 Calcium deficiency:

A dietary deficiency of calcium or phosphorous or lack of vitamin D which impair their absorption and utilization, if sufficiently sever or prolonged, result in abnormalities in the bone and teeth, subnormal growth, milk yield and egg production, depressed appetite and efficiency of feed use and develop of pica or deprived appetite (Underwood ,1981). In addition nutritional calcium deficiency is associated with weak, poor dairying ability, swollen joints, lameness, weak bone and a propensity for broken bone (Plus, 1994).

The deficiency of calcium,phosphorus and magnesium in fodder leads to lack of these elements in the blood and causes various disease such as milk fever which is a non febrile metabolic disease affecting milking cows in which acute calcium deficiency causes progressive neuromuscular dysfunction with flaccid paralysis, circulatory collapse and depression of consciousness (Oetzl and Goff, 1999).

John et al (2004) studied the effect of hypocalcaemia on reproductive performance of dairy cows. The author suggested that hypocalcaemia could reduce fertility of dairy cows due to in- appetence and prolong recumbence; moreover, cow serum calcium, inorganic phosphorus and magnesium may be responsible for retention of placenta (Bari et al, 1996) and it may lead to reproductive failure (Hidiroglou, 1979). The ovarian activities are most prone to mineral imbalance and

their deficiency affect ovarian activity in a negative manner (Haq et al, 1999)

2.2.2.8 Effect of age on blood calcium level:

In a survey of 260 cows of Pabna, 4-12 years of age, plasma calcium concentration was 9.04 ±1.34 mg /100 ml. No significant differences between groups were observed (Rahman and Baqi, 1985). However Kitchenham et al (1975) postulated that the concentration of mineral in the blood of cattle vary with age of animal, stage of pregnancy, milk yield, management factor and season of the year. Furthermore, Shafer et al (1981) confirmed that cow's age affect calcium, phosphorus and glucose level in dairy cattle.

The combination of age and stage of pregnancy or lactation significantly affect variations in all constituents. Glucose and inorganic phosphate decrease with age, while calcium and copper increased (Wiener et al, 1980). In contrast increasing age was associated with slight decrease in plasma calcium (Sarkar and Roy, 2000) and a decrease in the amount of calcium absorbed (Horst et al, 1990).

Naito et al, (1990) found that the concentration of plasma 1.25 dihydroxy vitamin D increase from calving to 3 days postpartum in five Holstein Friesian cows of age 3-5 years ,examined from 5 days before and 15 days after calving. The numbers of 1.25 dihydroxy vitamin D receptors significantly decline with increase age ,so the intestinal absorption of calcium decrease accordingly, resulting in decreased plasma calcium with increasing age (Goff et al, 1989). Mahala (1997) studied the effect of age on blood calcium of dairy cattle and found that lactating cows with 7-8 years have plasma calcium concentration as 8.13 mg /100 ml, while lactating cow with age of 3 - 4 years have plasma calcium concentration as 7.91 mg/100 ml.

2.2.2.9 Effect of Breed, parity and nutrition on blood calcium level:

In a study carried out to determine the association between breed, carcass composition and status of Cu, Zn, F, Ca and Mg of mature cows representing nine breeds of cattle, it has been observed that there were no breed differences in plasma concentration of Cu and Zn, but there were differences in plasma concentration of Ca and Mg (Little et al, 1995). However Suleiman (2005) reported that herd significantly affected blood calcium level in Sudanese cross bred dairy cattle.

In cows fed anonic prepartum diet, Perdo et al, (2004) found that cows with parity > 3 has significant lower plasma concentration of calcium and higher concentration of magnesium compared with cows with one or two parities. Yuko et al (2004) reported that there were no significant differences in plasma calcium and phosphorus concentrations between primiparous and multiparous Holstein cows.

Paletier et al, (1985) demonstrated that the blood mineral concentrations are not related to the level of mineral intake of the animal. This result support the findings of Underwood (1981) who stated that calcium in serum is less responsive to dietary changes in intake of the mineral than is inorganic phosphorus. However, Vijchulata et al, (1994) who investigated the association between mineral supplementation and blood calcium level showed that mineral supplementation was shown to increase calcium concentration in blood. On the other hand, maintaining lactating cows on low calcium diet for 21 days significantly reduces plasma calcium concentration as compared to non lactating ones (Elizabeth et al, 1987). Kida (2003) found that serum calcium indicated significant positive relationship to dry matter calcium in the stage of late lactation-dry period. This means that serum calcium changes reflect calcium intake. The serum calcium levels in

dairy cows are held under homeostatic control and seldom changed under common feeding management (Payne, 1987)

Generally, blood concentrations of macro-minerals don't reflect dietary status when the homeostatic system is functioning properly (Saba et al, 1987)

2.2.2.10 Effect of stage of lactation on blood calcium level:

Ostegeard and Larsen (2005) stated that there was no interaction between total blood calcium concentration and stage of lactation. Similarly Brent and Todd (2003) found that the stage of lactation didn't affect the blood calcium level in Ontario dairy herds, these result's supported the findings of Paletier et al (1985) who postulated that serum level of calcium weren't affected by stage of lactation.

In contrast, in a study carried to determine the association between stage of lactation and blood metabolic profile, it has been observed that jugular blood calcium and phosphorus were influenced by stage of lactation for Holstein dairy cows (Wohlt et al, 1984). Plasma calcium concentration was determined in 144 cows in late pregnancy, just after calving, in second month of lactation or in the fifth months of lactation. The lowest value was found in cows that had just calved and the highest value found in late pregnancy (Ivanov et al, 1990). These results were similar to those reported by Ballantine and Herbe, (1989) who reported that lactation onset parallel with movement of calcium from the plasma into milk which causes a decrease in concentration of calcium and phosphorus in plasma. However when examining the metabolic profile in plasma of dairy cows before and after parturition, it was found that calcium increase significantly by 1.2 mg/100 ml and Ca: p ratio by 0.4 after parturition (Flores et al, 1990). Suleiman (2005) noted that blood

calcium level is higher in mid lactation (7.4 ± 1.3 mg/dl) when compared with level in early lactation 5.9 ± 0.7 mg/dl.

