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METABOLIC AND ENDOCRINE PROFILE OF CROSSBRED PRE-RUMINANT CALVES UNDER EXTENDED COLOSTRUM FEEDING

By

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THESIS

Submitted in partial fulfilment of the requirement for the degree of

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DECLARATION

I hereby declare that the thesis, entitled **"METABOLIC AND ENDOCRINE PROFILE OF CROSSBRED PRE-RUMINANT CALVES UNDER EXTENDED COLOSTRUM FEEDING"** 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.

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CERTIFICATE

Certified that the thesis entitled **"METABOLIC AND ENDOCRINE PROFILE OF CROSSBRED PRE-RUMINANT CALVES UNDER EXTENDED COLOSTRUM FEEDING"** is a record of research work done independently by **Dr. Babitha .V.**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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Introduction

1. INTRODUCTION

Colostrum is a nutritious first lacteal secretion of the mammary gland soon after parturition and is of primary significance to the neonate. Compared to normal milk, bovine colostrum contains less lactose but more of other essential nutrients like proteins, notably immunoglobulins (Ig) as gamma globulins, fats, fat soluble vitamins, minerals and supplies passive immunity as well as more energy to the new born. Bovine colostrum is also rich in bioactive factors such as growth factors namely, insulin like growth factors I and II (IGF-I and IGF-II) and epidermal growth factor (EGF); hormones like insulin and prolactin, nucleotides, polyamines and cytokines (Rauprich *et al.*, 2000). Enzymes such as aspartate aminotrasferase (AST), alkaline phosphatase (ALP), gammaglutamyl transferase (GGT), lactate dehydrogenase (LDH) and glutamate dehydrogenase (GLDH) activities are also detected in casein precipitated colostrum (Hammon and Blum, 1998). Colostrum is a highly fortified source of nutrients having seven times protein, twice the total solids, higher content of vitamins and minerals than normal milk. Colostrum being a source of immunity in newborn is believed to have the ability of local protection against enteric disorders and thereby reduce many of the calfhood diseases and even mortality rate by providing high levels of serum immunoglobulins (Ig). Barnum *et al.* (1967) and Woode (1978) suggested that a specific agglutinating antibody in the colostrum may be effective in the

intestinal lumen for preventing the enteric form of colibacillosis and rota viral infections.

In commercial farms, day old weaning of calves is practiced after birth and calves are fed with colostrum according to their body weight. Apart from the needs for their own calves, healthy dairy cows produce 10 to 20 kg extra colostrum with an average dry matter content of 18 per cent, during the first few days of lactation. In the normal practice the first six postpartum milkings collected during the period of transition from colostrum to milk are unmarketable. The surplus colostrum thus available can be effectively preserved by any suitable method and can be used later for feeding the same calf or other neonates. Research works on the effective preservation and utilization of surplus colostrum in dairy farms for the improved growth performance in neonatal calves in Kerala are scanty. Preservation of colostrum by freezing abruptly prevents nutrient break down during storage and provides an indefinite shelf-life. Moreover, acceptability of thawed colostrum by calves was excellent as no appreciable changes occurred during storage by freezing in pH or percentage acidity, fat, total solids, total nitrogen or non-protein nitrogenous components. Thereby sufficient excess colostrum can be made available to feed heifer calves through four to five weeks of age (Foley and Otterby, 1978).

As far as the forestomach of the newborn ruminant is concerned it is rudimentary, small and non functional which allows the absorption of immunoglobulins (Ig) of the colostrum without much alteration and gets

pinocytotically absorbed by intestinal epithelium assuring sufficient passive immunity. About 24 to 48 h after birth, this property is lost due to gradual maturation of epithelial cells and its short lived phagocytotic mechanism.

Higher the proportion/availability of dietary proteins to the host through the total solids of colostrum, greater will be the amplitude of release of growth promoting hormones as somatotropin as well as insulin (Hammon *et al.*, 2000 and Kuhne *et al.*, 2000). Information regarding prolonged colostrum feeding and its effect on physiological parameters in calves are meagre. Surplus colostrum can be considered as a cost free liquid feed of calves since it is unmarketable for human consumption. The intake of colostrum rich in immunoglobulins (Ig) in newborn calves cause considerable metabolic changes depending up on the timing and quantity of colostrum ingestion. This study was undertaken to preserve the surplus colostrum of cows by a suitable method and to feed the preserved colostrum in order to evaluate and correlate the effect of enhanced colostrum feeding on growth, health status, haematological, hormonal and metabolic parameters of pre-ruminant crossbred calves during the first month of life.

Review of Literature

2. REVIEW OF LITERATURE

The first one month of calves' life is very critical, and is of great concern to dairy husbandry men. Colostrum, the vital liquid produced and secreted soon after parturition by dam is much more in quantity as compared to the requirement of calves. About 41 percentage remains unutilized, the potential of which can be exploited by feeding calves beyond the usual practice which is likely to leave the calves in better health. Bovine colostrum contains various essential nutrients, antibodies (gamma globulins), hormones, growth factors, vitamins and minerals that are important for nutrient supply, host defense, growth and for general neonatal adaptation. The available literature with respect to composition, quality and processing of colostrum and the impact of enhanced colostrum feeding on the health status, haematological, metabolic and endocrine profile of neonatal calves have been reviewed in this chapter.

2.1 Colostrum

2.1.1 Composition and quality

Parrish *et al.* (1950) opined that immunoglobulin-G (IgG) concentration in colostrum from Holstein cows at the beginning of their first, second or third lactation were similar, but older cows had more IgG concentration. They observed that dry matter, ash, total protein and whey protein concentrations decreased from first to the third milking (24 h) post partum. They also concluded that protein was the most variable constituent between cows at the

same post partum time. Larson and Kendall (1957) reported that total protein content of first milked colostrum of cattle was highest (1200 g) which was lowered (700 g) after one day of lactation. Roy (1969) and Roy (1970) opined that the first 24^{th} h post partum colostrum was a good source of proteins (particularly immunoglobulins), iron, fat soluble vitamins A, D, and E as well as vitamin B. Foley and Otterby (1978) recorded a decrease in total protein content in colostrum during the first four post partum milkings, with an increase in lactose content. They also suggested that the composition and physical characteristics of fresh colostrum varied with a number of factors, including individual, breed, parity, prepartum ration, length of dry period of cows and time post partum. They also observed faster weight gains for calves fed colostrum stored by freezing compared to calves fed whole milk on equal weight basis which justified the higher total solids of colostrum.

Oyeniyi and Hunter (1978) reported that colostrum had a high content of total solids than normal milk (25 per cent vs 13 per cent). They added that the concentration of lactose was lower in colostrum than normal milk and that the lower concentration of lactose in colostrum was an advantage because lactose can induce the young calf to scour with subsequent death or unthriftiness.

The total protein content in bovine colostrum decreased from 12 per cent on day one to 3.7 per cent on day five post partum (Kholand *et al.*, 1985) According to Grutter and Blum (1991a) bovine colostrum on comparison with milk, was characterized by higher concentrations of insulin like growth factor I (IGF-I), insulin and prolactin and similar concentration of glucagon, but it

contained lower amounts of growth hormone. Singh *et al.* (1993) analysed bovine colostrum and observed that with increasing post partum time, the mean total protein content decreased from 20.4 per cent (four h post partum) to 5.5 per cent (70 h post partum).

Koldovsky (1989), Campana and Baumrucker (1995) and Blum and Hammon (2000) reported that bovine colostrum contained proteins (including immunoglobulins, peptides), essential and non-essential fatty acids (EFA and NEFA), lactose, vitamins and minerals, as well as non-nutrient substances such as peptide hormones, growth factors, cytokines, biologically important proteins (such as lactoferrin), steroid hormones, triiodothyronine (T_3), thyroxine (T_4), nucleotides, polyamines and enzymes. Campana and Baumrucker (1995) reported that bovine colostrum contained less lactose, but more fat, proteins and peptides, fat-soluble vitamins, minerals, various enzymes, hormones, growth factors such as IGF-I and IGF-II, epidermal growth factor (EGF) etc., nucleotides, polyamines and cytokines than mature milk. According to Hammon and Blum (1998) bovine colostrum contained various essential nutrients and supplied newborn calves with energy, immunoglobulins and bioactive factors like growth factors such as IGF-I, IGF-II and EGF, hormones and cytokines.

