ASSESSMENT OF MINERAL STATUS DURING PREGNANCY IN CROSSBRED CATTLE

By
SHIBU K. JACOB

THESIS

Submitted in partial fulfilment of the requirement for the degree of

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University

Department of Physiology
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY, THRISSUR - 680651
KERALA, INDIA

2000

DECLARATION

I hereby declare that the thesis, entitled **"ASSESSMENT OF MINERAL STATUS DURING PREGNANCY IN CROSSBRED CATTLE"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Mannuthy
4-12-2000

Shibu K. Jacob

CERTIFICATE

Certified that the thesis, entitled **"ASSESSMENT OF MINERAL STATUS DURING PREGNANCY IN CROSSBRED CATTLE"** is a record of research work done independently by Dr. Shibu K. Jacob under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

4-12-2000

Dr. P.T. Philomina
(Chairperson, Advisory Committee)
Associate Professor and Head
Department of Physiology
College of Veterinary and
Animal Sciences, Mannuthy

Mannuthy
4-12-2000

ACKNOWLEDGEMENT

I wish to place on record my deep sense of gratitude to my major advisor, Dr. P.T. Philomina, Associate Professor and Head, Department of Physiology, for her personal attention, inspiring guidance, incessant encouragement and valuable advice given throughout this work.

I am deeply indebted to Dr. V. Ramnath, Assistant Professor, Department of Physiology, member of advisory committee for his wholehearted cooperation, personal attention and timely suggestions rendered during the course of this work.

I am grateful to the other members of the advisory committee Dr. Sisilamma George, Associate Professor and Head, Department of Biochemistry and Dr. K.V. Raghunandhanan, Associate Professor, Department of Animal Genetics and Breeding for their keen interest in the study and constructive criticism.

I am extremely grateful to the former members of my advisory committee, Dr. K.P. Surendranathan, Retired Professor and Head, Department of Physiology, Dr. K.P. Sreekumar, Associate Professor, Department of Physiology and Dr. K.M. Syam Mohan, Assistant Professor, Department of Animal Nutrition, not only for the guidance during the initial stages of this research work but also for the inspiration and encouragement rendered for the conduct of the work.

I am much obliged to Dr. K. Karthiayini, Assistant Professor, Department of Physiology for her profound encouragement and timely help during the study period.

I am grateful to Dr. S. Sulochana, Dean, College of Veterinary and Animal Sciences, Mannuthy and Kerala Agricultural University for the facilities provided for this research work.

I would like to express my sincere thanks to Dr. P. Sureshkumar, Assistant Professor, Radio-Tracer Laboratory, Kerala Agricultural University and Mr. V. Rajendra Prasad, Instrumentation Engineer, Department of Agricultural Chemistry and Soil Science for the timely assistance provided during the research work.

I would like to place on record my heartfelt thanks to the staff of Department of Physiology, Statistics, Nutrition and the staff of Kerala Agricultural University – Cattle Breeding farm, Thumburmuzhi for their cooperation rendered during the period of research work.

With great pleasure I acknowledge my colleagues and friends Dr. P. Vinu David, Dr. P. Ravindran, Dr. K.C. Bipin, Dr. Arun Shaju, Dr. J.B. Rajesh, Dr. Suresh N. Nair, Dr. Nigil Mathew and Dr. Srinivas Reddy for their support and help throughout the period of study.

No words can ever express my deep sense of gratitude to my beloved parents and brother for their love, prayers and moral support in successfully completing this task.

Above all, I bow my head before God Almighty for the blessings showered on me.

SHIBU K. JACOB

Dedicated to my
Loving Parents

CONTENTS

LIST OF TABLES

LIST OF FIGURES

Introduction

1. INTRODUCTION

The physiological activities in an animal's body as growth, pregnancy, parturition, lactation etc. require a proper synergism between the anabolic and catabolic reactions. Minerals are having important role in the body for maintaining various metabolic processes. Minerals influence reproductive performance in ruminants either singly or in combination with other elements as reported by Hidiroglou (1979). Electrolytes have multifaceted functions in an animal's body including maintenance of plasma osmolarity and volume, acid-base balance, nerve impulse propagation and cofactor for various enzymes.

According to the nutritional requirements of minerals in the body, they are classified as macro and microelements. Accordingly calcium, phosphorus, magnesium, sodium, potassium, sulphur and chlorine are considered as the macroelements and iron, copper, cobalt, manganese, zinc, iodine, selenium, molybdenum etc. are considered as micro elements. Each one of these minerals have its own function in the body. Mineral requirement depends upon age, level of production, chemical form of elements, interrelationship with other nutrients, mineral intake, breed and animal adaptation as recorded by McDowell and Conrad (1990). To promote efficient and profitable livestock production in tropical countries like India, adequate dietary supplementation of essential macro and microminerals are necessary.

Alterations in the haematological, enzymatic and biochemical indices occur in the body in and around parturition and during peak lactation. Thus high producing dairy cattle are always on the verge of risk due to the high turnover of fluids, minerals and organic matters in the body during pre and postpartum periods thereby, disturbing the homeostatic mechanism which eventually lead to an abnormal clinical situation as reported by Blood and Radostits (1989).

Meagre studies had been carried out in the crossbred cattle to evaluate the fluctuations in the mineral content of blood during pregnancy and lactation which are considered to be periods of physiological stress for the animal. No scientific information is available regarding the status of major elements like calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na), potassium (K) and minor elements like iron (Fe), copper (Cu), zinc (Zn) as well as haematological parameters such as erythrocyte count, haemoglobin (Hb) content and volume of packed red blood cells (VPRC) in the crossbred cattle of Kerala state especially during pregnancy and early lactation periods.

Monitoring of certain blood minerals may help in the maintenance and improvement of productive health of farm animals through effective management and thus may aid in timely correction of such deficiencies in animals exposed to various stressors like gestation and lactation. Similarly the knowledge about haematological indices is essential to establish the normal and variations caused by infectious and non-infectious agents. The values of above mentioned parameters could also serve as a tool in the hands of nutritionists to formulate a feed to improve the biological/nutritive value of feeds given especially during the periods of gestation and lactation. Hence this study was assigned to elucidate the variations in the serum mineral profile (Ca, P, Mg, Na, K, Fe, Cu, Zn), haematological values (erythrocyte count, Hb content, VPRC) and erythrocyte indices of crossbred heifers before pregnancy, during different periods of pregnancy and early lactation.

Review of Literature

2. REVIEW OF LITERATURE

Minerals like calcium, phosphorus, magnesium, sodium, potassium, iron, copper and zinc play basic key roles in the maintenance of various metabolic processes of the body either in elementary form or incorporated into specific compounds. They have multifarious functions especially during pregnancy and lactation. Hidiroglou (1979) explained that minerals regulate reproductive performance in ruminants either singly or in combination with other elements. McDowell and Conrad (1990) were of opinion that mineral requirement of an animal depended upon age, level of production, chemical form of elements, interrelationship with other nutrients, mineral intake, breed and animal adaptation. Blood and Radostits (1989) found that high producing dairy cattle are always on the verge of risk of facing deficiencies due to the high turnover of fluids, minerals and organic matters in the body during pre and post partum periods thereby disturbing the homeostatic mechanism which eventually lead to an abnormal clinical situation. Information regarding these minerals during gestation in the crossbred cattle of Kerala is scanty. The available literature with respect to the mineral status and the haematological parameters namely erythrocyte count, haemoglobin content, volume of packed red cells and erythrocyte indices during pregnancy and lactation are reviewed in the chapter.

2.1 Mineral profile in the serum:

2.1.1 Calcium, phosphorus and magnesium:

Early in 1957, Hackett *et al.* reported that estrogens can elevate the phosphorus concentration of blood. Patel *et al.* (1966) justified that the higher concentration of serum inorganic phosphorus during the fifth month of pregnancy may be due to its requirement for fetal growth. Symonds *et al.* (1966) opined that there was greater demand of calcium for the development of fetal skeleton during the advanced pregnancy period. Harper (1969) observed that levels of serum calcium and phosphorus were inversely correlated. Bar *et al.* (1970) reported that serum concentration of calcium and magnesium in cattle during pregnancy and lactation remained relatively constant while, total phosphorus decreased during the advanced stages of pregnancy. However, in Haryana cows, Murtuza *et al.* (1979) recorded a higher concentration of serum calcium and phosphorus in the late pregnancy than in early lactation with a higher magnesium concentration in early lactation than in late pregnancy. Whereas, in Holstein cows, McAdam and O'Dell (1982) observed that calcium and phosphorus concentration were depressed just prior to calving while magnesium concentration was elevated at calving.

Tainturier *et al.* (1984) observed that serum inorganic phosphorus remained unchanged during pregnancy and early lactation with slight, irregular and transient variation for calcium. Sahukar *et al.* (1984) reported that the levels of serum calcium, inorganic phosphorus and magnesium in crossbred cows increased from first month of pregnancy and attained maximum value at seventh month for calcium and phosphorus and at parturition for magnesium which might be due to the fact that calcium might be utilised for fetal skeletal growth whereas, phosphorus and magnesium for the enhanced carbohydrate metabolism. They also found that the values for calcium and phosphorus gradually declined from seventh month till ninth month due to an increase in estrogen in the system and a highly significant fall ($P<0.01$) after parturition due to the sudden drainage of calcium in colostrum.

Baranow-Baranowski *et al.* (1988) also confirmed that there was a rapid decrease in the concentration of serum calcium after parturition and a slight increase in the serum magnesium level at parturition. However, the serum magnesium level returned to normal after 6-12 hours of parturition. Hang-Poung (1989) opined that those Holstein cows with a service period of less than 60 days showed higher concentrations of calcium and magnesium in comparison to non-pregnant cows. Sen *et al.* (1989) found that in dairy cows, concentration of serum calcium was significantly lower upto 40 days postpartum when compared to prepartum levels. Concentration of magnesium was found significantly lowered only at about 30-40 days of lactation without any variation in the value immediately after parturition when compared with that of prepartum cows. Ivanov *et al.* (1990) reported a lowest value of serum calcium level of 2 mmol/litre in just calved cows and a highest value of 3.42 mmol/litre in late pregnant cows. However, Dolezel *et al.* (1991) opined that in pluriparous cows the average serum calcium and phosphorus values increased until 20 days of lactation while magnesium content increased from 25th day of lactation.

Bahga and Singh (1992) observed that magnesium level decreased significantly ($P<0.05$) from day three pre-partum upto the beginning of parturition followed by an elevation to prepartum values from day four postpartum onwards. Sangha *et al.* (1993) reported that high serum calcium level observed during ninth month of pregnancy may be because of the role of calcium in sensitizing the tubular genitalia for the action of hormones involved in parturition. Riond *et al.* (1995) concluded that in Brown Swiss and Red Holstein crossbreds, a transient increase in the magnesium concentration associated with a transient decrease in the serum concentration of calcium and inorganic phosphorus occurred during periparturient period. Nazifi and Sami (1997) observed that serum concentration of calcium and inorganic phosphorus got significantly lowered ($P<0.05$) at parturition when compared to their values at two months before and two months after parturition and a higher magnesium concentration at parturition. Bigras-Poulin and Tremblay (1998) indicated that in the dairy herds serum calcium and phosphorus values were lower on the first day postpartum than a week later whereas serum magnesium concentration decreased from the first to seventh day.

Deshpande *et al.* (1998) observed that in crossbred cows serum magnesium level gradually increased during first six months of pregnancy till the day of parturition, but it showed a decreasing trend during postpartum period due to lactational demand. They also reported that serum calcium exhibited irregular and transient changes upto 224-225 days of gestation in crossbred cows, but the level decreased from 269-270 days of gestation till the day of calving. A marked decline in phosphorus values during the late pregnancy and on the day of parturition and a significant elevation in calcium level from the day of parturition to first three weeks postpartum were also observed. Rajora and Pachauri (1998) noticed that serum level of calcium was significantly lower in lactating cows when compared to non-lactating cows and in mid pregnancy than in non pregnant cows in early lactation. The pregnant non lactating cows showed significantly higher serum concentration of inorganic phosphorus and magnesium in late gestation than the lactating cows in early gestation. Calcium: phosphorus as well as calcium: magnesium ratio showed a decreasing trend with the advancement of gestation which means that calcium levels decreased and phosphorus as well as magnesium level increased with gestation. Sarmah *et al.* (1999) observed that serum magnesium level in pregnant and lactating Jersey crossbreds increased significantly ($P<0.05$) which might be due to an increased bone resorption as well as increased absorption from gastro-intestinal tract.