2.2.3 Blood phosphorous:

2.2.3.1 Blood phosphorus level:

Normal level of plasma phosphorous is 4 – 6 mg/dl for adult and some what higher, often 6 – 8 mg/dl for young animal (Underwood, 1981). This finding is in accordance with the report of Whitaker (1995) who stated that optimum range of plasma phosphorous is between 4.3-7.6 mg/100ml.

Harris et al (1993) reported that normal serum blood level of inorganic phosphorous normally range between 3-8 mg/dl in cattle. However, Goff (1989) stated that phosphorous concentration in blood plasma normally is 4-8 mg/dl for adult animals. Bredon and Dugmore, (2005) reported 15 mg phosphorus/100ml of blood in cattle.

2.2.3.2 Phosphorous tissue distribution:

About 80% of the inorganic phosphorous is found in bone and teeth; such that bone formation and maintenance are quantitavely the most important functions of inorganic phosphorus in the animal's body. The 20% of phosphorous not present in the skeletal tissue is the widely distributed in the fluids and soft tissues of the body where it serves a range of essential functions (Underwood, 1981).

The phosphorous in the skeleton is present as crystal, while that in soft tissues is present mostly in organic form (Forbes et al, 1956). In blood plasma, phosphorous exist in both inorganic and organic forms, the later as a constituent of lipids, about 10% of the inorganic phosphorous is bound to plasma proteins and 50-60% ionized. Phosphorous in red blood cells is present as inorganic phosphorous,

organic phosphorus, acid- soluble phosphorus, lipid phosphorus and ribonucleic acid phosphate, the proportion varying with age and species (Widdowson and Dickerson, 1964).

2.2.3.3 Phosphorus requirements:

Phosphorus is required by lactating cows for bone formation, maintenance, milks secretion, building muscle tissue, energy metabolism, fatty acid transport, phospholipids synthesis, amino acid metabolism and protein synthesis (Katharine and Rick, 2001).

The NRC, (2001) guidelines concluded that feeding a diet of 0.32-0.42% phosphorous is sufficient for the entire lactation and there is no added benefit to feeding phosphorus greater than 0.42% of the ration dry matter. Phosphorous requirements are high during lactation, early growth and reproduction. High producing cows need much more phosphorus than cows producing at average or low level. Dry cows that aren't producing milk need less phosphorous than lactating cows due to high content of phosphorus in milk. A cow producing 100 pounds of milk daily would require 100g of phosphorus and secret 43g of phosphorus in milk because of high level of phosphorus in milk, a continuous supply is needed in the ration in order to allow high level of milk production (Harris et al, 1993).

2.2.3.4 Phosphorous functions:

As with calcium, the most important functions of phosphorus is as a component of the skeleton. It's a component of phospholipids which is essential in lipid transport and metabolism as well as cell membrane structure, also it had an obvious role in energy metabolism as a component of adenosinmonophosphate(AMP), adonsinediphosphate (ADP) and adonisinetriphosphate (ATP) and creatinine phosphate

(Church and Pond, 1988) besides that, 0.91% of the phosphorous is present in milk (Schroder, 2004).

Phosphorous is required by ruminal micro- organisms for digestion and synthesis of microbial protein. In addition, it helps in combination with other elements to maintain osmotic and acid base balance and it plays a vital role as a host of metabolic functions, including energy utilization and transfer, phospholipids formation and therefore fatty acids transport amino acid formation and protein formation (Underwood, 1981). It is implicated in carbohydrate metabolism, forming compounds with certain metabolic products, this phosphorous compound take part in the storage and release of energy for muscular work and maintaining body temperature.

Utilization of energy from feeds, buffering of blood and other fluids as well as many enzymes require phosphorous for proper function and protein metabolism (Mc Dowell et al, 1993).

2.2.3.5 Phosphorus absorption:

The primary site for phosphorous absorption is the small intestine (Care, 1994), while very little phosphorous absorption occur in the rumen (NRC, 2001). Phosphorous absorbed at varying rates across the walls of the small intestine by active transport and passive diffusion (Church and Pond, 1988) .

The regulation of phosphorous absorption is influence by vitamin D (NRC, 1989). When vitamin D3 enter the blood stream it is converted into 1.25 dihydroxy vitamin D (1.25-(OH)2D) by the mitochondria of the liver (Horst and Reinhardt, 1983), while phosphorus is low in the blood; (1.25-(OH)2 D) synthesis is activated which causes an increase in efficiency of absorption of phosphorus in the small intestine (Mc Kay, 1995).

2.2.3.6 Phosphorous excretion:

The primary route of phosphorous excretion is fecal (Morse et al, 1992b). This finding is supported by the report of Challa et al (1989) who stated that feaces represents the major route of phosphorous excretion in ruminants.

Wu et al (2001) reported strong relationship between phosphorous intake and fecal phosphorus concentration. Similarly Weiss and Wyatt (2004) mentioned that the concentration of fecal phosphorous increases as intake of phosphorous increase.

In a study to evaluate the effect of dietary phosphorus, it was found that reducing phosphorus from 0.42% to 0.33% resulted in approximately 25% less estimated fecal phosphorous excretion (Wu et al, 2001.These findings is in line with those reported by Dave, (2003) who observed that using imported feed supplements with lower phosphorus concentration will help reduce phosphorous in manure.

2.2.3.7 Phosphorus deficiency:

Signs of phosphorus deficiency aren't easily recognized except in sever cases when fragile bone, general weakness, weight loss, emaciation, stiffiness, reduced milk production and chewing of weed rocks, bone and other object (pica) may be noticed (Williams et al, 1991 a).

The predominant effect of low dietary phosphorus are associated with diminished appetite and it's result effects. Depressed feed intake, poor growth and weight loss are common with phosphorus deficient diets. Longer term phosphorus deficiency result in impaired reproductive performance, diminished immune function, bone abnormalities and pica (Dave, 2003)

In a study carried out to determine the effect of zinc, copper and phosphorus dietary intake on delayed puberty in Sudanese heifers, it was found that phosphorus deficiency delay puberty and cause stunted growth and infertility (Ahmed et al, 2004). Legel (1970) demonstrated in a definitive experiment that phosphorus deficiency decrease total feed intake which cause lower energy supply and lower weight gain in heifers.

Gerloff and Swensen , (1996) found that phosphorus deficiency in diets of lactating cows in early lactation cause recumbent, sever lameness, bone ash, ca ,p and mg concentration were lower than published ranges for healthy ones.