2.1.2 Quantity

Swannack (1972) and Swannack (1974) reported that average yield of colostrum/milk for the first four days post partum for Holstein cows ranged between 39 to 52 kg. According to Huber (1974) average colostrum yields for heifers and cows were 32.7 kg and 41.7 kg respectively. Yu *et al.* (1976) recorded that average colostrum yield for Holstein heifers was 24 kg and that for Holstein cows was 54 kg.

2.1.3 Processing of colostrum

2.1.3.1 Preservation by deep freezing

Owen *et al.* (1970) opined that when colostrum stored by freezing was fed to calves they gained more body weight when compared to calves fed with whole milk. Carlson and Muller (1976) reported that freezing virtually prevented nutrient breakdown in colostrum during storage. They could not observe any appreciable change in pH or percent acidity, fat, total solids, total nitrogen or non protein nitrogen content in the colostrum stored by freezing. Anon (1977) suggested that cold storage facilities were common in dairy farms and the practice of colostrum storage by freezing and its further use had increased.

Foley and Otterby (1978) reported that sufficient excess colostrum would be available to feed calves through 28 to 35 days of life and opined that freezing was an excellent method for storing surplus colostrum and that it virtually eliminated nutrient loss during storage, providing an indefinite shelf

life for colostrum. Acceptability of thawed colostrum was found to be excellent. They concluded that colostrum could be frozen for future use and remained palatable and nutritious feed for calves. Haines *et al.* (1992) observed that freeze-thawing did not adversely affect immunoglobulin levels in colostrum. Blum and Hammon (1999) reported that colostrum intake was of prime importance in that it transfers passive immunity, nutrients, minerals, vitamins and biologically active substances to the neonatal calves. They opined that colostrum should be ingested immediately after birth for sufficient absorption of immunoglobulins, fatty acids and fat soluble vitamins. Effects on insulin, insulin like growth factor-I (IGF-I) and insulin like growth factor binding proteins (IGFBP) were found to be dependent upon the time and amount of colostrum fed.

Mondal and Verma (2000) opined that even after sufficiently feeding the new-born calves with the nutritious colostrum, an average of 66.85 per cent of it was found as remaining surplus and they suggested setting up of colostrum banks and developing technology to transform it into innovative dairy products.

2.1.3.2 Sterilization by ultraviolet irradiation

Chumachenko *et al.* (1977) observed that ultraviolet (UV) treatment reduced total bacterial count in whole milk and the count of coliforms, staphylococci and enterococci in artificially infected skim milk, depending on the dose applied. Sarkin (1977) also pointed out that sterilization of milk by ultraviolet irradiation was simpler and cheaper than the conventional

pasteurization methods. Caserio *et al.* (1978) opined that ultraviolet treatment of milk, greatly improved its keeping quality by destroying most of the bacterial flora, and concluded that UV irradiation could be used industrially in soft cheese factories that do not have pasteurizers. While, Filipov *et al.* (1986) opined that ultraviolet irradiation for 40 seconds reduced total bacterial count of bulk milk from 41,27,000 to 8,95,700 and was adequate to prevent gastrointestinal disorders associated with milk feeding.

2.2 Physiological importance of colostrum feeding

2.2.1 Effect of colostrum feeding on body weight and health status

Smith and Little (1922) suggested that calves consuming inadequate amounts of colostrum were at increased risk of diseases while Sutton and Kaeser (1946) observed that calves that were fed with colostum for seven days showed faster weight gain upto seven days of age than calves switched from colostrum to whole milk feeding after four days of age. Allen (1948) opined that those calves which received colostrum for 63 days of age gained body weight much faster than calves fed whole and skim milk for 63 days.

Owen *et al.* (1970) found that when calves were fed colostrum continuously for a period of 21 days, body weight was improved by 60 per cent within three weeks, 40 per cent by six weeks and 25 per cent at twelve weeks of age and also observed an improved starter intake. They also reported that colostrum feeding reduced the incidences of calf scour and concluded that feeding colostrum once instead of twice daily, resulted in better weight gain and

reduced the calf scours with both colostrum and milk. Muller *et al.* (1974) reported that calves fed 3.6 kg of colostrum stored by freezing, gained 29 per cent more body weight than calves fed an equal amount of whole milk, in three weeks of age.

Nocek *et al.* (1984) found that calves fed with colostrum having high immunoglobulins had weight gain from birth to day four while those fed with low immunoglobulins had weight loss. According to their observations serum protein and immunoglobulin concentrations were higher for calves which were hand fed with colostrum. They concluded that a positive relationship developed between serum proteins and immunoglobulins at 12 to 24 h, and that mortality was low for all calves receiving colostrum. Contrary to this, Bradley and Niilo (1985) pointed out that there was no apparent increase in the concentrations of immunoglobulins and total proteins on second day post partum serum samples as well as weight gains at 42 days of age, when force-feeding was done with stored colostrum instead of suckling in beef calves of Hereford and Hereford-Angus breeds.

According to Verma *et al.* (1996) fresh and preserved colostrum provided local protection at the intestinal level by preventing enteric disorders and thus reduced calfhood diseases and mortality rate by maintaining higher serum immunoglobulin levels. They also pointed out that the maximum use of available colostrum in calf feeding program must be adopted to reduce the cost of calf-rearing effectively and to provide better growth and health status of calves. Hadorn *et al.* (1997) and Vermorel *et al.* (1998) reported that calves

reared under high density colostrum feeding were generally healthy, with normal cardio respiratory rates and rectal temperatures. Hammon *et al.* (2000) opined that intestinal absorptive capacity was decreased in neonatal calves fed no colostrum. Kuhne *et al.* (2000) explained that the rise of body weight gain in colostrum fed calves was probably due to high intake of energy and protein.

2.2.2 Effect of colostrum feeding on immune status

Howe (1921) found that globulins in colostrum were absorbed in large amounts during the first 24 to 36 hours after birth in calves. Kruse (1970) opined that the adequate passive antibody transfer in neonatal calves depended not only on the amount of colostrum and the age of the calf at the time of ingestion, but also on its immunoglobulin concentration. McCoy *et al.* (1970) suggested that in neonatal calves the gut was impermeable to colostral proteins by the 24^{th} h after birth. They found that gamma-globulin level was significantly higher by seven hours after birth in calves fed with pooled colostrum than calves nursed by their dams. But serum gamma-globulin levels in nursing calves were observed to be higher at 24 to 31 hours than in calves fed pooled colostrum. Corley *et al.* (1977) found that early feeding of colostrum was likely to benefit calves by preventing absorption of enteric pathogens. Foley and Otterby (1978) observed that colostral immunoglobulins exerted a local protective action in the intestinal tract of neonatal calves and that this form of protection can be effected in calves that were no longer able to absorb intact immunoglobulins. Stott *et al.* (1979) found that delay in feeding of colostrum resulted in a corresponding reduction in absorption of

immunoglobulins. They added that the rapid aging and post partum maturation of intestinal epithelial cells was considered to be the cause of a reduction in pinocytotic activity and subsequent reduction in immunoglobulin absorption. Stott *et al.* (1979) as well as Besser *et al.* (1985) reported that non selective transfer of immunoglobulins across the gut was influenced by time of first feeding and immunoglobulins concentration in colostrum. Corbeil *et al.* (1984) observed that calves were born agammaglobulinemic and therefore must receive colostral immunoglobulins to establish immunological protection and that insufficient serum immunoglobulins concentration predisposed calves to health problems. Geene (1984) opined that the total immunoglobulin content (Ig) in cow's colostrum decreased rapidly within 24 h after parturition and types of immunoglobulins as IgG_1, IgG_2, IgA and IgM decreased to 29, 39, 29 and 28 per cent respectively of the values immediately after parturition.