Pathak and Janakiraman (1987) recorded that pregnant Surti buffaloes showed lower values for serum calcium and magnesium and higher value for serum phosphorus than non-pregnant animals. Singh *et al.* (1995) also reported that the highest concentration of magnesium was encountered in non-pregnant buffaloe cows with a decline from oestrus to early, mid and late pregnancy and the levels then increased during and after parturition.

Jana *et al.* (1991) reported that in goats serum calcium was low only in final stages of pregnancy while serum phosphorus was found low during the later half of pregnancy. Whereas, Takarkhede *et al.* (1999) observed that there was no much difference in serum concentration of calcium and inorganic phosphorus between ewes at non-pregnant and pregnant phases. However, concentration of these elements significantly decreased just after parturition. Kaushik and Bugalia (1999) also recorded non significant variations in overall plasma calcium and inorganic phosphorus content from 2 to 20 weeks of pregnancy in goats.

Singh *et al.* (1999) found that plasma concentration of calcium and phosphorus in yaks decreased as pregnancy advanced.

2.1.2 Sodium and potassium:

Bar *et al.* (1970) reported that serum sodium concentration of pregnant and lactating cows fluctuated considerably while potassium concentration remained relatively constant. However, Belyea *et al.* (1975) opined that in the dairy cows the plasma concentration of sodium was fairly constant throughout pregnancy and lactation whereas, potassium fluctuated during lactation with an increase during the late preparturient period and a decrease during early postparturient period. Rowlands *et al.* (1975) observed that concentrations of sodium fell significantly during the first two months of lactation. Murtuza *et al.* (1979) noticed significantly lower values ($P<0.01$) in the serum level of sodium in late pregnant and lactating Haryana cows when compared to their heifers whereas, serum level of potassium showed significant differences ($P<0.05$)

between late pregnant and early lactating cows. Underwood (1981) concluded that decrease in sodium level was most likely to occur during lactation due to its drainage in milk. Tainturier *et al.* (1984) opined that in the dairy cows during pregnancy and early lactation, the serum potassium remained unchanged whereas, sodium exhibited slight, irregular and transient variations.

Baranow-Baranowski *et al.* (1988) recorded a variety of changes in the serum concentration of sodium and to a lesser degree that of potassium so as to maintain the osmotic potential in the body fluids of cows during periparturient period. Sen *et al.* (1989) observed that serum concentration of potassium significantly decreased only at early lactation stage (30-40 days after parturition) and for sodium no significant difference was observed at prepartum (2-3 days before parturition), postpartum (12-24 hours after parturition) and early lactation (30-40 days after parturition). Sikka (1992) reported that requirements of sodium and potassium were considerably increased during pregnancy. Bahga and Singh (1992) recorded that plasma level of sodium was lowest at the beginning of parturition (140.13 meq/l) but for plasma sodium and potassium, differences between three days prior to parturition to 48 days after parturition were non significant and concluded that this was in relation with no change in plasma volume of cows nearing parturition.

According to Nazifi and Sami (1997) serum concentration of sodium and potassium in the Holstein cows during two months before parturition, at the time of parturition and two months after calving had no significant variation. In contrast, Bigras-Poulin and Tremblay (1998) concluded that serum potassium values in dairy herds decreased from first to seventh day postpartum. Deshpande *et al.* (1998) opined that there was a gradual significant ($P<0.01$) increase in the serum concentration of sodium in Gir cows and its crossbreds during the advanced stages of gestation whereas potassium values during different phases of gestation remained almost same in both Gir and its crossbreds which may be due to an increased demand of these minerals for the fetus. They also observed that from the day of parturition the serum sodium and potassium levels declined significantly till 21st day of parturition.

Boestedt and Hausmann (1980) observed depressed sodium level during the post partum period of sheep. Krajnicakova *et al.* (1994) observed that potassium levels decreased significantly from the day of insemination in sheep. Likewise sodium levels also fluctuated and decreased significantly.

2.1.3 Iron, copper and zinc:

Butler (1963) justified that increased blood volume during pregnancy may be the reason for the low levels of copper during advanced pregnancy period. Soliman and El-amrousi (1965) reported that during pregnancy an increase in plasma volume was greatest resulting in an apparent decrease in haemoglobin and consequently the iron concentration. Gault *et al.* (1966) found that estrogen administration increases the serum copper and ceruloplasmin in many mammalian species. McLaurin and Cotter (1967) found that decrease in the serum concentration of iron at the end of pregnancy may be due to the incorporation of this element into the fetus. Maynard and Loosli (1969) observed that pregnancy resulted in an increased absorption of iron from the gut. Henkin *et al.* (1969) reported that during pregnancy the levels of serum copper and ceruloplasmin increased, as estrogen induces *denovo* synthesis of ceruloplasmin. Song and Adham (1978) concluded that prostaglandin secretions when increased made an elevated level of zinc in the serum as zinc was required for the transport of prostaglandin. Hidiroglou and Knipfel (1981) observed that the low level of copper in pregnancy may be due to the utilisation of maternal copper for the development of fetal nervous system.

Deshpande *et al.* (1981) as well as Sikka and Mudgal, (1988) had stated that copper had an important role to play in the fertility of animals. Georgievskii *et al.* (1982) reported that zinc and cupric ions act as specific activators for a number of enzyme systems like oxidases that assist in maintaining the activity of labile hypophyseal hormones in blood. Carlson *et al.* (1982) opined that zinc can interfere with PG receptor mediated phase and consequently the leuteolytic process. Mehta and Gangwar (1983) reported that higher zinc levels in pregnant goats than in non-pregnant ones might be due to an increased concentration of enzyme carbonic anhydrase in which zinc forms an integral component. Tainturier *et al.* (1984) observed that serum iron concentration in dairy cows was relatively high from third to seventh month of pregnancy which decreased steadily thereafter which might be due to the incorporation of iron into the fetus and the value of iron further decreased to a minimum level during first month of lactation also. Barnea *et al.* (1985) found that copper is an integral component of metalloenzymes and it can modulate PGF_3 receptor binding thus regulating release of LH releasing hormone. Lavingonzalez *et al.* (1987) reported that serum copper decreased with advancing gestation and was lower in last third trimester of gestation. Sikka (1992) stated that both serum iron and copper concentrations showed same trend of variations during pregnancy.

Setia *et al.* (1994) reported that blood plasma concentration of zinc and iron in cows and buffaloes declined progressively during the last month of pregnancy and at parturition whereas the value of copper increased during the same period. After parturition and with the advancement of lactation the concentrations of copper and zinc showed an increasing trend but the level of iron showed a little variation when lactation progressed. Saxena and Gupta (1995) opined that plasma level of copper and zinc were always significantly ($P<0.01$) higher for cows conceiving within 120 days postpartum than those not conceiving in the same period and concluded that higher levels of copper and zinc in the system were required for earlier conception. Nazifi and Sami (1997) recorded that at parturition the serum concentration of iron in Holstein cows was lower ($P<0.05$) when compared to pregnant and lactating cows. However, Rajora and Pachauri (1998) found that in crossbred cows the serum concentration of copper, iron and zinc had a decreasing tendency with advancement of gestation and the serum iron value decreased significantly ($P<0.05$) during the late pregnancy than in the early pregnancy. Sarmah *et al.* (1999) noticed that in lactating crossbred cows the serum concentration of copper was higher (2.08 ppm) than the pregnant animals (1.91 ppm) and their heifers (1.69 ppm).

According to them serum concentration of zinc of calf, heifer, pregnant and lactating crossbred animals were not significantly different. However, they observed a lower serum iron content in pregnant crossbred cows when compared to their lactating ones.

Singh *et al.* (1991) observed that in buffaloes whole blood and plasma concentrations of zinc decreased as pregnancy progressed, while copper concentration in blood and plasma increased as pregnancy advanced but declined just before parturition.

Bhattacharyya *et al.* (1995) reported that serum concentration of iron and copper was significantly lower whereas the zinc value was higher during pregnancy in goats compared to non-pregnant ones.

2.2 Haematological parameters:

2.2.1 Erythrocyte count, haemoglobin content, volume of packed red cells and erythrocyte indices:

Johnson *et al.* (1990) opined that erythrocyte count and haemoglobin values of non pregnant and pregnant dairy cows showed no significant difference. However, Mulei (1991) indicated that in dairy cows there was a significant variation in the values of haemoglobin content and packed cell volume during prepartum and postpartum periods. Stilinovic *et al.* (1992) reported that haemoglobin concentration had a tendency to decrease during two months before parturition and the same trend continued till 75 days after parturition. Rajora and Pachauri (1994) opined that the packed cell volume and haemoglobin profile of crossbred cows were significantly ($P<0.05$) higher during late pregnancy when compared to non-lactating period. Gupta *et al.* (1995) observed that in cattle and buffaloes haemoglobin content and total erythrocyte count significantly increased immediately after calving which were then slightly lowered after a week and one month postpartum respectively and this might be due to the continuous stress inflicted by lactation.

According to Prabhakaran *et al.* (1997) pregnancy had no influence on the packed cell volume of Jersey cattle whereas the values for haemoglobin and erythrocyte count were higher. Klinkon and Nemec (1998) reported that in Holstein dairy cows the erythrocyte count was significantly higher at parturition than during 4 or 10 days after parturition and the haemoglobin concentration was lower during 4 or 10 days after parturition than at parturition. However, the mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were not significantly affected during these stages of observations. Wang Qung Lan *et al.* (1998) found that in dairy cows the packed cell volume decreased from 90 days before calving and reached the lowest value by a day before parturition.

Prabhakar *et al.* (1999) recorded higher PCV levels in buffaloes on the day of calving as compared to prepartum days and the levels then declined gradually during the early postpartum period. However, haemoglobin levels declined from day 2 prepartum to the day of calving which later improved on subsequent days.

Ramnath and Leela (1997) registered significantly higher haemoglobin concentration, erythrocyte count and packed cell volume in pregnant Tellicherry goats than the lactating and dry goats.

Materials and Methods

3. MATERIALS AND METHODS

3.1 Experimental animals:

Twenty, healthy crossbred sexually mature heifers maintained under identical managemental conditions in a single herd at Kerala Agricultural University (KAU) – Cattle breeding farm, Thumburmuzhi, were selected for the study. The animals were fed individually as per KAU Package of practises recommendations (1994) and water was provided *ad libitum*.

Blood was collected from all the twenty animals by jugular vein puncture in order to estimate the basal value (control group) of serum minerals, haematological parameters and erythrocyte indices before artificial insemination.

The selected animals (20 numbers), were then inseminated during estrus and pregnancy was confirmed by rectal palpation method, after three months of insemination. Of the twenty animals inseminated, eleven got conceived which formed the experimental group.

Blood was then collected at monthly intervals from third month viz., third, fourth, fifth, sixth, seventh, eighth and ninth month of pregnancy as well as first month of lactation from the experimental group and parameters were estimated. The health status of the experimental animals were regularly monitored throughout the experiment.

3.2 Collection of blood samples:

Three ml of whole blood was collected in clean, dry, labelled vials using sodium salt of Ethylene diamine tetra-acetic acid (EDTA) at a concentration of 1-2 mg/ml of blood as anticoagulant for the estimation of haematological parameters.

Simultaneously 25 ml of whole blood was collected in labelled test tubes for serum separation. Clear serum was transferred in clean, dry, labelled vials and stored at –20°C until, the analysis of minerals was carried out.

3.3 Mineral profile in the serum:

The serum profile of minerals like calcium (Ca), magnesium (Mg), iron (Fe), Copper (Cu) and Zinc (Zn) were estimated using Atomic Absorption Spectrophotometer (Perkin-Elmer 2380). Standard conditions were set in the atomic absorption spectrophotometer for the estimation of various elements were shown below (Beaty and Kerber, 1993).

Element	Wavelength (nm)	Slit (nm)	Flame-gases	Sensitivity* check (mg/litre)
Ca	422.7	0.7	Air-acetylene	3.5
Mg	285.2	0.7	Air-acetylene	0.3
Fe	248.3	0.2	Air-acetylene	6.0
Cu	324.7	0.7	Air-acetylene	4.0
Zn	213.9	0.7	Air-acetylene	1.0

* Concentration of an element in mg/litre required to produce approximately 0.2 absorbance units.