2.2.3.8 Homeostasis of calcium and phosphorous:

The blood level of calcium and phosphorus are important both in relation to deficiency state and metabolic disorders such as milk fever. The plasma calcium content in most animals is normally around a total of 14-25 mg/100ml (Arthur and Martin, 1973). Although the blood contain a total of 14- 15 mg/100 ml, the most important fraction for diagnostic purposes is the inorganic phosphate which normally represents 4 - 7 mg/100m (Todd, 1976). The blood levels depend on the balance between absorption and excretion on one hand and between mobilization from and deposition in the bone on the other hand. Hormonal control plays an important part through feed back mechanism involving parathyroid gland (Charles, 1980); this can be noticed when there was decrease in calcium intake which stimulates secretion of parathyroid hormone from parathyroid gland. Parathyroid hormone enhance renal re absorption of calcium (Capen and Rosol, 1989) and promote the synthesis of 1.25-dihydroxycholecaliceferol (1.25-(OH)2D) from 25 hydroxycholecalciferol in the kidney (Allen and Sanson, 1985).

As result of stimulated 1.25-(OH)2 D and parathyroid secretion, bone calcium resorbtion and intestinal calcium absorption increase (Horst et al, 1994). The action of parathyroid hormone is counteracted by calcitonin which is secreted by thyroid cell. Calcitonin replenishes the body store of bone calcium at the time of calcium adequancy, it decrease the calcium concentration in the blood plasma by reducing the rate of bone resorption (Allen and Sanson, 1985).

Parathyroid hormone is responsible for controlling phosphorus metabolism through it's re-absorption in the skeletal system as well as in stimulation the emission of phosphate through the kidney during the status of inorganic phosphorus excess in the body fluid (Jorgensen, 1974).

2.2.3.9 Effect of nutrition on blood phosphorus level:

Mineral supplementation imposes minor changes in plasma metabolite concentration. Klimiene et al (2005) found only minor changes in phosphorous, magnesium and sodium in the serum of healthy dry and post calving cows fed on silage and mineral and vitamin supplementation compared to control cows.

Kida (2003) reported a positive relationship between serum phosphorus and dry matter intake. He also interpreted that serum inorganic phosphorus concentration increase with intake of dietary phosphorus. Furthermore Payne and Payne (1987) found that serum inorganic phosphorus concentration relatively reflects dietary phosphorous intake. The first- known response to deficiency of phosphorous is a fall in the inorganic phosphate fraction of the blood plasma and withdrawal of calcium and phosphorus from the reserves in the bone (Underwood, 1981).

Inorganic phosphorus tends to increase with high energy diet during early stage of lactation in dairy baffaloes (Bertoni et al, 1994).

2.2.3.10 Effect of age on blood phosphorus level:

Mahala (1997) found that phosphorous concentration in the blood of dairy cattle decrease with increase in age especially in young lactating cows. The same author found the phosphorus concentration in lactating cows of age 7 - 8 years was 5.74 mg/100 ml while phosphorus concentration in lactating cows aged 3 – 4 years was 6.48mg/100ml. Similarly Doornebal et al (1988) stated that blood serum calcium and phosphorus decrease with cow age from birth to the age of ten years. This result coincides with the report of Roussel et al (1982) that serum phosphorus and calcium decrease with age in Jersey cows.

Serum inorganic phosphorous was lower on the day of calving for older cows and then was over 6 mg/dl from week two through 22 month of lactation, while in younger cows serum inorganic phosphorous was always over 6 mg/dl.

2.2.3.11 Effect of stage of lactation, parity and breed on blood phosphorus level:

Aeberhard et al (2001) reported that the concentration of metabolites, minerals, hormones and enzymes activities were in normal range in both high yielding Swiss dairy cattle and the corresponding control in early and mid lactation.

Serum levels of inorganic phosphorous weren't affected by stage of lactation (Paletier et al, 1985). Similarly Brent and Todd (2003) found that stage of lactation didn't affect the blood phosphorous level in Ontario dairy herds. However, the blood phosphorous concentration in early lactation was 5.1±1.1 mg/dl, while phosphorus concentration in

mid lactation was 4±05 mg/dl (Suleiman, 2005). The study of Amir et al (2003) reflected no differences in the serum concentration of phosphorous, calcium and magnesium between Holstein Friesian and Jersey cows. In contrast Suleiman (2005) noted that breed had a significant effect on plasma phosphorus level in Sudanese cross bred-dairy cattle, the author found phosphorus concentration as 3.7± 0.5 mg/dl to 4.8 ± 0.6 mg/dl in different five crossed Sudanese breeds.

Chapter Three

Material and methods

3.1 Experimental site:

The current study was conducted in two commercial dairy farms during the period December 2005 to April 2006. The first farm is located at Shambat area on the North Khartoum with crossbred dairy cattle. The second one is located at Soba area South of Khartoum, the herd of which is composed of pure Holstein Friesian.

3.2 Experimental animal's management and housing:

Eighteen crossbred cows were selected from farm (1). The cows were kept under semi intensive system and were housed in traditional corrals build up from local material and partially shaded. The animals were fed on sorghum Bioclor (Abu 70) throughout the summer months and concentrate mix (Table 3.1) was offered only during milking time. The cows were hand- milked twice daily (morning and evening). Routine vaccination policy was adopted and weaning of calves was practice at age of 2- 3 months. The cows were subdivided as fallow:

3 cows were at their early lactation of first parity

3 cows were at their mid lactation of first parity.

6 cows were at their early lactation of fourth parity.

6 cows were at their mid lactation of fourth parity.

Table 3.1

Concentrate mixture for cross bred cows

Ingredients	Percentage (%)	Quantities (Ton)
Sesame cakes	15	2 sacks(150 kilo)
Sorghum	37.5	3 sack (375 kilo)
Wheat bran	26	6 sacks (260 kilo)
Groundnut hulls	6	2 sacks (60 kilo)
Molasses	12	120 kilo
Sodium chloride	1	10 kilo
Calcium carbonate	2.5	25 kilo

The other group of cows comprised 18 pure Holstein Friesian cows chosen from farm (2) and was managed through an intensive husbandry system with zero grazing (in door feeding). The cows were housed in modern corrals, completely shaded and build up from iron bars and galvanized zinc cover with earth bedding. The concentrate mix (Table 3.2) was offered during milking. Other management practices are similar to farm (1). The cows were further sub grouped into the following:

6 cows were at early lactation of their first parity.