White and Andrews (1986) concluded that the immunoglobulins derived from colostrum was the most important factor in determining the immune status of calf. Sridhar *et al.* (1988) recorded that the globulin concentration increased with the age of calves. Joshi *et al.* (1993) found out that colostrum feeding resulted in significant increase in total serum proteins, serum gammaglobulins as immunoglobulin G (IgG) and immunoglobulin M (IgM) concentrations with their maximum values recorded at 48 h after colostrum feeding in buffalo calves. The serum IgG and IgM concentrations were observed to increase rapidly after colostrum feeding from negligible values of 0.078 g/dl and 0.023 g/dl at birth to maximum values of 1.849 g/dl and 0.27 g/dl respectively within

48 h. Perino *et al.* (1995) reported that plasma protein and IgG concentrations were similar for single and twin crossbred calves at 10 h after birth, but IgG concentration at 24 h were higher in twin calves. They opined that age of dam was associated with plasma proteins and IgG concentrations at 10 h, but had no effect at 24 h. Sex of calf, dam's body condition score and birth weight were not related to plasma protein or IgG values in calves. Todd and Whyte (1995) concluded that the practice of feeding a relatively small volume of colostrum with a high concentration of Ig, to calves within eight hours of birth, successfully prevented hypogammaglobulineamia. They also observed that rapid aging and maturation of intestinal epithelial cells post partum was considered to be the cause of reduction of pinocytotic activity and subsequent reduction of immunoglobulin absorption.

Mee *et al.* (1996) concluded that measurement of total serum protein was one of the most convenient methods of indirectly evaluating the humoral immune status of calves because significant correlation existed among serum total protein content, serum IgG concentration and the risk of neonatal diseases. Morin *et al.* (1997) and Hammon and Blum (1998) reported that neonatal calves fed increased amounts of colostrum had higher concentration of total proteins and globulins on seventh day of age.

Kuhne *et al.* (2000) reported that feeding of increased quantity of colostrum in new born male calves of different breeds as Simmental, Holstein-Friesian, Limousine, Bravnvich and Brown-Swiss resulted in greater supply of nutrients to the body together with greater amounts of bioactive and growth

promoting substances. It had influenced neonatal metabolism and growth in a greater manner than a high density milk replacer feed containing only small amounts of bioactive and growth promoting substances. They concluded that colostrum was important for providing sufficient passive immunity, enhanced developmental changes and improved postnatal metabolism of proteins and fat in calves.

2.2.3 Effect of colostrum feeding on haematological traits

Dobsinka *et al.* (1977) and Sridhar *et al.* (1988) recorded that haematological parameters including total erythrocyte (RBC) count, volume of packed red blood cells (VPRC) and haemoglobin (Hb) values were significantly higher in the new born but 40 h after feeding colostrum, the values were reduced, and justified that this was possibly due to the intake of fluids in the form of colostrum. Sridhar *et al.* (1988) reported that haematological traits in new born healthy calves at fixed time intervals showed decreasing trends especially in the values of Hb concentration, VPRC and RBC count till one week of age.

Knowles *et al.* (2000) concluded that at birth,VPRC, Hb content and RBC count in the calves with high total WBC count group had higher values than the reference range, but the values were lowered within few days after birth. They pointed out that count of neutrophils and WBC roughly

paralleled each other in calves at birth and tended to be above the upper limit of the reference range. Kuhne *et al.* (2000) and Rauprich *et al.* (2000) justified that the decrease of plasma haemoglobin concentration in neonatal calves fed increased amounts of colostrum was probably as a result of haemodilution after colostrum intake.

2.2.4 Effect of colostrum feeding on certain biochemical parameters

2.2.4.1 Protein profile

Sridhar *et al.* (1988) reported that the serum concentrations of total proteins and globulin showed an increasing trend, with the initial decrease in albumin-globulin ratio but later an increase upto one week of age. According to them, plasma globulin level alone cannot be considered as a measure for identifying the future susceptibility of new born calf scours. Hadorn *et al.* (1997) also pointed out that there was an increase in plasma albumin concentration on seventh day in calves fed with increased amounts of colostrum which was probably due to an enhanced hepatic protein synthesis. Morin *et al.* (1997) and Hammon and Blum (1998) reported that there was an increase in the concentration of total proteins and globulins in the neonatal calves at seventh day of age when the neonatal calves were fed increased quantity of colostrum. Lents *et al.* (1998) opined that plasma protein concentration in Hereford and Hereford Angus neonatal calves was increased after ingestion of colostrum. They also observed that consumption of milk soon after birth increased protein

concentration in blood plasma and enhanced survival of the calves maintained under standard farm conditions.

Hammon *et al.* (2000) observed that total plasma protein content increased from first to third colostrum intake within 24 h and remained high during first week of life. Rauprich *et al.* (2000) noticed a higher plasma protein, immunoglobulins and urea levels on enhanced colostrum feeding which had reflected not only higher protein intake but also higher protein synthesis and turnover in male calves of Simmental, Holstein, Brown Swiss and Holstein-Friesian. Knowles *et al.* (2000) also recorded that the plasma protein level in calves increased from the lower value of 10 g/l to 61 to 81 g/l soon after the intake of colostrum and the values were lowered in 13 to 70 days of age. Later, Zanker *et al.* (2000) believed that higher serum total protein concentration as a result of high colostrum intake in calves could be due to enhanced absorption of immunoglobulins, indicating an overall improved protein status and its prolonged effect.

2.2.4.2 Lipid profile

Webb *et al.* (1969) opined that an inverse relationship existed between blood glucose and serum non-esterified fatty acids (NEFA) and NEFA are mobilized, when glucose and other energy substrates were less available before the first colostrum intake. Kurz and Willett (1991) found that triglycerides and cholesterol concentration increased from birth to 24 h in both groups of calves fed colostrum within one hour (first group) as well as 12 hours (second group)

after birth and the values continued to increase in the first group until 144 hours. Blum *et al.* (1997) concluded that a delayed rise of triglyceride and cholesterol concentration in new born calves was observed if colostrum was fed with a delay of 24 h after birth and they interpreted that it would be due to the consequence of reduced fat digestion and decreased fatty acid absorption. Hammon and Blum (1998) concluded that various metabolic and endocrine traits were influenced by colostrum feeding in male calves during the first week of life. They also observed that plasma triglyceride concentration on day two increased significantly more in the group in which calves were fed colostrum twice daily for three days (where colostrum was fed to calves only as their first meal) when compared to the group where only milk replacer was fed to calves. Lents *et al.* (1998) concluded that concentrations of non-esterified fatty acids (NEFA) were greatest on day one but decreased by day two of age (850 vs 283 meq/l for day one and day two respectively) and remained unchanged through 14 days of age in Hereford, Hereford x Angus calves maintained under standard farm conditions. Blum and Hammon (2000) speculated that bioactive components such as insulin like growth factor – I (IGF-I) and insulin in colostrum, modified digestion and absorption of fatty acids by possibly allowing lipase activity or fatty acid binding proteins.

Hammon *et al.* (2000) found that plasma non-esterified fatty acids (NEFA) concentrations were decreased during the first week of life when colostrum intake was delayed in neonatal calves. Rauprich *et al.* (2000) investigated the effects of enhanced first colostrum feeding on growth, health

status and metabolic and endocrine traits in male calves (Simental, Holstein, Brown Swiss, Holstein-Friesian) during their first week of life and noticed that there was higher lipid concentration in calves on enhanced colostrum feeding which had reflected not only higher fat intake but also higher lipid synthesis and turnover. They also observed an improved lipids status in neonatal calves as a consequence of intake of large amounts of first colostrum during the first week of life. Knowles *et al.* (2000) found that the non-esterified fatty acids (NEFA) concentration at birth were above the upper reference range, the normal range being 0-600 μmol/l; but the concentration decreased rapidly to about the middle of the reference range for cattle. According to them the levels of triglycerides in the calves were always within the reference range for cattle (0-20 mmol/l) and pointed out that the levels were highest at birth, at 0.4-0.5 mmol/l, but after three days they remained roughly in the range 0.2 to 0.3 mmol/l. Kuhne *et al.* (2000) explained that the higher fat content of first colostrum and intake of great amounts of colostrum immediately after birth markedly improved fat absorption and fatty acid status in neonatal calves. They observed a high non-esterified fatty acids (NEFA) concentration in neonatal calves soon after birth followed by a rapid decline after the first meal of colostrum in these calves because there was mobilization of fat reserves for energy demands, but after receiving nutrients in the form of colostrum or milk, NEFA concentration reduced due to lesser mobilization of fat for energy purposes.