The serum inorganic phosphorus was analysed spectrophotometrically at 340 nm (Daly and Ertingshausen, 1972) using kits supplied by Sigma diagnostics.

The concentration of serum sodium and potassium were estimated by using flame photometer as per the method of Oser (1976).

3.4 Haematological parameters:

Haematological parameters such as erythrocyte count, haemoglobin (Hb) content and volume of packed red cells (VPRC) were determined on the day of blood collection as per standard methods (Jain, 1986).

3.5 Statistical analysis:

All the data obtained during pregnancy and early lactation periods for the above mentioned parameters were compared using paired 't' test and statistically analysed for significance with controls according to the methods of Snedecor and Cochran (1994). Variations between months of pregnancy and early lactation were also analysed.

Results

4. RESULTS

4.1 Mineral profile in the serum:

4.1.1 Calcium, inorganic phosphorus and magnesium:

The serum concentrations of calcium, inorganic phosphorus and magnesium of non-pregnant (control), pregnant (from third month to term, at monthly intervals *viz.*, 3, 4, 5, 6, 7, 8 and 9 months) and early lactating (first month of lactation) crossbred heifers are shown in the table 1 and fig.1.

4.1.1.1 Serum calcium:

A great deal of fluctuations were noticed in the serum calcium levels of crossbred heifers during the period of study. A significant reduction ($P<0.01$) in the serum calcium level was noticed during third to sixth month of pregnancy and the value was lowest (5.59 ± 0.16 mg/dL) at fifth month of pregnancy as compared to pre conception value (7.33 ± 0.28 mg/dL). From the sixth month of pregnancy onwards the calcium level showed an increasing trend and the corresponding values during eighth and ninth month of pregnancy as well as at first month of lactation were (7.30 ± 0.12 mg/dL, 8.11 ± 0.35 mg/dL and 7.47 ± 0.17 mg/dL respectively) significantly ($P<0.01$) higher when compared to values obtained at fifth month of pregnancy (5.59 ± 0.16 mg/dL) and the maximum value during pregnancy was attained at ninth month (8.11 ± 0.35 mg/dL) vide table 1.

When the serum calcium content of pregnant and early lactating cows were compared within, it was found that the levels during fourth, fifth and ninth month of pregnancy were significantly ($P<0.01$) varied with third month level. While comparing the value recorded during fourth month of pregnancy, the levels at eighth and ninth month of pregnancy and first month of lactation increased significantly ($P<0.01$).

When serum calcium level of sixth month of pregnancy was compared with seventh, eighth and ninth month of pregnancy and first month of lactation these values were significantly ($P<0.01$) higher than the sixth month of pregnancy. Same was the case for serum calcium level at seventh month of pregnancy when it was compared with the values recorded during advanced months of pregnancy and first month of lactation. While comparing with the level of serum calcium recorded during eighth month of pregnancy, the value obtained during the ninth month pregnancy was significantly ($P<0.01$) higher and the respective values were 7.30 ± 0.12 mg/dL and 8.11 ± 0.35 mg/dL. During pregnancy the value of serum calcium was highest (8.11 ± 0.35 mg/dL) in the ninth month and lowest (5.59 ± 0.16 mg/dL) at fifth month of pregnancy.

4.1.1.2 Serum inorganic phosphorus:

Alternations were noticed in the concentration of serum inorganic phosphorus of crossbred heifers during the period of study. Comparatively a higher value was recorded during third month of pregnancy (7.66 ± 0.43 mg/dL) when compared to controls (6.69 ± 0.45 mg/dL). Then the value dropped during fourth month of pregnancy (7.15 ± 0.49 mg/dL) and significantly ($P<0.01$) higher value was observed during the fifth month of pregnancy (8.29 ± 0.51 mg/dL). However, from the sixth month onwards the inorganic phosphorus levels dropped and a value of 6.66 ± 0.30 mg/dL was encountered during ninth month of pregnancy which dropped further to 6.20 ± 0.27 mg/dL during first month of lactation.

During sixth, seventh and eighth month of pregnancy and first month of lactation the inorganic phosphorus levels were significantly ($P<0.01$) lower when compared to the value obtained during third month of pregnancy. On comparison with the inorganic phosphorus level recorded during fifth month of pregnancy, the levels at sixth, seventh, eighth, ninth month of pregnancy and first month of lactation were significantly lower ($P<0.01$).

The lowest inorganic phosphorus level (6.02 ± 0.26 mg/dL) was recorded during the eighth month of pregnancy and the highest value during fifth month of pregnancy (8.29 ± 0.51 mg/dL).

4.1.1.3 Serum magnesium:

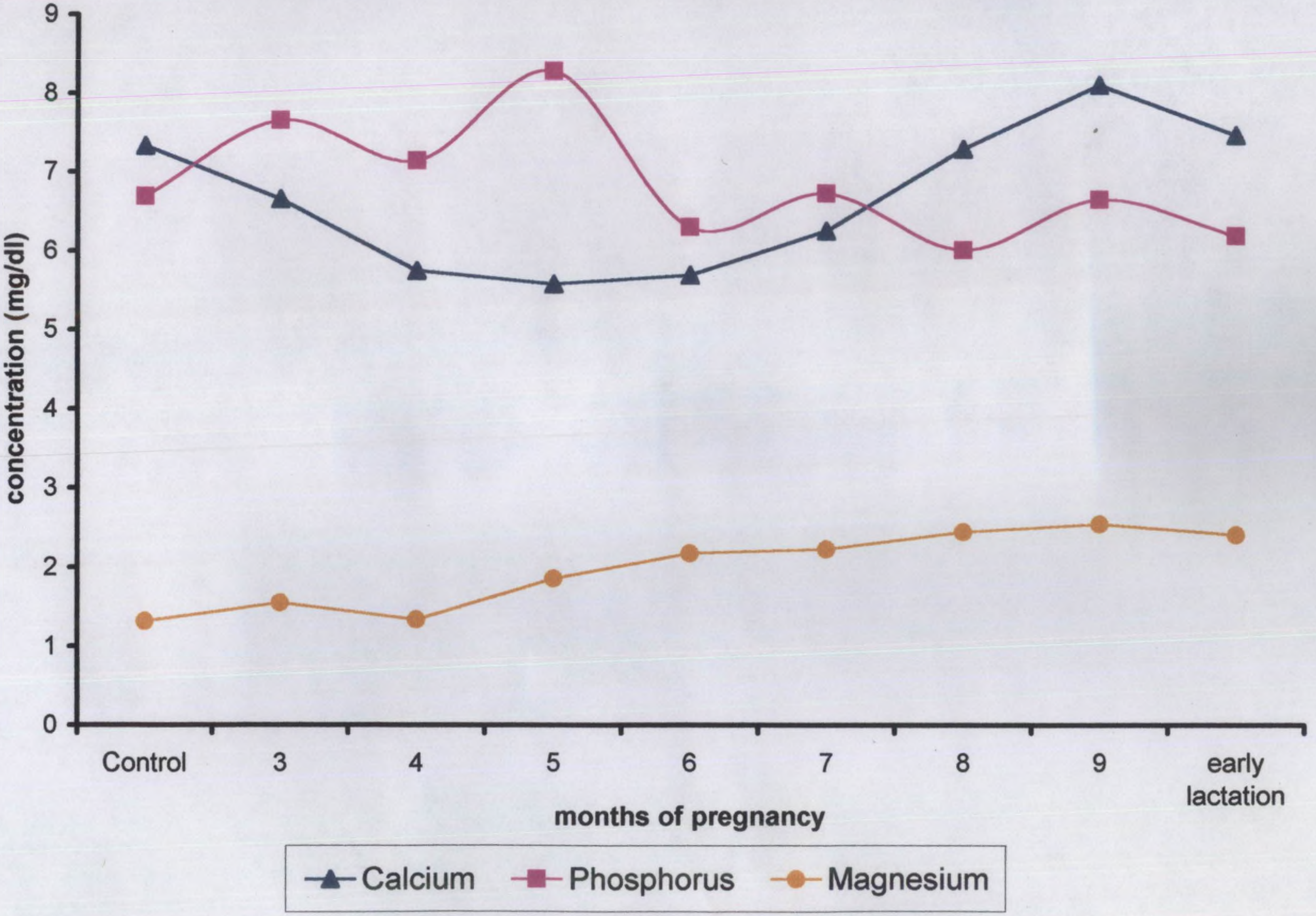
The level of serum magnesium during third month of pregnancy (1.55 ± 0.14 mg/dL) was higher than that of control (1.32 ± 0.10 mg/dL) which then dropped during fourth month of pregnancy (1.34 ± 0.16 mg dL). The level of magnesium steadily increased from the fifth month of pregnancy (1.86 ± 0.27 mg/dL) and thereafter, from the sixth month significantly ($P<0.01$) higher serum magnesium levels were recorded upto first month of lactation compared to control.

Table 1
The serum concentration of calcium, inorganic phosphorus and magnesium before and after conception and in early lactation in crossbred heifers (n=11)

Period/month of pregnancy	Calcium (mg/dl)	Inorganic phosphorus (mg/dl)	Magnesium (mg/dl)
Non pregnant (control)	7.33 [a] ± 0.28	6.69 [a] ± 0.45	1.32 [a] ± 0.10
3	6.67 [b] ± 0.30	7.66 [ab] ± 0.43	1.55 [ab] ± 0.14
4	5.76 [c] ± 0.23	7.15 [abc] ± 0.49	1.34 [ab] ± 0.16
5	5.59 [cd] ± 0.16	8.29 [bc] ± 0.51	1.86 [abc] ± 0.27
6	5.70 [bcd] ± 0.37	6.31 [acd] ± 0.45	2.18 [cd] ± 0.18
7	6.26 [abcd] ± 0.37	6.74 [acde] ± 0.31	2.23 [cde] ± 0.12
8	7.30 [abe] ± 0.12	6.02 [acdef] ± 0.26	2.45 [def] ± 0.22
9	8.11 [a] ± 0.35	6.66 [abcdefg] ± 0.30	2.55 [cdefg] ± 0.24
First month of lactation	7.47 [abe] ± 0.17	6.20 [acdefg] ± 0.27	2.41 [cdefg] ± 0.15

Values bearing similar superscripts in the column did not differ significantly ($P<0.01$)

Fig.1 Concentration of serum Ca, P and Mg of crossbred heifers before and after conception and in early lactation



The serum magnesium level was highest during ninth month of pregnancy (2.55 ± 0.24 mg/dL) which was further lowered to 2.41 ± 0.15 mg/dL during first month of lactation.

It was also found that serum magnesium levels recorded during sixth, seventh, eighth, ninth month of pregnancy and first month of lactation were significantly higher ($P<0.01$) when compared to the value encountered during third month of pregnancy. While considering the serum magnesium level of fourth month of pregnancy, it was found that significantly higher ($P<0.01$) values were obtained from fifth to ninth month of pregnancy as well as at first month of lactation. While comparing with the value of serum magnesium level during fifth month of pregnancy, the corresponding value observed during eighth month of pregnancy was significantly higher ($P<0.01$).

4.1.2 Sodium and potassium:

The serum concentration of sodium and potassium of non-pregnant (control), pregnant (from third month of pregnancy to term at monthly intervals viz. 3, 4, 5, 6, 7, 8, 9 months) and early lactating (first month of lactation) crossbred heifers are shown in table 2 and fig.2.

4.1.2.1 Serum Sodium:

A great deal of fluctuations in the serum sodium levels of crossbred heifers were observed during the period of study. A significant reduction ($P<0.01$) in the serum sodium concentration from the basal value of 188.07 ± 13.23 meq/L was noticed during third month of pregnancy where the serum sodium level was 158.73 ± 10.61 meq/L. From the fourth month onwards the serum sodium level increased and reached a peak value of 216.74 ± 8.23 meq/L nearing the term, ie. by ninth month of pregnancy. The serum sodium level recorded during first month of lactation was slightly lower than the ninth month value and was found to be 215.94 ± 8.28 meq/L.

When the data recorded during the period of pregnancy and early lactation were compared and statistically analysed it was found that the serum sodium levels observed during seventh, eighth, ninth month of pregnancy and first month of lactation were significantly higher ($P<0.01$) when compared to the value obtained during third month of pregnancy.