6 cows were at mid lactation of their first parity.

3 cows were at early lactation of their fourth parity.

3 cows were at early lactation of their fourth parity.

3.3 Blood sampling:

Ten mls of the blood were collected from each of the 36 experimental animals according to Payne et al (1970).Collection of the blood was performed following the evening milking. The blood was then divided into two equal portions (5 mls each). The first 5 mls were transferred to capped test tube containing fluoride oxalate and centrifuged at 3000 r.p.m for 15 minutes and the collected serum was kept pending glucose determination. The remaining 5 mls were transferred to hepranized tubes and centrifuged at 3000 r.p.m for 15 minutes and the collected plasma used for immediate phosphorus and calcium determination

Table 3.2

Concentrate mixture for Holstein Friesian

Ingredients	Percentage	Quantities (Ton)
Sorghum	36	4 sack(360 kilo)
Wheat bran	16	4 sacks (160 kilo)
Ground nut cake	36	4 sacks (360 kilo)
Molasses	10	100 kilo
Sodium chloride	1	10 kilo
Calcium carbonate	1	10 kilo

3.4 Blood analysis:

3.4.1 Determination of blood glucose concentration:

Blood glucose level was determined according to Cromatest (2005) method. Glucose is oxidized to D- gluconate by glucose oxidase with formation of hydrogen peroxide. In the presence of peroxidase, a mixture of phenol and 4- aminoantipyrine (4AA) is oxidize by hydrogen peroxide, to form red qunoneimine dye proportional to the concentration of glucose in the sample according to the following equation:

$$\text{B- D glucose} + H_2o + O_2 \xrightarrow{\text{Glucose oxidase}} \text{D- Gluconate} + H_2O_2$$

$$4\text{ AA} + \text{phenol} \xrightarrow[\text{Peroxidase}]{H_2O_2} \text{Quinoneimine} + H_2o$$

Reagent:-

Mono reagent: A solution composed of:

Phosphate buffer/ PH: 7.5	100 mmol/L
Phenol	5 mmol/L
Glucose oxidase	> 10 KU/L
Peroxidase	> 2 KU/L
4- aminoantipyrine	0.5 mmol/L

Standard:

This is a solution of glucose	100 mg/dl

Procedure

1- The reagent and sample were brought to room temperature.

2- In a set of three tubes representing blank, standard and tested sample, 1.0 ml of mono reagent was added to each of the labeled tubes. 10 µL of the sample were added to the sample tube.
3- Standard solution (10µL) was added to standard tube.
4- The tubes were then mixed, let to stand for 10 minutes at room temperature.
5- The colorimeter is adjusted to zero with blank solution or distilled water.
6- Finally the absorbance of sample (A) and the standard were read at 500 ± 20 nm) against the reagent blank.

- The color is stable for two hours.

Calculation:-

$$\text{Glucose (mg/ dl)} = \frac{A\ \text{sample}}{A\ \text{standard}} \times \text{Standard Conc. (100) mg/ dl).}$$

3.4.2 Determination of phosphorus concentration:

Blood phosphorus concentration was determined according to (Spinreact, 2005) method. Inorganic phosphorus reacts with molybdic acid forming a phosphomolybdic complex. It's subsequent reduction in alkaline medium originated a blue molybdenum color. The intensity of color formed depends on concentration of inorganic phosphorus in the sample.

Reagent:-

Reagent 1 (Molypdic): R1

This is buffer solution which composed of

Molybdate- Borate 1.2/m mol/L

Reagent 2 (catalyzer): R2

A catalyst solution, which is composed of:

1, 2 phenylenediamine 2.59m mol/L

Standard:

Phosphorus aqueous primary standard 5 mg/ dl

Working Reagent (WR): mixing equal volumes of R1 and R2.

Procedure:

1- In a set of three tubes representing blank, standard and tested sample, 1.5 ml of working reagent was added to each of the labeled tubes, 50 µL of sample were added to the sample tube.
2- Also 50 µL of the standard were added to the standard tube.
3- The tubes were then mixed and incubated for 10 minutes at 37Cº or 30 minutes at room temperature.
4- The colorimeter was adjusted to zero with distilled water or blank.
5- The absorbance (A) of sample against blank was read at weave length (710 nm).

Color is stable for at least 2 hours.

Calculation: $\frac{A \text{ sample}}{A \text{ standard}} \times$ Standard concentration (5 mg/dl).

3.4.3 Determination of plasma calcium concentration:

The plasma calcium level was measured according (Spinreact, 2005) method. The measurement of calcium in the sample is based on formation of color complex between calcium and o- cresolphtalein in alkaline medium.

Ca^{++} + 0- cresolphtalien → colored complex

The intensity of the color formed is proportional to the calcium concentration in the sample.

Reagents:-

Reagent 1 (R1):

A buffer solution which is composed of:

Ethanolamine 500 mmol/L

Reagent 2 (R2)

Chromogen composed of:

0- cresolphtalien	0.62 mmol/L
8- Hidroxyquinolien	69.00 mmol/L

Standard (Calcium Cal):

Calcium aqueous primary standard 10 mg/dl

Procedure

1- In a set of three tubes representing blank, standard and tested sample, 2.1 ml of R1 was added to each of the labeled tubes.
2- One drop of R2 is added to each labeled tubes.
3- Twenty µL of the sample were added to the sample tube.
4- Twenty µL of the standard were added to the standard tube.
5- The tubes were then mixed and incubated for 5 minutes at 37Cº/ 15 minutes at 25Cº.
6- The colorimeter was adjusted to zero with distilled water or blank solution.
7- The absorbance (A) of the sample against the (A) blank was read (at wave length 570 nm).

Calculation: $\frac{(A)\ \text{Sample}}{(A)\text{Standard}} \times$ Standard concentration (10 mg/dl).

3.5 Statistical analysis:

The Split plot design was adopted as the expermintal design of the study. Statistical Package for Social Science computer (version 10.5) model for data analysis was used.