2.2.4.3 Blood glucose

Ratcliff *et al.* (1958) reported that the blood glucose values in female Holstein neonatal calves which were fed with whole milk declined upto five weeks of age and increased thereafter whereas, the values for their male counterparts declined for upto eight weeks. Young *et al.* (1970) observed that both glucose and reducing sugars were significantly affected by age. They found that at one to two hours after birth, glucose and reducing sugars in whole blood averaged 62 and 127 mg/100 ml, respectively. The glucose in both plasma and corpuscles was found to be increased after birth and reached the peak level at day two and thereafter reduced with age.

Daniels *et al.* (1974) observed an increase in blood glucose concentration of calves during first 30 min of life and a subsequent decline. Massip (1980) investigated the endocrine and metabolic changes in calves of Blue Belgian breed, at the time of birth. He opined that mean plasma glucose concentration increased significantly within one hour of birth. The plasma glucose concentration at one hour after birth showed a negative linear relation with pH and a significant positive linear relation with cortisol. He concluded that at the time of birth there was an increased activity of the adrenal gland.

Girard (1986) opined that the increase in blood glucose level (BGL) after colostrum feeding despite a smaller lactose intake in calves during their first week of life could be due to the enhanced gluconeogenesis by glucagon, the concentrations of which was enhanced by colostrum intake.

Kurz and Willett (1991) conducted an experiment on two groups of calves, i.e., one group fed colostrum at one hour after birth and another group fed colostrum at 12 h after birth. They observed that plasma glucose concentration were lower at birth for both groups, but increased substantially from birth to 24 h. Increased insulin concentration was found associated with first feeding.

Grutter and Blum (1991b) and Tivey *et al.* (1994) observed that colostrum intake in high amounts and immediately after birth were shown to have prolonged positive influence on plasma glucose levels during the first week of life in calves which may be due to the stimulation of small intestinal lactase activity, thereby enhanced lactose digestion and absorption of glucose and lactose. Swenson and Reece (1996) observed that excessive glucose is stored as inactive glycogen mainly in the liver and little in the muscles. Hammon and Blum (1998) concluded that various metabolic and endocrine traits were influenced by colostrum feeding and the duration of colostrum feeding in the first week of life of male calves. They observed that post prandial glucose concentration on day two increased significantly more in the group in which calves were fed colostrum twice daily for three days where colostrum was fed to calves only as their first meal when compared to that group where only milk replacer was fed to calves and no colostrum. Lents *et al.* (1998) found out that plasma glucose concentrations increased from day one to day two of age (77 vs 108 mg% for day 1 and 2) but did not differ from 2 to 14 days

of age in Hereford, Hereford x Angus calves maintained under standard farm conditions.

Hammon *et al.* (2000) reported that the plasma glucose concentration was increased in delayed colostrum intake in neonatal calves from first to third colostrum intake. Knowles *et al.* (2000) found that the level of blood glucose was always above the upper reference range at birth but tended to decrease with age (2.8-3.6 mmol/l).

2.2.4.4 Metabolites

Carlson and Muller (1976) justified that the increase of serum urea nitrogen after first colostrum intake was due to the higher protein degradation and amino acid deamination, probably a consequence of the high intake of crude protein and amino acids that could not be utilized for protein synthesis. Kurz and Willett (1991) observed that the serum concentration of creatinine was decreased whereas that of bilirubin increased from birth to 24 h in calves fed colostrum at one hour and 12 hours after birth.

According to Kaneko *et al.* (1997) creatinine determination had one advantage over urea determination, that it is not affected by a high protein diet. Knowles *et al.* (2000) opined that the level of creatinine was roughly twice the upper limit of the reference range (44-165 µmol/l) at birth, but had decreased to within the reference range for adult cattle within 24 h. They found that the mean level of blood urea was always below the reference range for cattle (3.4-7.3 mmol/l) but showed a distinctive pattern of change. They also

observed that there was a rapid decrease of the concentration from birth to six days of age.

2.2.5 Effect of colostrum feeding on hormones

2.2.5.1 Thyroid hormones

Nathanielsz (1969) opined that the level of thyroxine in the blood represented the algebraic sum of thyroxine secretion and peripheral utilization and it varied considerably between one and six and a half days of age in the young calf. Hoch (1974) opined that thyroid hormones stimulate the basic metabolic rate via the metabolism of carbohydrates, lipids and proteins. Kahl *et al.* (1977) demonstrated that the concentration of thyroid hormones in plasma decreased during the first four weeks of life of both male and female calves from an early postnatal peak and reached a nadir at about six weeks of age. They conducted an experiment to compare changes in the plasma concentration of T_3 (triiodothyronine) and T_4 (thyroxine) between male and female Holstein calves from birth to 22 weeks of age. They observed that calves had increased concentration of T_3 and T_4 at birth (140 µg/l and 5.48 µg/l respectively) which declined rapidly to $1/5^{th}$ of initial concentration at one week of age

Kahl and Bitman (1983) observed that thyroxine and triiodothyronine concentration in blood plasma of male and female pure bred dairy calves exhibited a diurnal rhythm and were positively correlated with body weight during initial growth period upto 22 weeks of age. Hammon and Blum (1998) studied effects of colostrum feeding for different durations, on selected

metabolic and endocrine traits in the first week of life in male calves and observed that plasma triiodothyronine (T_3) and thyroxine (T_4) concentrations were reduced in the first week of life in all calves. However, Blum and Hammon (2000) opined that plasma thyroid hormones concentration were not influenced either by feeding different amounts of colostrum or by delaying colostrum feeding or by fasting.

2.2.5.2 Insulin

Young *et al.* (1970) indicated that plasma insulin showed a linear relationship with blood glucose and total reducing sugar levels, but there was no significant relationship between age and plasma insulin levels of Holstein neonatal calves maintained in farm condition. Guerino *et al.*, (1991) opined that increased insulin secretion would be utilized for protein anabolism and triglyceride synthesis. They also demonstrated a positive relationship between amino acid absorption and pancreatic insulin secretion. Mears (1993) believed that greater insulin responses in colostrum fed calves could be a consequence of enhanced insulin secretion as a result of greater nutritional intake during the entire week. According to Hadorn *et al.* (1997) factors such as feeding, energy intake, protein intake and gastro-intestinal hormones modified insulin secretion in colostrum fed calves.

Hammon and Blum (1998) concluded that various metabolic and endocrine traits were influenced by the duration of colostrum feeding and that

the plasma insulin concentration on day two significantly increased in male calves fed colostrum twice daily for three days as their first meal.

Hammon *et al.* (2000) reported that plasma concentrations of IGF-I and insulin like growth factor binding proteins were markedly dependent on the time of first colostrum feeding and IGFBP-2/IGFBP-3 ratio was higher and that plasma concentration of IGF-I and insulin were lower as there was a delay of 24 h in the first colostrum feeding. According to Kuhne *et al.* (2000) calves on intensive colostrum feeding had an elevated serum concentration of insulin due to the accelerated pancreatic development.

Materials and Methods

3. MATERIALS AND METHODS

3.1 Colostrum and milk

3.1.1 Collection and preservation of colostrum

Sufficient amount of colostrum was collected from recently calved healthy cows for the first three days (first six milkings) and pooled in hygienic conditions (two to three months ahead of the period of study). Colostrum so collected was sieved and transferred into cleaned, dried, sterile plastic containers (1.5 litre) and preserved air tight in deep freezer at –20° C, until fed to the calves.

3.1.2 *Analysis of pooled colostrum for crude protein and microbial count*

Samples of pooled colostrum and milk were evaluated for their crude protein content by Kjeldahl method (A.O.A.C., 1990) and tested for microbial count in $\log_{10}$ value of colony forming units per millilitre of colostrum ($\log_{10}$ *cfu/ml*) using pour plate method prescribed by American Public Health Association (APHA, 1976). Microbial count of irradiated colostrum was also checked.

3.2 Experimental animals

The study was conducted in twelve numbers of clinically healthy crossbred neonatal calves of either sex of University Livestock Farm (ULF), College of Veterinary and Animal Sciences, Mannuthy for a period of thirty days.

Calves were selected from those born as singles from cows with pregnancies of normal duration and parturition. They were separated from their dams immediately after birth, weighed and held on straw litter in individual boxes. The new born calves were randomly divided into two groups; Group I (control) and Group II (experimental), comprising six numbers each with equal number of both sexes. Calves weighing more than 20 kg at birth were assigned for the study.