While comparing the serum sodium level observed during fourth month of pregnancy, the levels obtained at seventh, eighth, ninth month of pregnancy and first month of lactation were significantly higher ($P<0.01$). Same was the case for serum sodium level at fifth month of pregnancy (eventhough the value was slightly lower than the fourth month) when it was compared with the values recorded during, seventh, eighth and ninth month of pregnancy and first month of lactation. While comparing with the serum sodium level at sixth month of pregnancy, the values obtained during seventh, ninth month of pregnancy and first month of lactation were significantly higher ($P<0.01$).

4.1.2.2 Serum Potassium:

The serum potassium concentration during third (4.98 ± 0.39 meq/L) and fourth month of pregnancy (4.45 ± 0.36 meq/L) were significantly ($P<0.01$) lower than the control levels (5.43 ± 0.34 meq/L). While potassium level reached to the lowest value of 4.10 ± 0.31 meq/L by the sixth month of pregnancy, the seventh month level (4.35 ± 0.19 meq/L) was also significantly ($P<0.01$) lower than the control level. Then the potassium level exhibited an increasing trend from seventh month and during ninth month of pregnancy reached the highest value of 5.66 ± 0.27 meq/L. During first month of lactation serum potassium level dropped to 5.45 ± 0.30 meq/L which was almost equal to the preconception level.

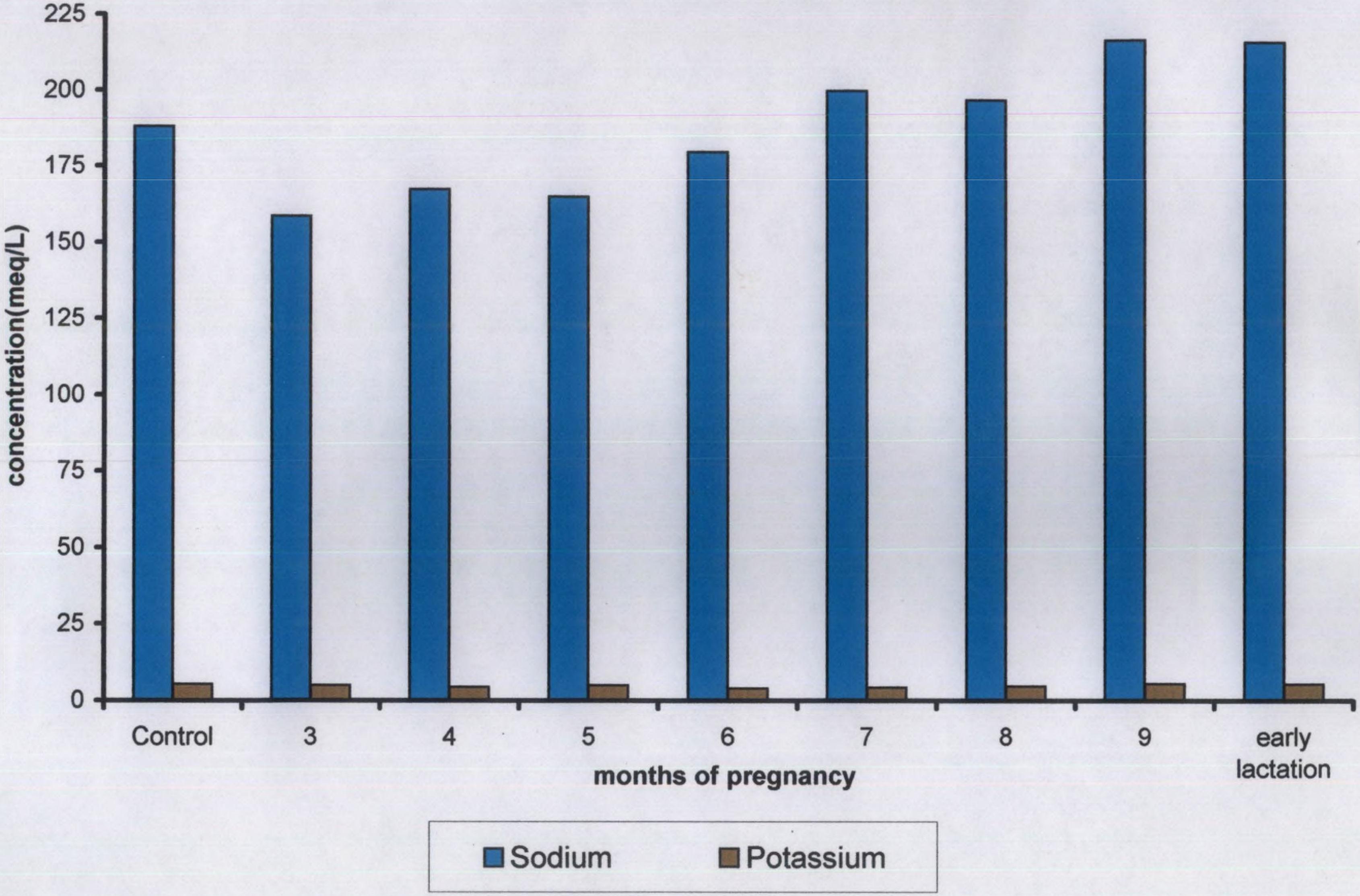
It was also found that the serum potassium level recorded during sixth month of pregnancy was significantly lower ($P<0.01$) to the value encountered during third month. On comparing with the serum potassium level observed during the fourth month of pregnancy, the levels at ninth month of pregnancy and first month of lactation were significantly higher ($P<0.01$). While comparing with the value of serum potassium recorded during sixth month of pregnancy, the corresponding values obtained during eighth and ninth month of pregnancy as well as first month of lactation were significantly higher ($P<0.01$). On comparing the serum potassium level of seventh month of pregnancy, values obtained during ninth month of pregnancy and first month of lactation were found to be significantly higher ($P<0.01$). While comparing with the value of serum potassium level recorded during eighth month of pregnancy, the corresponding

Table 2
The serum concentration of sodium and potassium before and after conception and in early lactation in crossbred heifers (n=11)

Period/month of pregnancy	Sodium (meq/L)	Potassium (meq/L)
Non-Pregnant (control)	188.07^{a} ± 13.23	5.43^{a} ± 0.34
3	158.73^{b} ± 10.61	4.98^{b} ± 0.39
4	167.54^{abc} ± 8.77	4.45^{bc} ± 0.36
5	165.21^{abcd} ± 8.77	4.98^{abcd} ± 0.44
6	179.80^{abcde} ± 8.50	4.10^{cde} ± 0.31
7	199.99^{af} ± 4.69	4.35^{bcdef} ± 0.19
8	196.83^{aefg} ± 6.76	4.82^{abcdfg} ± 0.18
9	216.74^{afgh} ± 8.23	5.66^{abdh} ± 0.27
First month of lactation	215.94^{afgh} ± 8.28	5.45^{abdgh} ± 0.30

Values bearing similar superscripts in the column did not differ significantly ($P<0.01$)

Fig.2 Concentration of serum Na and K of crossbred heifers before and after conception and in early lactation



value obtained during ninth month of pregnancy was found to be significantly higher ($P<0.01$).

4.1.3 Iron, copper and zinc:

The serum concentration of iron (Fe), copper (Cu) and zinc (Zn) of non-pregnant (control), pregnant (from third month to term at monthly intervals viz. 3, 4, 5, 6, 7, 8 and 9 months) and early lactating (first month of lactation) in crossbred heifers are given in table 3 and fig.3.

4.1.3.1 Serum iron:

The concentration of iron in the serum of crossbred heifers during third month of pregnancy was higher (1.53 ± 0.16 ppm) than the non pregnant (control) animals (1.44 ± 0.10 ppm). The value continued to increase upto fifth month of pregnancy (1.68 ± 0.14), later decreased upto seventh month of pregnancy (1.30 ± 0.20 ppm). However, during ninth month the value was as low as 1.23 ± 0.07 ppm which further dropped to 1.13 ± 0.09 ppm during first month of lactation. The serum iron level recorded during first month of lactation was significantly lower ($P<0.01$) when compared to the value observed during the third month of pregnancy. While comparing the values recorded during fourth and fifth months of pregnancy, the values obtained during ninth month of pregnancy and first month of lactation were significantly lower ($P<0.01$).

4.1.3.2 Serum copper:

A significantly higher ($P<0.01$) level of serum copper was recorded during third month of pregnancy (0.87 ± 0.02 ppm) when compared to control value of 0.72 ± 0.04 ppm. The serum copper level then started to increase and reached significantly ($P<0.01$) higher/peak level (1.16 ± 0.15 ppm) during fifth month of pregnancy. At sixth month of pregnancy also serum copper value (0.92 ± 0.09 ppm) was significantly higher ($P<0.01$) than the control and later it exhibited a decreasing trend upto eighth month of pregnancy (0.76 ± 0.05 ppm). During ninth month of pregnancy the serum copper level was elevated to a value of 0.81 ± 0.04 ppm which further increased to 0.89 ± 0.07 ppm in the first month of lactation. On comparing the serum copper level observed during fifth month of pregnancy, the levels encountered at eighth and ninth months of pregnancy were significantly lower ($P<0.01$).

4.1.3.3 Serum zinc:

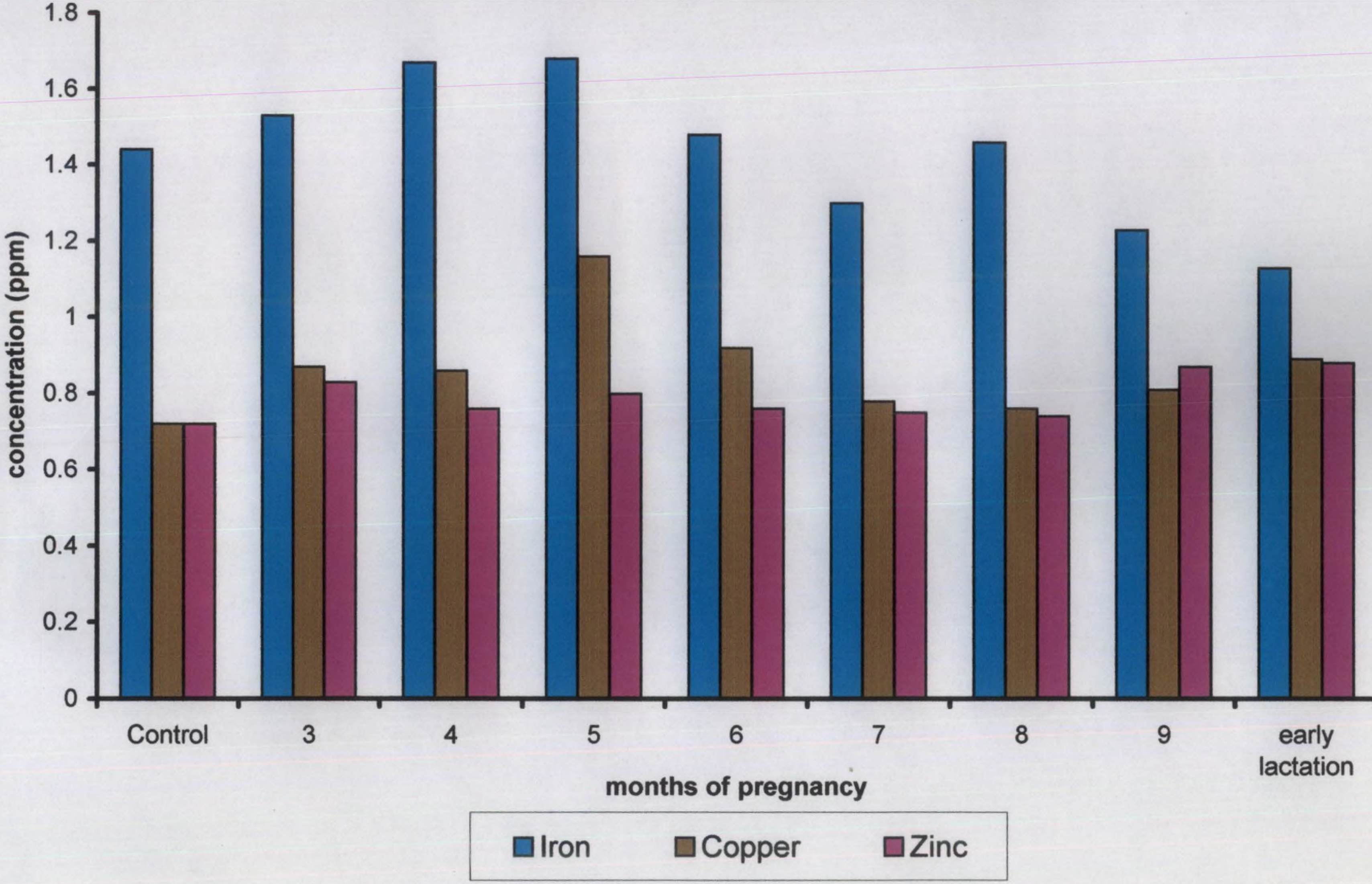
Marked fluctuations were noticed in the serum zinc level of crossbred heifers during the period of study. A significantly higher ($P<0.01$) value was obtained during third month of pregnancy (0.83 ± 0.05 ppm) when compared to the control level (0.72 ± 0.04 ppm). The serum zinc level then decreased during fourth month of pregnancy (0.76 ± 0.04 ppm) and again increased during the fifth month of pregnancy (0.80 ± 0.08 ppm). Thereafter a decreasing trend was noticed upto eighth month of pregnancy (0.74 ± 0.08 ppm). During ninth month of pregnancy a significantly ($P<0.01$) higher value of 0.87 ± 0.04 ppm was obtained.