Chapter Four

Result

4.1 The overall means of serum glucose and plasma phosphorus and calcium:

The overall means ± SE of the investigated metabolites are presented in table (4.1). The serum glucose concentration was 46.96 ± 1.42 mg/ dl , while the plasma phosphorus and calcium concentration attained an overall means of 5.07± 0.20 mg/ dl and 8.17± 0.24 mg/ dl respectively.

4.2 Effect of breed on blood metabolites:

The data in table (4.2) shows the blood metabolites values for Friesian and crossbred. Serum glucose, plasma phosphorus and plasma calcium levels for Friesian cows were 47. 58± 2.01 mg/dl, 5.56 ± 0.27 mg/dl and 7.78 ± 0.33 mg/dl respectively, while the values of serum glucose, plasma phosphorus and plasma calcium for crossbred were 46.54 ± 2.01 mg/dl, 4.58 ± 0.27 mg/ dl and 8.57 ± 0.33 mg/dl, respectively. The serum glucose and plasma phosphorus levels of Friesian cows tend to be higher than those of crossbred cows while plasma calcium level was higher in crossbred than that of Friesian cows.

The breed had no significant effect on serum concentration of glucose and plasma calcium ($p \geq 0.05$). However, the breed had significantly affected plasma phosphorus concentration ($p \leq 0.05$).

4.3 Effect of stage of lactation on blood metabolites:

The effect of lactation stage on blood metabolites concentration is shown in table (4.3). The blood metabolites concentration in early lactation for serum glucose, plasma phosphorus and plasma calcium were 46.50 ± 1.95 mg/ dl, 5.22 ± 0.27 mg/dl, 8.11 ± 0.33 mg/dl respectively, while the blood metabolites concentration in mid lactation for serum glucose, plasma phosphorus and plasma calcium were 47.42 ± 1.95 mg/dl, 4.92 ± 0.27 mg/dl and 8.24 ± 0.33 mg/dl, respectively. Serum glucose and plasma calcium levels tend to be lower in early lactation when compared with those in mid lactation. However plasma phosphorus level was higher in early lactation.

There was no significant difference between serum glucose concentration, plasma phosphorus concentration and plasma calcium concentration in early and mid lactation ($p \geq 0.05$).

4.4 Effect of parity order on blood metabolites:

The result in table (4.4) summaries the changes in blood metabolites level due to parity. The level of serum glucose, plasma phosphorus and plasma calcium in the first parity were 46.63 ± 2.01 mg/dl, 4.88 ± 0.27 mg/dl and 8.40 ± 0.33 mg/ dl, respectively. The level of serum glucose, plasma phosphorus and plasma calcium in fourth parity were 47.29 ± 2.01 mg/dl, 5.26 ± 0.27 mg/dl and 7.95 ± 0.33 mg/dl, respectively. Both Serum glucose and plasma phosphorus secured higher values during fourth parity compared to first parity. Plasma calcium showed different pattern and secured higher concentration in first parity compared to fourth parity.

Non significant differences could be detected in serum glucose level, like wise this was found to be true for plasma phosphorus and plasma calcium levels.

4.5 The impact of breed and parity interactions on blood metabolites:

The result in table (4.5) indicates the impact of breed and parity interactions on blood metabolites. The serum glucose level of Friesian at first parity and fourth parity were 48.17 ± 2.32 mg/dl and 47.00 ± 3.28 mg/dl respectively, while the serum glucose level of crossbred cows at first and fourth parity were 45.10 ± 3.28 mg/dl and 47.58 ± 2.32 mg/dl, respectively. In crossbred cows, the concentration of serum glucose increases with increasing parity, while in Holstein Friesian cows it decreases with increasing parity.

Plasma phosphorus levels for Friesian at first and fourth parity were 5.75 ± 0.32 mg/dl and 5.37 ± 0.45 mg/dl respectively while the plasma phosphorus level for cross bred at first and fourth parity were 4.00 ± 0.45 mg/dl and 5.16 ± 0.32 mg/dl, respectively. Plasma phosphorus concentration in crossbred increases with increasing parity however, it decrease with increasing parity in Holstein Friesian

Plasma calcium levels for Friesian cattle at first and fourth parity were 7.89 ± 0.39 mg/dl and 7.67 ± 0.55 mg/ dl, respectively, while plasma calcium level for cross bred at first and fourth parity were 8.90 ± 0.55 mg/dl and 8.23 ± 0.39 mg/ dl, respectively. Plasma calcium concentrations tend to decrease with increasing parity in both breed.

There were non significant differences due to breed and parity interaction on serum glucose and plasma calcium levels. However,

plasma phosphorus level was significantly affected by the interaction between breed and parity ($p \leq 0.05$).

4.6 The impact of breed and stage of lactation interactions on blood metabolites:

The result in table (4.6) indicates the impact of breed and stage of lactation interaction on blood metabolites. Serum glucose levels for Friesian at early and mid lactation were 46.67 ± 2.80 mg/dl and 48.50 ± 2.80 mg/dl, respectively. Serum glucose level for cross bred in early and mid lactation was 46.34 ± 2.80 mg/dl in both lactation stages. The result suggests that serum glucose tends to increase with increasing parity in the Holstein Friesian breed while such trend was not observed in the cross bred cows. The level of serum glucose maintained the same value at both investigated lactation stages.

Plasma phosphorus level for Friesian in early and mid lactation were 6.03 ± 0.38 mg/dl and 5.09 ± 0.38 mg/dl, respectively, while plasma phosphorus level for cross bred in early and mid lactation were 4.4 ± 0.38 mg/dl and 4.75 ± 0.38 mg/dl, respectively. In cross bred, the plasma phosphorus level increase with progress of lactation stage while in Holstein Friesian plasma phosphorus level decrease with increasing lactation stage. The interaction between breed and stage of lactation was significantly affecting the plasma phosphorus level.

Plasma calcium level for Friesian at early and mid lactation were 7.46 ± 0.47 mg/dl and 8.10 ± 0.47 mg/dl, respectively, while the plasma calcium level for cross bred at early and mid lactation were 8.76 ± 0.47 mg/dl and 8.37 ± 0.47 mg /dl, respectively. Plasma calcium level tends to increase with increasing lactation stage in Holstein Friesian cows and vise versa for crossed bred cows.

There were no significant differences observed between values of serum glucose, and plasma calcium as a result of the breed and lactation stage interactions.