About two to three hours before feeding time, containers having colostrum were submerged in warm water bath (47°C), for thawing and temperature of the thawed colostrum was 40°C. The amount of colostrum computed as per the body weight of individual calves was taken in a stainless steel tray and irradiated under ultraviolet (UV) lamp (10 cm/watt; height – 2 cm; thickness of sample – 1 cm) for 30 min in order to control microbial population. Calves of Group I and II were fed fresh colostrum (one-tenth of the body weight) in feeding pail, twice daily for the first three days. Thereafter Group I calves were fed with whole milk (one-tenth of body weight) twice daily as under standard farm conditions for 30 days of age whereas, thawed, UV irradiated colostrum was fed (one-tenth of body weight) to the calves of Group II twice daily (08.00 h and 15.00 h), till 30 days (one month) of age. Daily intake of colostrum and milk in calves of both groups were recorded. From 15 days of age, calf starter (tables a and b) at the rate of 250 g was made freely accessible to all the calves in both the groups daily. The calves were provided with water *ad libitum* during the period of study and were maintained under standard farm conditions.

Table a. Composition of the calf starter fed to the neonatal crossbred calves during the experimental period

Sl. No.	Ingredients	Kilogram
1.	Finely ground maize	45
2.	Groundnut cake	35
3.	Fish meal	8
4.	Wheat bran	10
5.	Mineral mixture	2
	Total	100

To every 100 kg of mixture added 0.5 kg common salt and 25-30 g of Vit. A, B_2, D_3 supplements

Table b. Chemical composition of the calf starter (on DM basis) fed to the neonatal crossbred calves during the experimental period

Sl. No.	Nutrients	Percentage
1.	Crude protein	23
2.	Crude fat	4
3.	Crude fibre	7
4.	Total ash	5
5.	Nitrogen free extract	61
	Total	100

The entire experiment was carried out in a span of four months during the cooler parts of the year (November, 2001 to February, 2002)

3.3 Evaluation of clinical health status and body weight of calves

The calves were daily watched for their health status. Heart rate (pulse), respiratory rate and rectal temperature of calves in Group I and II were recorded daily in the morning at a fixed time (07.30 h). The body weight of calves in both groups were recorded initially on the day of birth and thereafter at weekly intervals (7, 14, 21 and 28 days of age), till the end of the experimental period and body weight gain was calculated.

3.4 Blood collection

From all animals of Group I and II, blood samples (10 ml each) were collected with and without anticoagulant by jugular *venepuncture* immediately after birth (within 10 min before colostrum was fed) and repeated on day 1, 6, 12, 18, 24 and 30. Except for the first blood collection (just after birth), all other blood collections were made during a fixed time (14.00 h).

Blood samples collected with anticoagulant (heparin 20 U/ml of blood) were subjected to the estimation of various haematological parameters. Blood samples collected with anticoagulant sodium flouride (10 mg/ml of blood) were subjected for the estimation of blood glucose level. Serum was separated from blood samples collected without anticoagulant, by centrifuging at 3000 r.p.m. for 30 min. Serum samples were aliquoted and stored at –20°C till further analysis.

3.5 Estimation of haematological parameters

Total erythrocyte (RBC) count, total leucocyte (WBC) count and volume of packed red blood corpuscles (VPRC) were determined on the day of blood collection as per the standard procedures (Jain, 1986).

The concentration of haemoglobin (Hb) was estimated by cyanmethaemoglobin method as suggested by Van Kampen and Tijlstra (1965), using Haemocheck Kit (M/s Agappe Diagnostics, India).

Erythrocyte indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated using standard formulae (Swenson and Reece, 1996).

3.6 Estimation of biochemical parameters

3.6.1 Protein profile

3.6.1.1 Total protein: Total protein content of serum was estimated by Biuret method, as suggested by Henry *et al.* (1957) using Ecoline® Kit (M/s E. Merck (India) Limited, Mumbai).

3.6.1.2 Albumin : Concentration of serum albumin was estimated by Doumas method, as described by Doumas *et al.* (1971) using Ecoline® Kit (M/s E. Merck (India) Limited, Mumbai).

3.6.1.3 Globulin and Albumin and globulin (A:G) ratio

The serum globulin content was determined by substracting serum albumin level from the total serum protein content and subsequently, A:G ratio was calculated.

3.6.1.4 Fractionation of serum protein by Agarose Gel Electrophoresis

The serum samples were subjected to gel electrophoretic fractionation of different plasma proteins using 1% agarose in Tris-HCl buffer (pH 8.6) for constructing the gel. After subjecting for 2 h electrophoretic run (30 mA, 150 V), the gel was fixed in methanol (30 min) and air dried (80°C, overnight). The slides were stained with 1% amido black (30 min) and destained using 5% acetic acid till the protein bands were clearly visible (Talwar, 1983).

3.6.2 Lipid profile

3.6.2.1 Total lipids: Concentration of serum total lipids was estimated by phosphovainilline method, as described by Chabrol (1961) using Labkit® Kit (M/s Labkit, Spain).

3.6.2.2 Cholesterol: The concentration of total serum cholesterol was estimated by cholesterol phenol aminoantipyrine (CHOD-PAP) method as suggested by Richmond (1973) using Ecoline® Kit (M/s E. Merck (India) Limited, Mumbai).

3.6.2.3 Triglyceride: Concentration of serum triglyceride was estimated by the method suggested by Schettler and Nussel (1975) using Ecoline® Kit (M/s E. Merck (India) Limited, Mumbai).

3.6.2.4 Non-esterified fatty acids (NEFA): Serum NEFA concentration was estimated by a method suggested by Faholt *et al.* (1973) using copper soap formation.

Principle

Serum is extracted with a chloroform-heptane-methanol mixture in the presence of a phosphate buffer to eliminate interference from phospholipids and the extract was shaken with a high density copper reagent (pH 8.1). The copper soaps remain in the upper organic layer from which an aliquot is removed and the copper content determined colorimetrically with diphenyl carbazide.

Reagents required

1. Extraction solvent containing chloroform, heptane and methanol (5:5:1) was prepared.
2. Phosphate buffer (pH 6.4, 33 mmol/l). 2 volumes of potassium dihydrogen phosphate (4.539 g/l) were mixed with 1 volume of disodium hydrogen phosphate dihydrate (5.938 g/l) to prepare the buffer.
3. Stock copper solution (500 mmol/l). 12.07 g of copper nitrate trihydrate $(CuNO_3)_2.3H_2O$) was dissolved in distilled water and the volume was made to 100 ml with distilled water.
4. Triethanolamine solution (1 mol/l). 10 ml of triethanolamine was diluted to 100 ml with distilled water to prepare 1 mol/l solution.

5. Sodium hydroxide solution (1 mol/l). 4 g of sodium hydroxide was dissolved in distilled water and the volume was made to 100 ml using distilled water.

6. Copper reagent: 10 ml of stock copper solution, 10 ml of triethanolamine solution and 6 ml of sodium hydroxide solution were mixed and diluted to 100 ml with distilled water to which, 33 g of sodium chloride was added and the pH was adjusted to 8.1, using 1 mol/l sodium hydroxide solution.

7. 1, 5 Diphenylcarbazide solution (4 g/l in ethanol). 40 mg of diphenylcarbazide was dissolved in 10 ml ethanol to which 0.1 ml of triethanolamine solution was added (prepared immediately before experiment).

8. Stock standard palmitic acid solution (2 mmol/l). 51.2 mg of palmitic acid was dissolved in the extraction solvent and the volume was made to 100 ml using extraction solvent. This solution was stored in a tightly stoppered container.

9. Working standard palmitic acid solution. 5 ml of stock standard palmitic acid solution was diluted to 20 ml with extraction solvent to give a solution containing 500 µmol/l (prepared freshly).

Procedure

1. To 50 µl serum in a suitable stoppered centrifuge tube, 1 ml phosphate buffer and 6 ml extraction solvent were added. At the same time, 50 µl

working standard palmitic acid solution was also prepared in another centrifuge tube in the same fashion.