Table 3
The serum concentration of iron, copper and zinc before and after conception and in early lactation in crossbred heifers (n=11)

Period/month of pregnancy	Iron (ppm)	Copper (ppm)	Zinc (ppm)
Non pregnant (control)	1.44^{a} ±0.10	0.72^{a} ± 0.04	0.72^{a} ± 0.04
3	1.53^{ab} ± 0.16	0.87^{b} ± 0.02	0.83^{b} ± 0.05
4	1.67^{abc} ± 0.11	0.86^{abc} ± 0.11	0.76^{abc} ± 0.04
5	1.68^{abcd} ± 0.14	1.16^{bcd} ± 0.15	0.80^{abcd} ± 0.08
6	1.48^{abcde} ± 0.17	0.92^{bcde} ± 0.09	0.76^{abcde} ± 0.04
7	1.30^{abcdef} ± 0.20	0.78^{abcdef} ± 0.08	0.75^{abcdef} ± 0.03
8	$1.46^{abcdefg}$ ± 0.20	0.76^{abcefg} ± 0.05	$0.74^{abcdefg}$ ± 0.08
9	1.23^{abcfgh} ± 0.07	$0.81^{abcefgh}$ ± 0.04	0.87^{bcdegh} ± 0.04
First month of lactation	1.13^{aefgh} ± 0.09	$0.89^{abcdefgh}$ ± 0.07	$0.88^{abcdefgh}$ ± 0.08

Values bearing similar superscripts in the column did not differ significantly (P<0.01)

Fig.3 Concentration of serum Fe, Cu and Zn of crossbred heifers before and after conception and in early lactation



The value recorded during first month of lactation was still higher as 0.88 ± 0.08 ppm and it was the highest value obtained for serum zinc concentration during the period of study. On comparison with the serum zinc level observed during seventh month of pregnancy (0.75 ± 0.03 ppm), the ninth month value was found to be significantly ($P<0.01$) higher.

4.2 Haematological parameters:

The haematological parameters like erythrocyte count, haemoglobin (Hb) concentration and volume of packed red cells (VPRC) that were recorded in non-pregnant (control), pregnant (from third month of pregnancy to term at monthly intervals viz. 3, 4, 5, 6, 7, 8 and 9 months) and early lactating (first month of lactation) in crossbred heifers are shown in table 4.

4.2.1 Erythrocyte count:

The erythrocyte count during third month of pregnancy ($5.80 \pm 0.16 \times 10^6/\mu L$) was significantly ($P<0.01$) lower than the control levels ($6.40 \pm 0.38 \times 10^6/\mu L$) and the value started to decline gradually and reached to the lowest value of $5.48 \pm 0.24 \times 10^6/\mu L$ during the ninth month of pregnancy. The erythrocyte count still dropped during first month of lactation ($5.22 \pm 0.23 \times 10^6/\mu L$) which was significantly lower ($P<0.01$) than the control level.

4.2.2 Haemoglobin (Hb) content:

The haemoglobin content during the third month of pregnancy was (9.96 ± 0.60 g%) lower than the control level (10.09 ± 0.46 g%). Then the value gradually

Table 4
Certain haematological parameters before and after conception and in early lactation in crossbred heifers (n=11)

Period/month of pregnancy	Erythrocyte count (millions/μL)	Haemoglobin (Hb) content (g%)	Volume of packed red cells (VPRC) (%)
Non pregnant (control)	6.40 [a] ± 0.38	10.09 [a] ± 0.46	32.27 [a] ± 1.03
3	5.80 [b] ± 0.16	9.96 [a] ± 0.60	30.46 [b] ± 0.81
4	5.86 [a] ± 0.25	9.98 [a] ± 0.63	32.18 [a] ± 1.43
5	5.69 [a] ± 0.22	10.17 [a] ± 0.36	30.36 [a] ± 0.58
6	5.64 [a] ± 0.20	8.64 [b] ± 0.26	28.91 [b] ± 0.53
7	5.85 [a] ± 0.26	8.77 [b] ± 0.23	30.55 [a] ± 0.78
8	5.63 [a] ± 0.29	8.96 [b] ± 0.34	30.64 [a] ± 0.72
9	5.48 [a] ± 0.24	8.23 [b] ± 0.18	29.27 [a] ± 0.94
First month of lactation	5.22 [b] ± 0.23	8.18 [b] ± 0.35	28.82 [a] ± 1.05

Values bearing similar superscripts in the column did not differ significantly ($P<0.01$)

increased to reach a value of 10.17 ± 0.36g% by fifth month of pregnancy. During rest of the period of study, i.e., from the sixth month of pregnancy, the Hb content was significantly lower (P<0.01) than the control level. During pregnancy the lowest value for Hb concentration was encountered in the ninth month of pregnancy (8.23 ± 0.18 g%). During the first month of lactation the Hb content further dropped to 8.18 ± 0.35g%.

4.2.3 Volume of Packed red blood cells (VPRC):

In the present study it was found that during third month of pregnancy VPRC was significantly (P<0.01) lower (30.46 ± 0.81%) than the control levels (32.27±1.03%). It further lowered to reach a significantly lower (P<0.01) value of 28.91 ± 0.53% by the sixth month of pregnancy, which later increased to 30.64 ± 0.72% by eighth month of pregnancy and then dropped to 29.27 ± 0.94% during ninth month of pregnancy. During the first month of lactation VPRC value further declined to 28.82 ± 1.05%.

4.3 Erythrocyte indices:

The erythrocyte indices like mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated in non-pregnant (control), pregnant (from the third month of pregnancy to term at monthly intervals viz. 3, 4, 5, 6, 7, 8 and 9 months) and early lactating (first month of lactation) in crossbred heifers are shown in table 5.

Table 5
Erythrocyte indices before and after conception and in early lactation in crossbred heifers (n=11)

Period/month of pregnancy	Mean corpuscular volume (MCV in fl)	Mean corpuscular haemoglobin (MCH in pg)	Mean corpuscular haemoglobin concentration (MCHC in g%)
Non pregnant (control)	51.92 ^{a} ± 3.26	16.33 ^{a} ± 1.16	31.72 ^{a} ± 2.03
3	52.79 ^{a} ± 1.81	17.29 ^{a} ± 1.12	32.97 ^{a} ± 2.34
4	55.92 ^{a} ± 3.40	17.06 ^{a} ± 0.88	31.72 ^{a} ± 2.58
5	54.43 ^{a} ± 3.00	18.07 ^{a} ± 0.93	33.67 ^{a} ± 1.50
6	51.85 ^{a} ± 1.96	15.58 ^{a} ± 0.95	29.98 ^{a} ± 1.14
7	52.72 ± 1.30	15.25 ^{a} ± 0.78	28.89 ^{a} ± 1.01
8	55.65 ^{a} ± 2.84	16.34 ^{a} ± 1.18	29.29 ^{a} ± 1.10
9	54.13 ^{a} ± 2.45	15.22 ^{a} ± 0.70	28.38 ^{a} ± 1.05
First month of lactation	55.95 ^{b} ± 2.59	15.89 ^{a} ± 0.90	28.85 ^{a} ± 1.80

Values bearing similar superscripts in the column did not differ significantly ($P<0.01$)

4.3.1 Mean corpuscular volume (MCV):

The means corpuscular volume (MCV) varied throughout the various stages of study. The MCV value during third month of pregnancy was 52.79 ± 1.81 fl whereas that of control was 51.92 ± 3.26 fl. The MCV during ninth month of pregnancy was 54.13 ± 2.45 fl, which later increased to 55.95 ± 2.59 fl during first month lactation.

4.3.2 Mean corpuscular haemoglobin (MCH):

The MCH during third month of pregnancy was 17.29 ± 1.12 pg, whereas that of control was 16.33 ± 1.16 pg. Variations were observed in the MCH levels throughout the period of study which attained a value of 15.22 ± 0.70 pg during ninth month of pregnancy and 15.89 ± 0.90 pg during first month of lactation.

4.3.3 Mean corpuscular haemoglobin concentration (MCHC):

The MCHC level during third month of pregnancy was 32.97 ± 2.34 g% whereas that of control was 31.72 ± 2.03 g%. The MCHC value showed variation throughout the period of study which reached to the lowest value of 28.38 ± 1.05 g% during ninth month of pregnancy.

Discussion

5. DISCUSSION

5.1 Mineral profile in the serum:

5.1.1 Calcium, inorganic phosphorus and magnesium:

Normal physiological functions of the body including growth, pregnancy, parturition and lactation require a proper synergism between the anabolic and catabolic reactions in which minerals play a vital role. The variations in the concentration of macroelements as calcium, phosphorus and magnesium in the animal body during different phases of reproduction upset the proper functioning of reproductive organs. Calcium is a structural component of skeleton which is absolutely essential for the formation of fetal skeleton. Phosphorus being part of energy rich phosphate bonds is absolutely essential for metabolic activity of living tissues including those of endocrine glands and reproductive organs. Magnesium is involved in many of the enzymatic reactions leading to ATP generation and also influences the absorption of calcium and phosphorus. Hence the fluctuations that can occur in the concentration of these macroelements in different stages of gestation are of utmost importance.

5.1.1.1 Serum calcium:

In the present investigation it was recorded that during early stages of pregnancy there was a decline in the serum calcium level with a negative calcium balance (Fig.1) and there was an increasing trend from the sixth month to ninth month of pregnancy (table 1). These recordings are in consonance with the observations of Pathak and Janakiraman (1987) who explained that it may be due to the demand of physiological situation when the fetation and organogenesis takes place. Symonds *et al.* (1966) were also of same opinion that there is a great demand of calcium for the development of fetal skeleton during the advanced pregnancy period.

In the present study the highest serum calcium level was recorded during ninth month of pregnancy, where peak estrogen activity occurs. Thereafter the level of serum calcium was slightly lowered during the first month of lactation. These observations corroborate with the findings of Sahukar *et al.* (1984). The enhanced serum calcium level observed during ninth month of pregnancy may be because of the role of calcium in sensitizing the tubular genitalia for the action of hormones involved in parturition (Sangha *et al.*, 1993). Besides minerals like calcium and phosphorus play a vital role in the action of hormones and enzymes at subcellular levels in an integrated fashion especially the reproductive aspects of young growing heifers. Symonds *et al.* (1966) attributed the low calcium recorded in the first month of lactation was due to a sudden demand of calcium for the production of colostrum/milk. Murtuza *et al.* (1979) also observed that serum calcium was higher in cows during late pregnancy than in early lactation, which confirmed the findings of the present study.

5.1.1.2 Serum inorganic phosphorus:

During early pregnancy, the serum calcium concentration was negatively correlated with serum inorganic phosphorus level which showed higher values (fig.1). This observation is closely in consonance with the findings of Harper (1969) who reported that levels of serum calcium and phosphorus were inversely correlated. In the present investigation serum phosphorus had a higher value during fifth month of pregnancy and then the value fluctuated to fall towards the basal value during ninth month of pregnancy (table 1). This observation was in accordance with those made by Pathak and Janakiraman (1987). Phosphorus being part of energy rich phosphate bonds is absolutely essential for metabolic activity of living tissues including those of endocrine and reproductive organs. Phosphorus is an integral component of nucleic acids, nucleotides, phospholipids and certain proteins and this macroelement is essential for transfer and utilization of energy in cells as well as an integral part of many of the enzymes. Patel *et al.* (1966) justified that the higher concentration of serum inorganic phosphorus during fifth month of pregnancy may be due to its requirement for fetal growth.