4.7 The impact of parity and stage of lactation interactions on blood metabolites:

Table (4.7) shows the impact of parity and stage of lactation interaction on blood metabolites. The serum glucose level in first parity at early and mid lactations were 47.27 ± 2.80 mg/dl and 46.00 ± 2.80 mg/dl respectively, while serum glucose level in fourth parity at early and mid lactation were 45.74 ± 2.80 mg/dl and 48.84 ± 2.80 mg/dl respectively. Serum glucose concentration in early lactation stage of first parity seems to be slightly higher than that in mid lactation (47.27 ± 2.80 vs 46.00 ± 2.80 mg/ dl) while in the fourth parity the pattern was reversed , whereby the serum glucose was higher in mid lactation stage compared to early lactation stage(48.84 ± 2.80 vs 45.74 ± 2.80 mg/dl).

Plasma phosphorus levels in first parity at early and mid lactation were 4.95 ± 0.38 mg/dl and 4.80 ± 0.38 mg/dl, respectively. Plasma phosphorus level in fourth parity at early and mid lactation were 5.50 ± 0.38 mg/dl and 5.03± 0.38 mg/dl, respectively. Plasma phosphorus levels in early lactation stage of first parity seem to be slightly higher than that in mid lactation (4.95 ± 0.38 vs 4.80 ± 0.38 mg/dl). However, plasma phosphorus in fourth parity in early lactation stage seemed to be higher compared to that in mid lactation stage (5.50 ± 0.38 vs 5.03± 0.38 mg/dl).

Plasma calcium levels in first parity at early and mid lactation were 8.36 ± 0.47 mg/dl and 8.44 ± 0.47 mg/dl, respectively, while plasma calcium levels in fourth parity at early and mid lactation were

7.86± 0.47 mg/dl and 8.04 ± 0.47 mg/ dl, respectively. Plasma calcium levels in the first parity at early lactation stage tend to be slightly lower than that in mid lactation stage (8.36 ± 0.47 vs 8.44 ± 0.47) mg/dl. However in the fourth parity the pattern was oposite, whereby plasma calcium level was higher in the mid lactation stage compared to early lactation stage (8.04 ± 0.47 vs 7.86± 0.47 mg/ dl)

Serum glucose levels, plasma phosphorus levels and plasma calcium levels weren't significantly affected by the impact of parity and lactation stage interactions.

Table (4.1)

The overall means of glucose, phosphorus and calcium levels (mg/dl) in the two dairy breeds

Blood metabolites	**Overall concentration means (mg/dl)**
Glucose	46.96 ± 1.42
Phosphorus	5.07 ± 0.20
Calcium	8.17 ± 0.24

Table (4.2)

Effect of breed on blood metabolites levels

Blood Metabolites	**Friesian**	**Cross bred**	**F value**	**Sig**
Glucose (mg/dl)	47.58 ± 2.01	46.54 ± 2.01	0.66	NS
Phosphorus (mg/dl)	5.56 ± 0.27	4.58 ± 0.27	0.02	*
Calcium (mg/dl)	7.78 ± 0.33	8.57 ± 0.33	0.11	NS

NS: not significant ($p > 0.05$).

*: mean significant ($p<0.05$).

Table (4.3)

Effect of stage of lactation on blood metabolites levels

Blood metabolites	**Early lactation stage**	**mid lactation stage**	**F value**	**Sig**
Glucose (mg/dl)	46.50 ± 1.95	47.42 ± 1.95	0.74	NS
Phosphorus (mg/dl)	5.22 ± 0.27	4.92 ± 0.27	0.41	NS
Calcium (mg/dl)	8.11 ± 0.33	8.24 ± 0.33	0.78	NS

NS: not significant different ($p > 0.05$).

Table (4.4)

Effect of parity on blood metabolites levels

Blood metabolites	**First parity**	**Fourth parity**	**F value**	**Sig**
Glucose (mg/dl)	46.63 ± 2.01	47.29 ± 2.01	0.82	NS
Phosphorus (mg/dl)	4.88 ± 0.27	5.26 ± 0.27	0.34	NS
Calcium (mg/dl)	8.40 ± 0.33	7.95 ± 0.33	0.35	NS

NS: not significant ($p > 0.05$).

Table (4.5)

The impact of breed and parity interactions on blood metabolites levels

Blood metabolites	**Breed**	**Parity**	**Mean ± S.E (mg/dl)**	**F value**	**Sig**
Glucose	Friesian	First parity	48.17 ± 2.32	0.53	NS
		Fourth parity	47.00 ± 3.28		
	Cross bred	First parity	45.10 ± 3.28		
		Fourth parity	47.58 ± 2.32		
Phosphorus	Friesian	First parity	5.75 ± 0.32	0.05	*
		Fourth parity	5.37 ± 0.45		
	Cross bred	First parity	4.00 ± 0.45		
		Fourth parity	5.16 ± 0.32		
Calcium	Friesian	First parity	7.89 ± 0.39	0.64	NS
		Fourth parity	7.67 ± 0.55		
	Cross bred	First parity	8.90 ± 0.55		
		Fourth parity	8.23 ± 0.39		

NS: not significant different ($p > 0.05$).

*: significant ($p < 0.05$)

Table (4.6)

The impact of breed and stage of lactation interactions on blood metabolites levels

Blood metabolites	**Breed**	**Stage of lactation**	**Mean ± S.E (mg/dl)**	**F value**	**Sig**
Glucose	Friesian	Early	46.67 ± 2.80	0.75	NS
		Mid	48.50 ± 2.80		
	Cross bred	Early	46.34 ± 2.80		
		Mid	46.34 ± 2.80		
Phosphorus	Friesian	Early	6.03 ± 0.38	0.11	NS
		Mid	5.09 ± 0.38		
	Cross bred	Early	4.41 ± 0.38		
		Mid	4.75 ± 0.38		
Calcium	Friesian	Early	7.46 ± 0.47	0.28	NS
		Mid	8.10 ± 0.47		
	Calcium	Early	8.76 ± 0.47		
		Mid	8.37 ± 0.47		

NS: Not significant ($p > 0.05$).