2. The tubes were shaken vigorously for 90 sec, left undisturbed for 15 min and then, centrifuged at 4000 rpm for 10 min.
3. The buffer was carefully removed by suction and 5 ml of extraction solvent settled at the bottom of the tubes, was transferred to a similar dry centrifuge tube to which 2 ml of copper reagent was added.
4. The tubes were shaken vigorously for 5 min and then, centrifuged at 3000 rpm for 5 min.
5. Three ml of the upper layer was transferred to a tube containing 0.5 ml phenyl carbazide solution and mixed carefully.
6. The reading was taken after 15 min at 550 nm in a spectrophotometer (UV-VIS Spectrophotometer 118, Systronics)

Calculation

$$\text{Serum NEFA } (\mu\text{mol/l}) = 500 \times \frac{\text{Reading of unknown}}{\text{Reading of standard}}$$

3.6.3 Blood glucose level (BGL): Blood glucose level (BGL) was estimated by glucose oxidase peroxidase method (GOD-POD method), as suggested by Mayne (1994a) using Ecoline® kit (M/s E. Merck (India) Limited, Mumbai).

3.6.4 Serum urea nitrogen (BUN) content : Serum urea nitrogen (BUN) content was estimated by diacetyl monoxime (DAM) method, as suggested by Mayne (1994b) using Urea Kit® (M/s Dr. Reddy's Laboratories, Hyderabad).

3.6.5 Serum creatinine : Serum creatinine was estimated by kinetic method of Jaffe reaction without deproteinisation as described by Helger *et al.* (1974) using Merckotes® Kit (M/s E. Merck (India) Limited, Mumbai).

3.6.6 Serum bilirubin level: Serum bilirubin level was estimated by the method of Jendrassik and Grof (1938) using Merckotest® Kit (M/s E. Merck (India) Limited, Mumbai).

3.7 Hormonal profile

3.7.1 Thyroxine (T_4), Triiodothyronine (T_3) and Thyroxine: Triiodothyronine (T_4:T_3) ratio: Serum concentrations of T_4 and T_3 were estimated using the gamma coat T_4 and T_3 radioimmunoassay commercial kit (M/s Diasorin, Minnesota, USA). Thyroxine:Triiodothyronine (T_4:T_3) ratio was calculated.

3.7.2 Insulin: Serum insulin concentration was estimated using radio immuno assay commercial kit (INSIK-5®) (M/s Diasorin, Saluggia, Italy).

Inter and intra assay coefficients of variation while determining the above mentioned three hormones were found to be less than 10 per cent.

3.8 Statistical analysis

The data recorded were statistically analysed by employing students paired and unpaired *t* tests for comparing both within the group and between groups respectively (Snedecor and Cochran, 1989).

4. RESULTS

4.1 Colostrum and milk

4.1.1 Effect of ultra-violet irradiation on total viable count of pooled colostrum

Effect of ultra-violet irradiation on total viable count of pooled colostrum is given in table 1 and fig.1. Mean values of total viable count of pooled colostrum samples before UV irradiation reduced significantly ($P<0.05$) from 5.65 ± 0.50 $\log_{10}$ cfu/ml ($\log_{10}$ of colony forming units per ml) of colostrum to a value of 4.35± 0.29 $\log_{10}$ cfu/ml of colostrum after UV treatment.

4.1.2 Crude protein content of colostrum and milk

The crude protein content of fresh colostrum samples taken from healthy cows from first, third, fifth milkings, pooled colostrum (0, 1 and 2 days) sample and that of whole milk (ninth milking) analysed by Kjeldahl method are given in table 2. The mean values of crude protein content reduced from the first milking (14.23 ± 1.25 g%) through third (7.34 ± 1.35 g%) and fifth (5.15 ± 0.36 g%) milkings. Crude protein content of pooled colostrum sample was 9.51 ± 1.44 g%, and that of milk (fifth day) sample was 2.63 ± 0.28 g%. The crude

Table 1. Effect of ultra-violet irradiation on *total viable counts of pooled colostrum* (*n=6 per group*)

Sample No.	*log_{10} cfu/ml of colostrum	
	Before U.V. irradiation	After U.V. irradiation
1	5.13	4.81
2	5.65	4.45
3	5.47	3.97
4	5.18	4.15
5	6.05	4.44
6	6.40	4.29
Mean ± SD	$5.65^{a} \pm 0.50$	$4.35^{b} \pm 0.29$

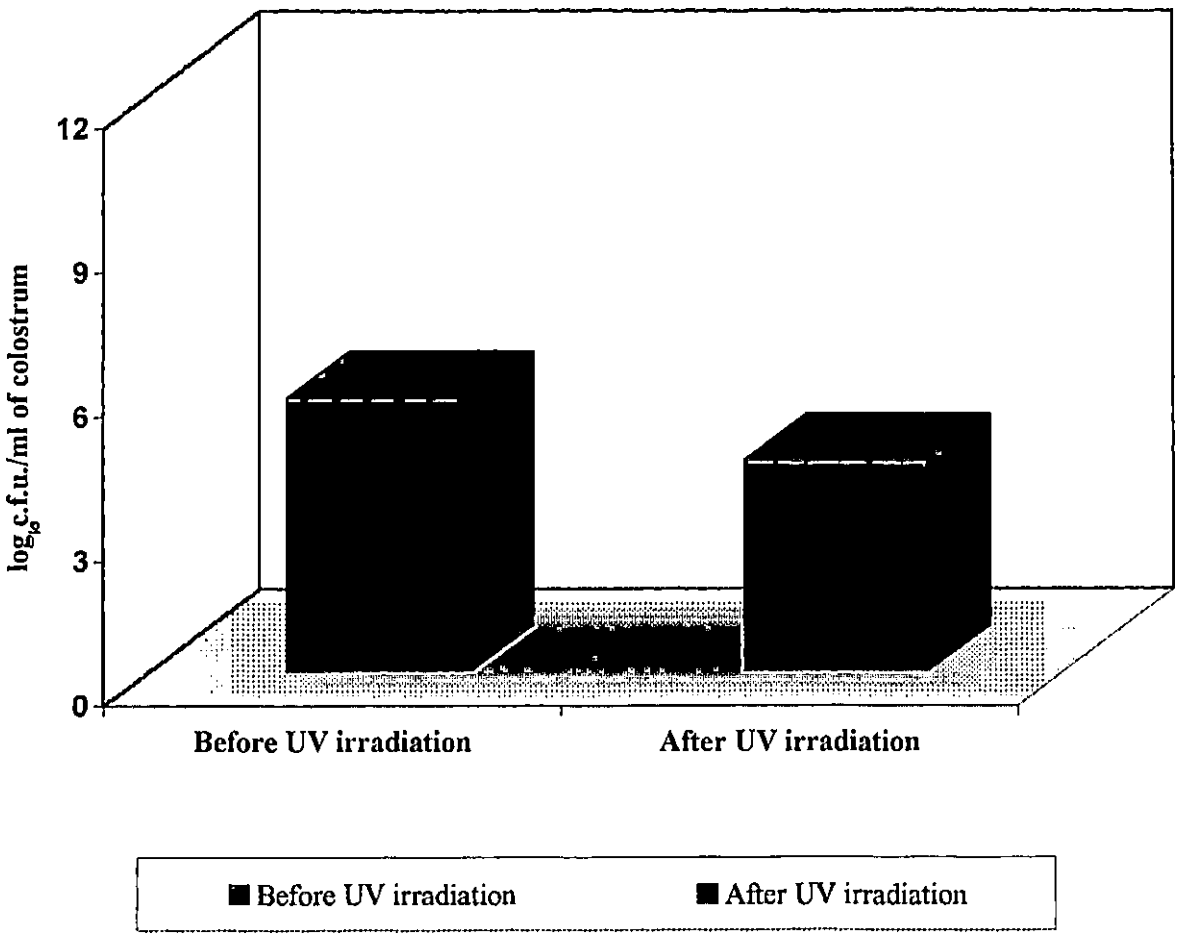
Mean ± standard deviation bearing different superscripts in the same row differ significantly ($P<0.05$)
*log_{10} value of colony forming units/ml of colostrum.