It is assumed that calcium and phosphorus might have been mobilised from dam's skeleton, where the calcium was drained for the fetal skeletal formation (leading to lower serum calcium value of 5.59 ± 0.16 mg/dl at mid gestation) leaving behind the phosphorus in the serum. The increase in serum phosphorus during ninth month of pregnancy than eighth month may be due to the effect of higher levels of estrogens in the advanced stage of gestation, since estrogens are found to raise phosphorus level as reported by Hackett *et al.* (1957). In the present investigation also a fall in the serum inorganic phosphorus level in the first month of lactation was noticed. This observation was in agreement with recordings of Murtuza *et al.* (1979) who found that early lactating cows had a significantly lower serum phosphorus value than the late pregnant cows. This decline in the serum phosphorus level may be attributed to its shift from blood to udder for milk synthesis.

5.1.1.3 Serum magnesium:

The trend of increase in the serum magnesium level during different stages of gestation which reached the highest level by ninth month of gestation in this study (Fig.1) was in close consonance with an earlier report made by Deshpande *et al.* (1998). Sarmah *et al.* (1999) reported that this may be due to an increased gastrointestinal absorption of magnesium to meet the increased metabolic demands during pregnancy. As metabolic rate of pregnant animals increase, the demands of magnesium for the body also getting increased since glycolytic enzymes like glucokinase, phosphofructokinase, enolase and citric acid cycle enzyme namely succinate thiokinase require magnesium as cofactor. In the present study magnesium values declined during first month of lactation (table 1), probably due to an increased lactational demand which was in close agreement with those recordings of Deshpande *et al.* (1998).

5.1.2 Sodium and potassium:

The major electrolytes of body such as sodium and potassium are having multifarious role in the maintenance of osmolarity of blood, fluid volume, acid-base/electrolyte balance, impulse transmission etc. Physiological conditions like pregnancy, parturition and lactation impose tremendous stress to the animal's body and animal must maintain homeostatis in order to survive where sodium and potassium are having potent role.

5.1.2.1 Serum sodium:

In the present study it was observed that serum sodium concentration at the third month of pregnancy was lower than basal value (fig.2) and then from third to sixth month of pregnancy eventhough showed an increasing tendency, the values were lower than the preconception levels (table 2). The lowered value of serum sodium from third to sixth month of pregnancy observed in the present study was in agreement with recordings of Singh *et al.* (1999) who observed lower sodium values in pregnant yaks from third to sixth month when compared with normal cycling ones. Sikka (1992) reported that requirements of sodium and potassium were considerably increased during pregnancy period. However, in the present study an increasing trend was noticed from the third to ninth month of gestation with the peak value at the ninth month of pregnancy. This observation was in close agreement with the findings of Deshpande *et al.* (1998) who observed a gradual significant increase in the serum sodium level in crossbred cows during advancing stages of gestation which may be due to an increasing demand of this mineral for the growing fetus. In the present study, peak value of serum sodium was recorded during ninth month of pregnancy. This observation was in corroboration with the findings of Singh *et al.* (1999) who found that in yaks, peak value of serum sodium was attained during third trimester of pregnancy.

In the study conducted it was found that serum sodium concentration decreased during first month of lactation than the advanced month of pregnancy which was in consonance with an earlier report of Underwood (1981) who concluded that decrease in sodium level was most likely to occur during lactation due to the drainage of sodium in milk. This kind of observation made during first month of lactation also agreed with the recordings of Rowlands *et al.* (1975) and Deshpande *et al.* (1998) who observed that serum sodium level was lowered from the day of parturition as in first two months of lactation. Similarly Murtuza *et al.* (1979) also reported that serum sodium levels in early lactating cows were lower than the late pregnant cows. Boestedt and Hausmann (1980) observed a depressed sodium level in the postpartum period of sheep.

5.1.2.2 Serum potassium:

In the present study, serum potassium level, showed gradual declining tendency from third to sixth month of pregnancy (fig.2). This observation was in agreement with the observation of Krajnicakova *et al.* (1994) who found that potassium level decreased significantly from the day of insemination in ewes. It was earlier mentioned that serum sodium level was lower than preconception values during three to six months of pregnancy and the dynamics of the potassium and sodium decrease in the first few days of pregnancy might be connected with an increased permeation of electrolytes into the uterine environment at the moment when cleavage of the fertilized ovum and zygote differentiation take place and later with the drainage of electrolytes into the fetoplacentary unit. Serum potassium level reached the highest value during ninth month of pregnancy whereas during first month of lactation, the value again declined (table 2). These observations made here were in close agreement with the observations of Belyea *et al.* (1975) who concluded that plasma potassium increased in late pregnancy period and then declined during early lactation. The decrease in plasma potassium during early lactation might be due to an increased transfer of this cation into milk. Sen *et al.* (1989) observed that the serum potassium levels declined significantly from the day of parturition till 21st day of lactation in crossbred cows.

5.1.3 Iron, copper and zinc:

The microelements like iron, copper and zinc are integral components of many of the structural proteins and enzymes involved in several biological functions including reproduction. Iron and copper are involved in many of the cellular metabolic actions especially in the formation of respiratory pigment. Copper can modulate PGE_3 receptor binding thereby regulating LH release. Zinc is an essential component of many of the enzymes involved in steroidogenesis and is required for ovarian mechanism involved in parturition. Therefore close monitoring of these microelements in the dairy cattle especially crossbred heifers in Kerala is essential.

5.1.3.1 Serum iron:

In the present study it was noticed that serum iron and copper showed an increasing trend upto fifth month of pregnancy and thereafter the values declined (table 3). This observation corroborates well with an earlier report made by Sikka

(1992) who had stated that both serum iron and copper showed the same trend of fluctuations especially during first trimester of pregnancy. The present finding of an increase in the serum concentration of iron from third to fifth month of pregnancy and thereafter declining to a minimum in the first month of lactation (Fig.3) was in accordance with the observations of Tainturier *et al.* (1984), who reported that serum iron concentration steadily increased from the third month of pregnancy which later decreased and reached a minimum value in the first month of lactation. Pregnancy stimulated an increased iron absorption from the gut to meet the needs of fetal growth as reported by Maynard and Loosli (1969). During the first trimester of pregnancy the developing fetal demands for iron may not be equal to the rate of iron absorption which resulted in an increased serum iron level. But as pregnancy advanced fetal demands overtook rate of absorption resulting in decreased serum iron concentration (from the sixth month of pregnancy) in the dam. The lower level of serum iron during late pregnancy encountered in this study was also in agreement with the observation of Bhattacharyya *et al.* (1995). The decrease in serum iron concentration at the end (ninth month) of pregnancy could be either due to the incorporation of this element into fetus as suggested by McLaurin and Cotter (1967) or due to a greater increase in plasma volume resulting in an apparent decrease in haemoglobin and consequently the iron concentration as described by Soliman and El-amrousi (1965).

5.1.3.2 Serum copper:

Copper has an important role to play in the fertility of animals as reported by Deshpande *et al.* (1981) as well as by Sikka and Mudgal (1988). In the present study the serum copper level increased from third to fifth month of pregnancy to reach the peak value (Fig.3). During pregnancy the levels of serum copper and ceruloplasmin get increased as reported by Gault *et al.* (1966) and Henkin *et al.* (1969). In the present study, serum copper level is found to have a decreasing trend from sixth to eighth month of gestation (Table 3) which is in consonance with the observations of Lavingonzalez *et al.* (1987). Butler (1963) justified that increased blood volume during pregnancy may be one of the reasons for low level of copper during advanced pregnancy period especially in the last trimester of pregnancy. The lower level of copper in this period may be due to the utilization of maternal copper for the development of fetal nervous system (Hidiroglou and Knipfel, 1981) and copper is an integral component of metalloenzymes and it can modulate PGE_3 receptor binding thus regulating release of LH releasing hormone (Barnea *et al.*, 1985). In the present study it was also recorded that an increase in the serum copper level especially during ninth month of pregnancy which could be attributed to the positive correlation between copper and estrogen, as the estrogen level increases consequently the serum copper level also rises (Henkin *et al.*, 1969). During the study, serum copper level increased slightly during the first month of lactation. This was more or less in accordance with the observations made by Rajora and Pachauri (1998) as well as by Sarmah *et al.* (1999) who reported that serum concentration of copper was higher in lactating crossbred cows than pregnant animals.

5.1.3.3 Serum zinc:

Serum zinc levels determined in the present study were more in pregnant animals than in non pregnant (control) animals (table 3). This observation was in close agreement with the recordings made by Bhattacharyya *et al.* (1995), who had reported higher zinc levels in pregnant goats than in non pregnant ones. Mehta and Gangwar (1983) explained that it might be due to an increased concentration of enzyme carbonic anhydrase of which zinc is an integral component. Georgievskii *et al.* (1982) has also reported that zinc and cupric ions can act as specific activators of a number of enzyme systems like carbonic anhydrase and oxidases that assist in maintaining the activity of labile hypophyseal hormones in the blood. In the present study conducted levels of serum zinc exhibited a decreasing tendency, from the third to eighth month of pregnancy (fig.3) which agreed with an observation made by Singh *et al.* (1991) and Rajora and Pachauri (1998) who reported that serum zinc had a decreasing tendency with the advancement of gestation. However, in the present investigation the serum concentration of zinc increased significantly during ninth month of pregnancy when compared with controls and the same was in agreement with the findings of Bhattacharyya *et al.* (1995) who observed a similar pattern for zinc concentration in goats in late pregnancy. Zinc acts as an integral part of many of proteins and enzymes. It is involved as essential component of enzymes involved in steroidogenesis. Song and Adham (1978) concluded that prostaglandin secretions when increased nearing parturition made an elevated levels of zinc in the serum as it was required for the transport of prostaglandins. Zinc can interfere with PG receptor mediated phase and consequently the leuteolytic process (Carlson *et al.*, 1982). These could be the reasons for a significantly high levels of zinc during ninth month of pregnancy observed during this study. In the present study level of serum zinc increased in the first month of lactation which closely agreed with observations of Setia *et al.* (1994) and Sarmah *et al.* (1999) who recorded that after parturition the plasma concentration of zinc showed an increasing tendency in cows as well as in buffaloes.

5.2 Haematological parameters:

Monitoring of haemogram may help in the maintenance and improvement of productive health of farm animals through effective management.

5.2.1 Erythrocyte count:

In the present study, it was recorded that the erythrocyte count fluctuated insignificantly between months and reached the lowest value during the ninth month of pregnancy (table 4). It was observed that this insignificant reduction in erythrocyte count was being compensated by an increase in MCV values. In contrast to this observation Prabhakaran *et al.* (1997) reported an increased erythrocyte counts whereas Johnson *et al.* (1990) did not observe any significant difference in erythrocyte count of pregnant and non-pregnant dairy cows. In the present investigation it was also found that the erythrocyte count further decreased during first month of lactation. This observation was in agreement with the report of Gupta *et al.* (1995) who found that total erythrocyte count was slightly lower in the postpartum period which might be due to the continuous lactational stress.

5.2.2 Haemoglobin (Hb) content:

In the present study the haemoglobin content varied to a greater extent between months of pregnancy and with a decreasing trend to reach a lower value during sixth month and least at ninth month of pregnancy and it continued to decline further during the first month of lactation (table 4). This observation was closely in consonance with the findings of Stilinovic *et al.* (1992) who reported that haemoglobin content had a tendency to decrease during two months before parturition and the trend even continued till 75 days after parturition. This observation made in the present study also agree with the findings of Klinkon and Nemec (1998) who recorded lower values during four days after parturition than at parturition. In the present investigation, it was noticed that the highest value of haemoglobin content was encountered during fifth month of pregnancy which coincided with the highest concentrations of serum iron and copper observed during the same period. In the present study the lower value of Hb content and erythrocyte count during first month of lactation than at ninth month of pregnancy may be due to the continuous stress of lactation. The lower Hb content in the specific periods observed in present study was coincided with the lower erythrocyte count as well as lower iron and copper levels.