Table (4.7)

The impact of parity and lactation stage interactions on blood metabolites levels

Blood metabolites	**Parity**	**Lactation stage**	**Mean ± S.E (mg/dl)**	**F value**	**Sig**
Glucose	First	Early	47.27 ± 2.80	0.45	NS
		Mid	46.00 ± 2.80		
	Fourth	Early	45.74 ± 2.80		
		Mid	48.84 ± 2.80		
Phosphorus	First	Early	4.95 ± 0.38	0.68	NS
		Mid	4.80 ± 0.38		
	Fourth	Early	5.50 ± 0.38		
		Mid	5.03 ± 0.38		
Calcium	First	Early	8.36 ± 0.47	0.92	NS
		Mid	8.44 ± 0.47		
	Fourth	Early	7.86 ± 0.47		
		Mid	8.04 ± 0.47		

NS: Not significant ($p > 0.05$).

CHAPTER Five
DISCUSSION

5.1 Metabolic profile:

In this study, selected metabolites included glucose; phosphorus and calcium were investigated in two dairy breeds at two parities and two different stages of lactation. The overall mean blood glucose level reported in this study in the two dairy breeds was 46.96 ± 1.42 mg/dl, Osman (1998) had studied glucose profile for cross bred dairy cows in Sudan and reported a higher value 53.9- 64.5 mg/dl) than the present result. Kappel et al (1984) reported average plasma glucose for Holstein cow as 62.00 ± 8 mg/dl. The lower values reported in this study for the two breeds may be attributed to the nutritional and managerial practices in the different studies. Moreover Kapple et al (1984) study was in the temperate region or Friesian homeland where environmental conditions favor the full dairying potential of the breed. Glucose is very critical for milk production and hence Friesian cattle are expected to have higher blood glucose level than their counterparts in tropical environment.

The overall mean of blood phosphorus level reported in this study was(5.07 ± 0.2 mg/dl) is very close to earlier reports of Whitaker et al (1995) who mentioned that optimum range of plasma phosphorus is between 4.3-7.6 mg/ 100 ml.

The findings of the present investigation regarding plasma calcium concentration (8.17 ± 0.24) mg/dl is comparable with the findings of Goff et al, (2005) who stated that blood calcium in adult cows is maintained at about 8.5-10 mg/dl.

The findings of this study and those of others indicated that the concentration of blood metabolites depend on many factors, which

include stage of lactation (Lee et al, 1978), milk yield (Payne et al, 1974), season of year(Lee et al, 1978), dietary system(Treacher et al 1976) and breed(Shafer et al, 1981).

5.2 Effect of breed on blood metabolites level:

Serum glucose concentration in the two studied breeds didn't maintain significant differences despite the higher level in Holstein Friesian. This result is in agreement with the work of Shalu et al, (1993) who stated that the breed and diet had no effect on glucose level.

The present results however reflected significant differences in plasma phosphorous levels in the two studied breeds. The plasma phosphorus level for Holstein Friesian was 5.56 ± 0.27 mg/ dl which is significantly ($p< 0.05$) higher than that of 4.58 ± 0.27 mg/dl for crossbred. This could be attributed to different feeding system adopted in the two farms. There is a positive relationship of serum phosphorus to dry matter phosphorous intake as claimed by Kida, (2003). Similarly Payne and Payne, (1987) found that serum inorganic phosphorous concentration relatively reflect dietary phosphorous intake.

Plasma calcium levels in the two breeds didn't show significant differences. This results contradicted the finding of little et al,(1995) who observed that there was no breed differences in plasma concentration of Cu and Zn, but there were differences in plasma concentration of Ca and Mg. The controversy between these results and the present study may be attributed to factors including management system and environment. Whitaker et al, (2005) stated that it's known from literature and practice that environmental and nutrition conditions in cows have great influence on diagnostic picture of the blood. However this result was in line with the findings of Lee et al, (1978) who postulated that variation due to herd origin causes variation on concentration of metabolites in Jugular blood of dairy cattle.

5.3 Effect of stage of lactation on blood metabolites:

Due to the high demands of energy for the process of lactation and maintenance, glucose has been shown to be depressed in several studies in the early lactation period.

In the present experiment serum glucose levels weren't significantly affected by stage of lactation which are very close to the finding of Dunshea et al,(2000) who reported that no significant variation in blood glucose during different stages of lactation, however glucose level during early lactation was less than that during mid lactation. Although there was no significant effect of stage of lactation on serum glucose, the serum glucose level in mid lactation tends to be higher than that in early lactation. The study of Suleiman, (2005) has provided evidence that blood glucose level in mid lactation was higher than early lactation, which is in line with the result of this study.

The plasma phosphorous levels in early and mid lactation weren't significantly affected by stage of lactation. This result is matchable with the report of Paletier et al, (1985) who claimed that serum level of inorganic phosphorous weren't affected by stage of lactation. Suleiman, (2005) however reported a contradicting evidence that blood phosphorous levels were significantly affected by the stage of lactation. The discrepancy between the results of this study and that of Suleiman, (2005) may be attributed to a variety of factors. The most important of which is the different nutritional management in the two studies. In Suleiman study, (2005) liberal amounts of grain were included in the diet and which may have affected the phosphorus plasma level due to phytic acid available in the grain.

The values of plasma calcium in early and mid lactation were 8.11±0.33 mg/dl and 8.24±0.33 mg/dl respectively. This result indicated

non significant differences due to stage of lactation, which agree with Ostegeard and Larsen, (2005) who stated that there was no interaction between total blood calcium concentration and stage of lactation. However Wholt et al, (1984) observed that Jugular blood calcium and phosphorous were significantly influenced by stage of lactation in Holstein Friesian dairy cows. These differences may be attributed to the assumingly hypothesis that in the latter study vitamin D fortified diets were used which play an important role in maintaining plasma calcium and phosphorus.

5.4 Effect of parity on blood metabolites level:

Serum glucose levels during first and fourth parities in this study didn't show significant differences. This result isn't in line with that of Shin-ich-kume et al, (2003) who observed that the plasma glucose of Holstein primiparous cows was higher than that of multiparous cows. These differences may be due to feeding during dry period. In a study conducted to investigate the effect of different feeding strategies of multiparous and primiparous high yielding dairy cows, it was concluded that dry off feeding period markedly affected the metabolites in blood and rumen and that the cows offered only straw during the dry-off were most affected (Odensten et al, 2005).