Table 2. Average crude protein content of colostrum and milk samples of cows at different days of post partum milkings (n=6 per group)

Milking	Post partum sampling day	Protein content (g%) (Mean ± SD)
1	0	$14.23^{a} \pm 1.25$
3*	1	$7.34^{b} \pm 1.35$
5*	2	$5.15^{c} \pm 0.36$
	Pooled samples (day 0, 1 and 2)	$9.51^{d} \pm 1.44$
9*	Milk 5th day	$2.63^{e} \pm 0.28$

*Morning
Mean ± standard deviation in columns bearing different superscripts differ significantly ($p<0.05$)

Fig.1. Effect of ultra-violet irradiation on total viable count of pooled colostrum



protein content of all the samples of colostrum and milk showed significant ($P<0.05$) variation between each other.

4.2 Experimental animals

4.2.1 Health status

Clinical parameters of all the animals irrespective of the groups, were within the normal range. Range (mean values) observed for respiratory rate per minute was between 32.11 ± 0.70 to 34.67 ± 0.67, heart rate per minute was between 90.39 ± 1.01 to 99.89 ± 1.51 and rectal temperature in °F was between 102.48 ± 0.27 to 103.31 ± 0.22 (table 3). There were no significant variation between periods or groups for any of the parameters as respiratory rate, heart rate and rectal temperature.

4.2.2 Weekly body weight

Effect of continued feeding of colostrum (group II) and milk (group I) on weekly body weight of neonatal crossbred calves for four weeks of age are given in table 4 and fig.2. The mean body weight of control animals of group I, (which were fed colostrum for the first three days post partum and then milk for 30 days of age) recorded soon after birth was 26.33 ± 3.93 kg and was lower than that of experimental calves of group II which were continuously fed colostrum from birth to 30 days of age (27.17 ± 3.31 kg). The body weight of calves of both group I and II showed a progressively increasing trend till the end of the experiment. The body weight

Table 3. Clinical health status of neonatal calves with normal and prolonged colostrum feeding for 30 days of age, Mean ± SD (n=6 per group)

Period (Age in days)	Respiratory rate (per min)		Heart rate (per min)		Rectal temperature (°F)	
	Group I	Group II	Group I	Group II	Group I	Group II
1 – 3	32.66 ± 1.76[a]	34.44 ± 2.83[a]	90.64 ± 2.20[a]	90.94 ± 5.03[a]	103.04 ± 0.43[a]	102.71 ± 0.30[a]
4-6	34.17 ± 0.29[a]	34.67 ± 0.67[a]	92.05 ± 4.35[a]	98.55 ± 1.68[a]	102.57 ± 0.17[a]	102.69 ± 0.10[a]
7-9	33.44 ± 0.10[a]	33.04 ± 0.45[a]	99.89 ± 1.51[a]	93.00 ± 4.73[a]	103.19 ± 0.09[a]	102.73 ± 0.35[a]
10-12	32.11 ± 0.70[a]	33.33 ± 1.67[a]	91.33 ± 5.37[a]	99.11 ± 3.96[a]	102.79 ± 0.33[a]	102.75 ± 0.18[a]
13-15	32.72 ± 0.67[a]	33.44 ± 1.26[a]	97.50 ± 5.39[a]	94.22 ± 4.53[a]	103.25 ± 0.36[a]	102.48 ± 0.27[a]
16-18	34.11 ± 1.67[a]	32.83 ± 0.76[a]	92.94 ± 5.83[a]	96.03 ± 5.53[a]	103.31 ± 0.22[a]	102.78 ± 0.12[a]
19-21	34.50 ± 1.02[a]	32.72 ± 0.75[a]	93.11 ± 7.02[a]	91.28 ± 10.27[a]	102.89 ± 0.11[a]	103.20 ± 0.20[a]
22-24	32.89 ± 1.35[a]	33.95 ± 0.75[a]	92.44 ± 5.55[a]	94.44 ± 6.35[a]	102.80 ± 0.15[a]	102.72 ± 0.31[a]
25-27	34.22 ± 1.84[a]	34.28 ± 0.79[a]	92.17 ± 5.10[a]	95.44 ± 7.46[a]	102.68 ± 0.07[a]	102.62 ± 0.27[a]
28-30	33.28 ± 1.21[a]	32.66 ± 0.58[a]	93.00 ± 5.14[a]	90.39 ± 1.01[a]	102.63 ± 0.27[a]	102.64 ± 0.38[a]

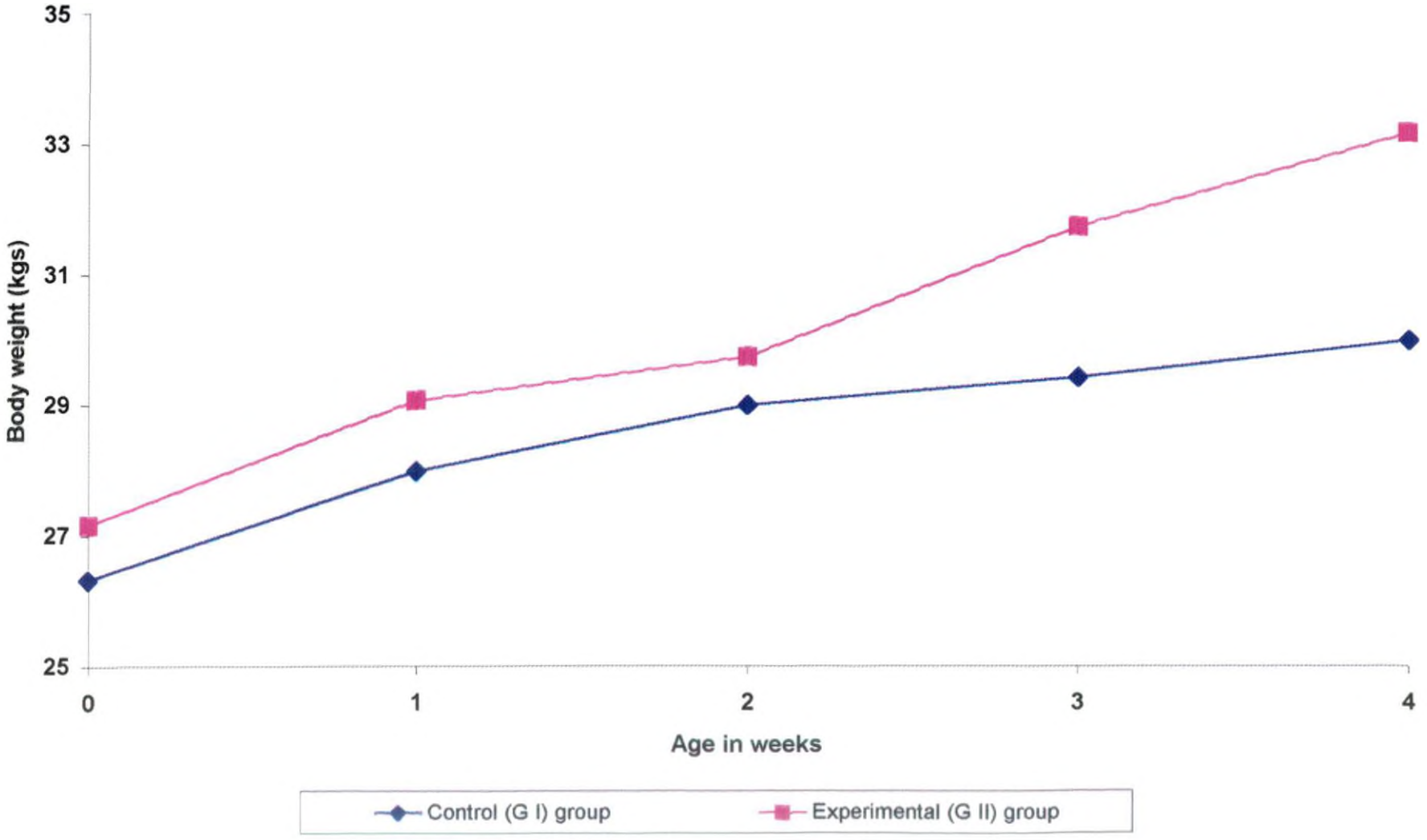
Means ± standard deviation (between groups) in rows bearing different superscripts for each parameter differ significantly ($p<0.05$)

Table 4. Effect of continued feeding of colostrum and milk on body weight of neonatal crossbred calves during the experimental period, Mean ± SD (n=6 per group)