5.2.3 Volume of Packed Red Blood Cells (VPRC):

The decreasing trend of volume of packed red blood cells as pregnancy advanced in the present study was in agreement with the observations of Wang Qung Lan *et al.* (1998) who reported that in dairy cows VPRC decreased from three months before calving and reached the lowest value on the day before parturition. The declining trend of VPRC during first month of lactation was also in agreement with the findings of Prabhakar *et al.* (1999) who reported that packed cell volume declined gradually during early postpartum period. In the present study, since the erythrocyte count declined during early lactation due to continuous lactational stress, correspondingly VPRC also decreased in the same period (table 4).

5.3 Erythrocyte indices:

In the present study, the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were not significantly different when compared to controls. This observation was in full agreement with the findings of Klinkon and Nemec (1998) who reported that the MCV, MCH and MCHC values were not significantly affected by pregnancy.

However, insignificant lower values for MCV, MCH, MCHC observed during ninth month of pregnancy and first month of lactation were positively correlated with the erythrocyte count, Hb concentration and VPRC observed during the corresponding period.

Summary

6. SUMMARY

Quantification of certain macro and microelements in serum and estimation of certain haematological parameters were carried out in the crossbred heifers stationed at Kerala agricultural University – Cattle breeding farm, Thumburmuzhi. The elements screened for this study include calcium, phosphorus, magnesium, sodium, potassium, iron, copper, zinc and haematological parameters that were determined being erythrocyte count, haemoglobin content and volume of packed red blood cells. From the hamatological values erythrocyte indices were calculated. The entire evaluations and estimations were carried out at Department of Physiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur.

Among the total number of twenty healthy crossbred heifers selected for the study, eleven animals conceived, which formed the experimental group. Blood was collected from all the animals before they got conceived (control group) and the blood collection was continued (experimental group) at monthly intervals from the third month viz., third, fourth, fifth, sixth, seventh, eighth and ninth month of pregnancy as well as first month of lactation.

The minerals such as calcium, magnesium, iron, copper and zinc were estimated using Atomic absorption spectrophotometer (Perkin-Elmer 2380) and serum inorganic phosphorus by spectrophotometric means using commercial kit whereas sodium and potassium were estimated by using the flame photometer. Erythrocyte count, haemoglobin (Hb) content and volume of packed red cells (VPRC) were determined by standard procedures on the day of blood collection

All the data recorded before and during pregnancy and first month of lactation were statistically analysed.

Serum levels of calcium and phosphorus showed a negative correlation during both the first and second half of pregnancy period. During first half the serum levels of calcium decreased with an increase in phosphorus. However, during second half of pregnancy serum calcium level had increased and then decreased during first month of lactation. High serum calcium during second half of pregnancy might be due to high physiological needs of body for this mineral so as to incorporate it into fetal skeleton and in colostrum. The serum inorganic phosphorus level which was increased during first half of gestation however, decreased during second half and thereafter, continued the same trend in the first month of lactation. Low serum concentration of inorganic phosphorus during pregnancy and lactation might be due to its greater incorporation for growth and production respectively.

From the beginning to end of pregnancy period serum magnesium level was increased. This increase might be due to an increase in the gastrointestinal absorption of magnesium and thereby an increased demand of magnesium for fetal growth as well as for the metabolic needs of the dam with the advancement of pregnancy were being met with.

The serum sodium level was increased from third month upto ninth month of pregnancy and thereafter declined during first month of lactation. The increase in serum sodium during gestation might be due to an increasing demand of this electrolyte for growing fetus and the lower value encountered during first month of lactation might be due to its drainage into milk.

The serum potassium level was gradually decreased from third to eighth month of pregnancy and then increased during ninth month and again decreased during first month of lactation. The decrease in serum potassium during third to eighth month of gestation might be due to an increased permeation of it into the uterus and thereby its drainage into the fetoplacentary unit.

The serum iron level which was increased till mid gestation, declined as pregnancy advanced as well as during early lactation. The increase in serum iron till mid gestation might be due to a greater absorption of iron from the gastrointestinal tract a further decrease could be due to an increase in plasma volume and thereby a haemodilution observed during advanced gestation.

The serum copper level which was increased during first half of gestation, declined during second half of gestation especially upto eighth month and thereafter increased during ninth month of pregnancy and first month of lactation. This fluctuation of serum copper might be due to the influence of estrogen related synthesis of ceruloplasmin.

The serum zinc level was declined from third month of pregnancy upto eight month and thereafter increased during ninth month of pregnancy and first month of lactation. This significant leap during last month of gestation might be due to the higher circulating levels of prostaglandins in the systems.

The erythrocyte count fluctuated insignificantly during different months of gestation to reach a lower value at ninth month of pregnancy and thereafter, further lowered during first month of lactation. This insignificant reduction in erythorcyte count during pregnancy was compensated by an increase in mean corpuscular volume during the same period.

Haemoglobin content exhibited marked variations between different months of gestation and a high value was recorded during fifth month of pregnancy. Then the value reached the lowest level during ninth month of pregnancy which later declined further during first month of lactation. The variations in the haemoglobin content during the period of study was in accordance with the level of iron and copper which were involved in haemoglobin synthesis.

The volume of packed red blood cells (VPRC) showed a declining trend throughout the period of gestation due to an increase in plasma volume and haemodilution observed during pregnancy. VPRC values declined further during first month of lactation which could be due to the stress of lactation. However, the erythrocyte indices were not significantly affected by different stages of pregnancy.

References

REFERENCES

Bahga, C.S. and Singh, N. (1992). Effect of stage of parturition on plasma electrolytes in normal parturient cows. *Int. J. Anim. Sci.* 169-170.

Bar, H.J., Seidel, H. and Muller, I. (1970). Mineral concentrations in the serum of clinically healthy cattle. *Arch. Exp. Vet. Med.* **24**: 889-982.

Baranow-Baranowski, S., Jankowiak, D., Klata, W., Orowicz, W. and Skrzypczak, W.F. (1988). Some physiological and biochemical indices in the blood serum of cows in the perinatal period and in the blood of their calves. II. Mineral elements and alkaline phosphatase activity. *Roczniki Nauk Rolniczych, B. (Zootechniczna)* **104** (1): 19-29.

*Barnea, A., Cho, G. and Colombani-Vidal, M. (1985). A role of extracellular copper in modulating prostaglandin E_2 (PGE_2) actions: facilitation of PGE_2 stimulation of the release of gonadotropin-hormone (LHRH) from median. *Endocrinology* **117**: 415.

Beaty, R.D. and Kerber, J.D. (1993). Concepts, instrumentation and techniques in Atomic Absorption Spectrophotometry. The Perkin-Elmer Corporation, Norwalk, C.T., USA.

Belyea, R.L., Coppock, C.E. and Lake, C.B. (1975). Effects of silage diets on health, reproduction and blood metabolites of dairy cattle. *J. Dairy Sci.* **58**: 1336-1346.

Bhattacharyya, B.N., Talukdar, S.C., Baruah, R.N., Baruah (Sr), K.K., Baruah (Jr), K.K. and Anubha Baruah (1995). Studies on circulatory levels of trace mineals at different reproductive status in goats. *Indian J. Anim. Reprod.* **16**(2): 96-98.

Bigras-Poulin, M. and Tremblay, A. (1998) An epidemiological study of calcium metabolism in non-paretic post parturient Holstein cows. *Prev. Vet. Med.* **35**(3): 195-207.

Blood, D.C. and Radostits, O.M. (1989). Veterinary medicine. A Textbook of Disease of cattle, sheep, pigs, goats and horses. 7th edn. (Asian). ELBS, Great Britain.

*Boestedt, Von, H. and Hausmann, C. (1980). Einfluss Von fortgeschrittener Graviditat, Geburt und fruher Laktationsperiode anf Elektrolytkon Zentrationen im Blutserum Von Schafen Unter Besonderer Berucksichtiguang des Festliegens ante partum. *Prak. Tierarzt. 61*: 482.

Butler, E.J. (1963). The infuence of pregnancy on the blood plasma and ceruloplasmin copper level of sheep. *J. Physiol.* **9**: 1-6.

*Carlson, J.C., Buhr, M.M., Wentworth, R. and Hansel, W. (1982). Evidence of membrane changes during regression in the bovine corpusluteum. *Endocrinology*. **110**: 1472-76.

Daly, J.A. and Ertingshausen, G. (1972). Direct method for determining inorganic phosphate in serum with the "centrifichem". *Clin. Chem.* **18**: 263.

*Deshpande, B.R., Velhankar, D.P., Jadnav, D.S. and Sane, C.R. (1981). *Indian J. Anim. Reprod.* 1:23.

Deshpande, S.M., Mantri, A.M., Talvelkar, B.A., and Deshmukh, B.T. (1998). Studies on macroelements during gestation and early postpartum period in Gir and crossbred cows. *Indian J. Dairy Sci.* **51**(5): 275-279.

Dolezel, R., Kudlac, E., Studencik, B. and Balastik, J. (1991). Biochemical changes in the peripheral blood of cows in the 45 days after parturition. *Veterinarni Medicina*. **36**(5): 265-271.

*Gault, M.H.., Stein, M.H.J. and Aronoff, A. (1966). Serum ceruloplasmin in hepatobiliary and other disorder, significance of abnormal values. *Gastroenterology* **50**: 8-18.

Georgievskii, V.I., Annenkov, B.N. and Samokhin, V.I. (1982). Mineral Nutritionof Animals. Butterworths, London.

Gupta, G.C., Pachauri, S.P. and Rajora, V.S. (1995). Haematological profile in peri-parturient cattle and buffaloes. *Indian J. Anim. Sci.* **65**(11): 1225-1227.

Hackett, P.L., Gazalar, D.W. and Busted, I.K. (1957). Blood constituents in Suffolk ewe and lambs. *Am. J. Vet. Res.* **18**: 338-43.

Hang–Poung, F. (1989). The relationship between the seasonal serum biochemical profile and reproductive performance in perinatal Holstein cows. *Taiwan Journal of Veterinary Medicine and Animal Husbandry* **54**, 1-19.

Harper, H.A. (1969). Review of Physiological Chemistry. 12th edn. Lange Medical Publications, Los Altos, California. 194-217.

Henkin, R.I.S., Meret, S. and Jacobs, J.B. (1969). Steroids dependent changes in copper and zinc metabolism. *J. Clin. Invest.* **48**: 38a.

Hidiroglou, M. (1979). Trace elëment deficiencies and fertility in ruminants – A review. *J. Dairy Sci.* **62**: 1195.

Hidiroglou, M. and Knipfel, J.E. (1981). Maternal foetal relationship of copper, manganese and sulphur in ruminants: A review. *J. Dairy Sci.* **64**: 1637-49.

Ivanov, I., Rajic, I., Jovanovic, M.J. and Lalic, M. (1990). Calcium concentration in the blood serum of cows in advanced pregnancy and during lactation under intensive conditions. *Veterinarki Glasnik.* **44** (5): 359–364.

Jain, N.C. (1986). Schalm's Veterinary Haemtology. Fourth edition. Lea and Febiger, Philadelphia. 36-48.

Jana, S., Bhattacharyya, B., Duttagupta, R. and Moitra, D.N. (1991). A note of some biochemical constituents of blood in pregnant goats reared on extensive management system. *Indian Vet. J.* **68**(6): 592-594.

Johnson, S.K., Johnson, A.R., Keefer, C.L. and Silcox, R.W. (1990). Blood constitutents during the estrous cycle and early pregnancy in dairy cows. *Theriogenology* **34**(4): 701-707.

Kaushik, H.k. and Bugalia, N.S. (1999). Plasma total protein, cholesterol, minerals and transaminases during pregnancy in goats. *Indian Vet. J.* **76**: 603-606.

Klinkon, M. and Nemec, M. (1998). Erythrocyte count in Friesian dairy cows at parturient period. *Veterinarske Novice* **24**(2): 47-50.

Krajnicakova, M., Bekeova, E., Maracek, I. and Hendrichovsky, V. (1994). Sodium and potassium concentrations and their relationship to ovarian hormones in ewes during oestrus synchronisation and pregnancy. *Veterinarni Medicina* **39**(9): 541-550.

*Lavingonzalez, S., Monseai Bosh, L., Abad Gavin, M. and Farnandaz Celadille, L. (1987). Copper values of plasma and hair of cattle in different reproductive states. *Medicine Veterinaria* **4**: 415-18.

Mc Adam, P.A. and O'Dell, G.D. (1982). Mineral profile of blood plasma of Lactating Dairy cows. *J. Dairy Sci.* **65**:1219-1226.