Plasma phosphorous levels in first and fourth parities were 4.88 ± 0.27 mg/dl and 5.26 ± 0.27 mg/dl respectively, while plasma calcium levels in first and fourth parties were 8.40 ± 0.33mg/dl and 7.95± 0.33 mg/dl respectively. Plasma phosphorus and calcium levels weren't significantly affected by parity, this result complies with the work of Yuko et al, (2004) who claimed non significant differences in plasma calcium and phosphorous concentration between primiparous and multiparous Holstein cows.

5.5 The impact of breed and parity interactions on blood metabolites:

Breed and parity interactions didn't exert significant effects on blood glucose level in the two studied groups. Serum glucose level of Holstein Friesian in first parity tend to be higher than that of cross bred which may be attributed to genetic variations in addition to the different dairying potentialities in the two breeds. Since glucose is a limiting factor in milk production thus high yielders should maintain higher blood glucose levels. While serum glucose level for crossbred in fourth parity tend to be slightly higher than that for Holstein Friesian.

The effect of breed and parity interaction on plasma phosphorus hadn't been reviewed. In this study plasma phosphorus tend to be affected significantly by the interaction of breed and parity. Plasma phosphorus levels of Holstein Friesian in both parites tend to be higher than that of cross bred which may be attributed to genetic variation between the two breeds.

The present study shows that, there was no significant effect of breed and parity interaction on plasma calcium. The level of plasma calcium had never been previously determined in Friesian and Sudanese cross breed dairy cow under Sudanese environment in that specific physiological condition. In first and fourth parities, plasma calcium level for crossbred was higher than that for Holstein Friesian in both parities. This result indicated that crossbred dairy cattle maintain high level of blood calcium through parity progress when compared to pure Holstein Friesian kept under Sudan environmental condition, this may be due to fact that Holstein Friesian release more of plasma calcium in milk and since they are high yielder much plasma calcium is withdraw in milk , resulting in decreased plasma calcium.

5.6 The impact of breed and stage of lactation interactions on blood metabolites:

Although there was no significant effect of breed and lactation stage interaction on serum glucose, plasma phosphorus and calcium, glucose level in early lactation tend to be lower than that in mid lactation for Frisian cows. This may imply the high demand of glucose for milk production at the beginning of the lactation cycle, beside that early lactation is associated with peak of milk yield and the voluntary dry matter intake during early lactation is low while energy demand for milk production is accelerating so the cow goes into negative energy balance. In crossbred cows serum glucose level was similar in the two stages of lactation.

This study has demonstrated that breed and lactation stage interaction didn't significantly affect the level of plasma phosphorus and plasma calcium, this may be due to similarity in seasoning sampling on which the blood samples were taken. Seasonal and physiological variations have to be taken into consideration for the correct interpretation of serum chemistry and elements status in cattle (Yokus and Cakir, 2006). Plasma phosphorus levels for Holstein Friesian was higher in the two stages of lactation when compared to that for crossbred in both stage of lactation. This difference may be due to percentage of phytic acid which was available in the grain fed to Holstein Friesian group. While plasma calcium levels for crossbred in the two stages of lactation were higher than that for Holstein Friesian which may be attributed to different husbandry practices.

5.7 The impact of parity and lactation stage Interactions on blood metabolites:

The finding of this research indicates that the interactions of parity and lactation stage hadn't significantly affected serum glucose, plasma phosphorus and plasma calcium. The level of these metabolites hadn't been ever measured under the condition of the present study. Generally, parity had a significant effect on blood glucose level in Holstein Friesian (Shin-ich- kume et al, 2003) while the lactation stage effect on blood glucose is inconsistent, Dunshea et al,(2000) reported that there is non significant variation in blood glucose during different stages of lactation however Mc Clure, (1977) postulated that there appeared to be an interaction between feed quality and stage of lactation in their effects on blood glucose concentration.

Plasma glucose level in first parity of early lactation stage seems to be higher than that in fourth parity; this can be due to age factor. Roussel, (1982) claimed that serum glucose was influenced by age of Jersey cows. While plasma glucose level in first parity of mid lactation stage seems to be lower than that of fourth parity. These results may be attributed to high milking yield during mid lactation stage (lactation curve reach the peak point) which is accompanied by increase appetite to feed.

In the fourth parity, plasma phosphorus levels seem to be higher in both stages of lactation when compared with that in first parity.

Plasma calcium levels in first parity at the two lactation stages tend to be higher than that in the fourth parity.

As previously reported in a study carried out to determine the effect of parity on blood metabolites such as total protein, albumin, urea, non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHB),

cholesterol, aspartate aminotransferase (AST), calcium, phosphorus and magnesium in Holstein Friesian in two months before parturition and three months after parturition, it was found that Primiparous cows showed a more unbalanced metabolic profile than multiparous cows in the two periods (D.Cavestany et al, 2005). Plasma phosphorus and plasma calcium levels in this study were not affected significantly by the interaction of parity and lactation stage. Differences among studies could be due to sample frequencies and different production systems.

Conclusion

The present study was carried out to investigate some blood metabolite (glucose, phosphorus and calcium) in pure Holstein Friesian and Sudanese cross bred cows as affected by breed, parity order and stage of lactation and their interactions with the aim to assess the performance of Pure Holstein Friesian cows compared to that of cross bred dairy cattle under Sudan environmental conditions.

It can be concluded that, parity and stage of lactation didn't significantly affect the level of investigated metabolites, Holstein Friesian have higher serum glucose and plasma calcium levels when compared to that of cross bred , while cross bred have higher plasma calcium level than of Holstein Friesian cows. The result of the study also pointed that, mid lactations are associated with higher serum glucose and plasma calcium levels when compared to early lactations however, plasma phosphorus seem to be higher in early lactation rather than mid lactation.

Serum glucose and phosphorus levels tend to increase with increasing parity whereas plasma calcium levels decrease with increasing parity.

Other valid conclusions that can be drawn include the interaction of breed x stage of lactation and interaction of parity x lactation stage both of which significantly affected all investigated blood metabolites while the interaction between breed and parity affected only plasma phosphorus level whereas, serum glucose level and plasma calcium weren't affected.

I recommended on the importance and necessity of metabolic profile test applying for dairy cattle in Sudan to assess the general performance.

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