Maynard, L.A. and Loosli, J.K. (1969). Animal Nutrition. 6th edn. McGraw-Hill Company. 246.

*McDowell, L.R. and Conrad, J.H. (1990). *Intl. J. Anim. Sci.*, **5**: 21.

*McLaurin, L.P. and Cotter, J.R. (1967). *Am. J. Obstet. Gynaecol.* **98**: 931-937.

Mehta, S.N. and Gangwar, P.C. (1983). Carbonic anhydrase and acid-base balance in relation to thermal stress in buffaloes. *Int. J. Biomet.* **27**: 41-46.

Mulei, C.M.. (1991). Changes in blood chemistry in late pregnancy and early lactation and their relationships to milk production in dairy cows. *Bulletin of Animal Health and Production in Africa* **39**(1): 77-81.

Murtuza, Md., Pandey, M.D. and Rawat, J.S. (1979). Concentration of certain minerals in the serum of Haryana cattle under various physiological states. *Indian Vet. J.* **56**: 95-99.

Nazifi, S. and Sami, M. (1997). Concentration of serum electrolytes in Holstein cows at late pregnancy, parturition and post-parturition periods. *Journal of Applied Animal Research* **11**(2): 189-193.

Oser, B.L. (1976). Hawk's physiological chemistry. Tata McGraw-Hill Publishing Co. Ltd., New Delhi. 1141.

Package of Practices Recommendations for Livestock, Poultry, Elephant, Rabbit, Dog, Pig and Pets. (1994). Directorate of Extension. Kerala Agricultural University. Mannuthy, Thrissur.

Patel, B.M., Memon, G.N. and Mistry, V.V. (1966). Inorganic phosphorus, calcium and magnesium in the serum of Gir cattle. *Indian Vet. J.* **43**: 795-800.

Pathak, M.M. and Janakiraman, K. (1987). Blood serum calcium, inorganic phosphorus and magnesium at different stages of pregnancy in Surti buffaloes. *Indian J. Anim. Sci.* **57**(5): 398-402.

Prabhakar, S., Nanda, A.S. and Ghuman, S.P.S. (1999). Sequential changes in some blood indices during peripartal period in buffaloes. *Indian Vet. J.* **76**: 1067-1070.

Prabhakaran, V., Chithravel, V., Kokilaprabhakaran, S. and Ravindranath, T.K. (1997). Haemogram of pregnant Jersey heifers. *Indian J. Anim. Hlth.* **36**(2): 185-186.

Rajora, V.S. and Pachauri, S.P. (1998). Monitoring of mineral profiles in a crossbred herd. *Indian J. Anim. Sci.* **68**(3): 251-253.

Rajora, V.S. and Pachauri,S.P. (1994). Blood profile evaluation in crossbred cows under different stages of lactation and gestation. *Indian J. Anim. Sci.* **64**(12): 1351-1353.

Ramnath, V. and Leela, V. (1997). Haematological studies in Tellicherry goats. *J. Vet. Anim. Sci.* **28**: 39-46.

Riond, J.L., Kocabagli, N., Spichiger, U.E and Wanner, M. (1995). The concentration of ionised Magnesium in serum during the periparturient period of non paretic dairy cows. *Vet. Res. Communs.* **19** (3): 195-203.

Rowlands, G.J., Manston, R., Rita, M. Pocock and Sally, M. Dew. (1975). Relationships between stage of lactation and pregnancy and blood composition in a herd of dairy cows and the influences of seasonal changes in management on these relationships. *J. Dairy Res.* **42**: 349-362.

Sahukar, C.S., Pandit, R.K., Chauhan, R.A.S. and Porwal, M.L. (1984). Electrolytes during various reproductive phases in crossbred cows. *Indian J. Anim. Sci.* **54** (10): 993-995.

Sangha, G.K., Sharma, R.K. and Guarya, S.S. (1993). Distribution of trace elements in blood and ovary during the oestrus cycle and pregnancy in house rat. *Indian J. Anim. Sci.* **63**: 1247-1257.

Sarmah, B.C., Kalita, D.J. and Bhattacharyya, B.N. (1999). Certain mineral profile and haemoglobin concentration in local non-descript and crossbred (NxJ) cattle. *Indian Vet. J.* **76:** 291-293.

Saxena, M.S. and Gupta, S.K. (1995). Variations in the plasma levels of copper and zinc in crossbred cows with early and delayed postpartum conception. *Indian J. Anim. Reprod.* **16**(1): 25-27.

Sen, M.M., Ahmed, M.J. and Rahman, A. (1989). Effects of parturition and lactation on blood minerals and glucose status of dairy cattle. *Bangladesh Veterinarian* **6** (1): 10-13.

Setia, M.S., Duggal, R.S., Singh, R. and Singh, R. (1994). Distribution of trace elements in whole blood and blood plasma during late pregnancy and different stages of lactation in buffaloes and cows. *Buffalo Journal* **10**(3): 213-220.

Sikka, P. (1992). Role of minerals in reproduction – A Review. *Indian J. Dairy Sci.*, **45**(4): 159-167.

*Sikka, P. and Mudgal, V.D. (1988). *Indian Dairyman* **40**: 175-77.

Singh, M., Nigam, J.M., Mohinder Singh and Sharma, K.B.. (1999). Blood plasma biochemical profile of pregnant and nonpregnant yaks in comparison with reported values in cattle. *Indian Vet. J.* **76**: 568-570.

Singh, R., Singha, S.P.S., Singh, R. and Setia, M.S. (1991). Distribution of trace elements in blood, plasma and erythrocytes during different stages of gestation in buffalo (*Bubalis bubalis*). *Buffalo Journal* 7(1): 77-85.

Singh, R., Singha, S.P.S., Setia, M.S.., Singh, R. (1995). Distribution of magnesium in whole blood, plasma and erythrocytes during different stages of pregnancy in buffalo (*Bubalus bubalis*). *Buffalo Journal* **11**(1): 71-75.

Snedecor, G.W. and Cochran, W.G. (1994). Statistical Methods. 8th edition, Iowa State University Press, Ames, Iowa.

Soliman, M.K. and El-Amrousi (1965). Blood iron and haemoglobin level in healthy Egyptian sheep, cattle, buffaloes and camels. *Indian Vet. J.* **42**: 832-836.

*Song, M.K. and Adham, N.F. (1978). Role of Prostaglandin F in zinc absorption in the animals. *Am. J. Physiol.* **234**: 99-103.

Stilinovic, Z., Lovrinov, D. and Kronja, M. (1992). Haemoglobin concentrations in the blood of dairy cows during pregnancy and lactation. *Veterinarski Arhiv.* **62**(6): 363-371.

*Symonds, H.W., Manston, R., Payne, J.M. and Sansom, F.B. (1966). Changes in the calcium and phosphorus requirements of dairy cows at parturition with particular reference to the amounts supplied to the foetus *in utero*. *British Vet. J.* **122**: 196-200.

Tainturier, D, Braun, J.P., Rico, A.G. and Thouvenot, J.P. (1984). Variations in blood composition in dairy cows during pregnancy and after calving. *Res. Vet. Sci.* **37**: 129-131.

Takarkhede, R.C., Gondane, V.S., Kolte, A.Y. and Rekhate, D.H.. (1999). Biochemical profile during different phases of reproduction in ewes in comparison to rams. *Indian Vet. J.* **76**: 205-207.

Underwood, E.J. (1981). The Mineral Nutrition of Livestock. Commonwealth Agriculture Bureaux, London.

Wang Qung Lan., Guo KeJun., Jiao Shuxian., Cheng DeMing and Wan Shaoyue (1998). Changes in the main haemorheological values in dairy cows during the perinatal period. *Chinese J. Vet. Sci.* **18**(6): 574-576.

* Originals not consulted

ASSESSMENT OF MINERAL STATUS DURING PREGNANCY IN CROSSBRED CATTLE

By

SHIBU K. JACOB

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the requirement for the degree of

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University

Department of Physiology

COLLEGE OF VETERINARY AND ANIMAL SCIENCES

MANNUTHY, THRISSUR - 680651

KERALA, INDIA

2000

ABSTRACT

Twenty, healthy crossbred heifers stationed at Kerala Agricultural University – Cattle Breeding Farm, Thumburmuzhi, were utilized for the study. The parameters estimated in the experimental animals comprise of serum profile of certain macro and microelements as calcium, phosphorus, magnesium, sodium, potassium, iron, copper, zinc and haematological parameters like red blood cells count, haemoglobin content, volume of packed red cells and erythrocyte indices before conception and at different stages of pregnancy viz., from third month to ninth month at monthly intervals and first month of lactation.

The serum calcium level showed significant variations during the period of study, which decreased from the control level till fifth month of pregnancy and then increased from sixth month of gestation and reached the highest level by ninth month of gestation thereafter, decreased during first month of lactation (table 1). The increased serum calcium level from sixth month of pregnancy and early lactation could be due to an increased demand of this element for the formation of fetal skeleton and organogenesis as well as for drainage into the milk. Serum inorganic phosphorus concentration was increased from third month of pregnancy and the highest level was attained by fifth month of pregnancy. Later the level of serum inorganic phosphorus gradually declined from sixth month to ninth month of pregnancy. These fluctuations in serum phosphorus during pregnancy might be due to the mobilisation of calcium and phosphorus from the dam's skeleton or due to an increased G.I tract absorption during the period .

Besides the utilisation of calcium for fetal growth, calcium and phosphorus play a vital role in the action of hormones and enzymes at subcellular levels in an integrated fashion in reproduction of young heifers. During first month of early lactation still lower content of serum inorganic phosphorus was observed which could be due to its drainage into milk. There was an increase in the serum magnesium concentration from the preconception level during different stages of pregnancy and the same was significantly higher from sixth to ninth month of pregnancy which later dropped a little during first month of lactation as observed in the case of serum calcium. The increased magnesium level during the period of gestation could be due to the increased gut absorption in order to meet increased metabolic demands of this mineral, as many of the enzymes of glycolysis and citric acid cycle require magnesium as a cofactor. The serum concentration of sodium showed an increasing tendency whereas that of potassium showed a decreasing tendency from third to ninth month of pregnancy. However the values of sodium encountered during third to sixth month and that of potassium from third to eighth month of pregnancy were lower than pre conception values which might be due to an increased permeation of these electrolytes into the uterus and finally drainage into the fetoplacentary unit. The serum sodium and potassium level reached highest values during ninth month of pregnancy which further decreased during first month of lactation due to an increased transfer of these electrolytes into milk. An increasing trend was observed for serum levels of iron and copper from third to fifth month of pregnancy. The increased level of serum iron during the first half of pregnancy might be due to an increased absorption from the gastrointestinal tract. It was observed that both these minerals reach their peak serum level by fifth month of pregnancy and towards late pregnancy the iron level decreased which might be either due to its incorporation into the fetus or an increase in plasma volume so that haemoglobin content of blood also got apparently decreased. Serum copper concentration which decreased during the period of sixth to eighth month of pregnancy might be due to an increased blood volume and further increase in serum copper level during the ninth month of pregnancy might be due to the increase in estrogen level during that period as estrogen induce *denovo* synthesis of ceruloplasmin and thereby increased serum copper value during this period. During the first month of postpartum, serum iron level was found decreased while copper concentration increased. As far as serum zinc levels was concerned it was noticed that throughout the pregnancy its level increased when compared to control value. This could be due to an increased concentration of several enzymes involved in steroidgenesis and carbonic anhydrase, a zinc containing enzyme encountered during pregnancy. Eventhough serum zinc content exhibited a decreasing trend from third to eighth month of gestation, eventually it got increased during ninth month which might be due to higher levels of circulating prostaglandins during this period as zinc is required for their transport. A further increase in serum zinc level was noticed during first month of lactation.

The erythrocyte count fluctuated insignificantly during different months of pregnancy which finally reached the lowest value by ninth month of gestation. This reduction was compensated by an increase in the value of mean corpuscular volume during the same period. Marked variations were noticed during pregnancy as far as haemoglobin content was concerned, the highest value of it was recorded during fifth month and lowest value during ninth month of pregnancy. The volume of packed red cells exhibited a declining trend throughout the period of gestation probably because of increased plasma volume and increased drainage of microelements like iron and copper into the fetoplacentary unit required in haemoglobin synthesis. The above mentioned haematological parameters showed a decline during first month of lactation due to stress of lactation. However, the erythrocyte indices were not significantly affected by gestation.