Group	Animal number	Body weight (kg)					Weekly body weight gain per animal (g)
		Before the trial (0 d)	At the end of 1st week	At the end of 2nd week	At the end of 3rd week	At the end of 4th week	
I	1149	26.00	27.50	29.50	30.00	31.00	1250.00
	1291	29.00	32.00	32.00	33.00	34.00	1250.00
	T-707	20.00	22.00	27.00	27.50	28.00	2000.00
	1010	24.00	26.00	26.50	26.50	27.00	750.00
	1294	31.00	32.00	31.50	31.50	31.50	125.00
	W02	28.00	28.50	27.50	28.00	28.50	125.00
Mean ± S.D		26.33 ± 3.93[a]	28.00 ± 3.81[a]	29.00 ± 2.37[a]	29.42 ± 2.52[a]	30.00 ± 2.63[a]	916.67 ±131.72[a]
II	1297	28.00	30.00	29.00	32.50	32.50	1125.00
	1140	27.00	28.00	29.00	30.00	32.00	1250.00
	662	21.00	23.50	26.00	27.50	30.50	1375.00
	TW04	30.00	30.50	31.00	34.00	35.50	1375.00
	693	27.00	31.50	31.00	33.50	34.50	1875.00
	1013	30.00	31.00	32.50	33.00	34.00	1000.00
Mean ± S.D		27.17 ±3.31[a]	29.08 ±3.00[a]	29.75 ±2.27[a]	31.75± 2.50[a]	33.17 ±1.84[b]	1333.33±302.77[a]

Mean ± standard deviation (between groups) in columns bearing different superscripts for each parameter differ significantly ($p<0.05$)

Fig 2. Effect of continued feeding of colostrum and milk on weekly body weight of neonatal crossbred calves during the experimental period



of group II calves recorded (33.17 ± 1.84 kg) at the end of fourth week of age was significantly ($P<0.05$) higher than the corresponding body weight recorded (30.00 ± 2.63 kg) for group I calves. Weekly body weight gain per animal for group I was recorded as 916.67 ± 131.72 g as against the higher value of 1333.33 ± 302.77 g for group II animals.

4.2.3 Haematological parameters

Effect of first colostrum intake and continued feeding of colostrum (group II) and milk (group I) on certain haematological parameters such as haemoglobin (Hb) concentration, total erythrocyte/red blood cell (RBC) count, total leucocyte/white blood cell (WBC) count and volume of packed red blood corpuscles (VPRC) of neonatal crossbred calves for a period of 30 days are given in the tables 5a, 5b, 5b1, fig.3 and 4.

The effect of first colostrum intake and continued feeding of colostrum (Group II) and milk (Group I) on erythrocytic indices viz., mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) for a period of 30 days are depicted in tables 5c, 5d, 5d1 and fig.5.

4.2.3.1 Haemoglobin (Hb) concentration

Haemoglobin (Hb) concentration in the control animals of group I which were fed colostrum and then milk as per standard feeding regime declined gradually from the value (13.87 ± 2.51 g%) recorded immediately after birth (zero day) and reached the lowest value (11.07 ± 2.53 g%) on 30th day of age.

A similar trend was observed in the experimental calves of group II which were continuously fed colostrum from birth to 30 days of age recorded the peak value (14.13 ± 1.16 g%) soon after birth (zero day) and the lowest value (10.37 ± 0.98 g%) on 30th day of age *vide* table 5a and fig.3.

There was no significant variation in haemoglobin (Hb) concentration between the two groups (table 5a) at different periods of age.

Animals of control group I failed to show any significant variation in the Hb concentration 18 h after birth (the first day of birth) or afterwards (30th day) from the value observed soon after birth (zero day). But the experimental animals of group II, soon after birth (zero day) showed a significant ($P<0.05$) variation (14.13 ± 1.16 g%) in the Hb concentration when compared with the values recorded 18 h after birth (13.15 ± 1.48 g%) and that of 30th day of age (10.37 ± 0.98 g%) *vide* table 5b.

4.2.3.2 Total erythrocyte (RBC) count

In the animals of control group I which were fed colostrum and then milk as per standard feeding regime, erythrocyte (RBC) count reached a peak value on 12th day of age (9.78 ± 2.05 x 10^6/µl) and then the value was lowered (8.78 ± 2.01 x 10^6/µl) on 30th day of age. The RBC count in the experimental animals of group II (fed colostrum for 30 days of age) increased gradually from the lowest recorded value (9.79 ± 1.06 x 10^6/µl) on the sixth day of age to the peak value (11.40 ± 1.00 x 10^6/µl) recorded on 30th day of age (table 5a and fig. 3).

While analyzing the RBC count of calves of group I and II from the day of birth to 30 days of age, there was significant ($p<0.05$) variation between the groups on 24th and 30th days of age (table 5a). The values on 24th day of age for group I and II were 8.97 ± 0.92 x 10^6/µl and 10.65 ± 1.52 x 10^6/µl respectively and that of 30th day of age for group I and II were 8.78 ± 2.01 x 10^6/µl and 11.40 ± 1.00 x 10^6/µl respectively (table 5a).

Group I (control) and group II (experimental) calves failed to show any significant difference in the RBC count on 18 hours after birth (first day) or even at the end of experimental period (30th day of age) from the value recorded immediately after birth (zero day) *vide* table 5b.

4.2.3.3 Total leukocyte (WBC) count

Total leukocyte (WBC) count in the animals of control group I, which were fed colostrum and then milk as per standard feeding regime of farms, increased steadily from the value (5117.00 ± 660.80/µl) recorded soon after birth, to reach the peak value (6275.00 ± 1270.33/µl) on 30th day of age. A similar trend was observed in the experimental calves of group II which were fed colostrum for 30 days of age, where the lowest value of 5225.00 ± 1190.84/µl was recorded immediately after birth and the peak value was noticed as 8750.00 ± 1295.76/µl on 30th day of age (table 5a and fig.4).

Total leucocyte (WBC) count of the calves of group I on 12th day (5533.33 ± 1531.88/µl), 18th day (6033.33 ± 488.54/µl), 24th day (6121.67 ± 880.69/µl) and 30th day of age (6275.00 ± 1270.33/µl) were significantly

($P<0.05$) lower than the corresponding values of group II calves. The corresponding values during 12th, 18th, 24th and 30th day of age for group II calves were 7458.33 ± 1156.90/µl, 7950.00 ± 151.66/µl, 8383.33 ± 1746.33/µl and 8750.00 ± 1295.76/µl respectively (table 5a).

In the animals of control group I, the WBC count recorded soon after birth (zero day) as 5117.00 ± 660.80/µl and the value at the 30th day of age (6275.00 ± 1270.33/µl) showed significant ($P<0.05$) variation when compared with the count (5167.00 ± 801.04/µl) observed at 18 h after birth (first day). The WBC count of the calves of the experimental group II, on 30th day of age was significantly ($P<0.05$) higher (8750.00 ± 1295.76/µl) than the values (6092.00 ± 1011.15/µl) obtained 18 h after birth (first day) and soon after birth (5225.00 ± 1190.84/µl) *vide* table 5b.

4.2.3.4 Volume of packed red blood cells (VPRC)

Volume of packed red blood cells in the animals of control group I which were fed with colostrum and then milk as per standard feeding regime of the farms, declined from the value (42.33 ± 5.75%) observed soon after birth (zero day) to reach the lowest value (32.52 ± 4.21%) on 30th day of age. In the experimental calves of group II, fed colostrum for 30 days of age, a fluctuating trend was observed with the highest value recorded (38.03 ± 2.58%) soon after birth (zero day) which was lowered further to reach the lowest value (31.82 ± 5.33%) on 24th day of age which was increased slightly (32.35 ± 5.90%) at the end of the experiment.

On comparing the values from the day of birth (zero day) to 30 days of age of calves of both groups, it was found that there was significant ($P<0.05$) variation between the values observed on 18th day of age for calves of group I and group II and the VPRC values were 39.83 ± 3.49% and 32.63 ± 4.84% respectively (table 5a).

In the calves of control group I, the VPRC value (42.33 ± 5.75%) observed immediately after birth (zero day) was significantly ($P<0.05$) higher than the value found (37.50 ± 4.23%) after first colostrum intake (first day) and at 30th day of age (end of the experiment) with a value of 32.52 ± 4.21%. However the experimental calves of group II did not have any significant differences in the VPRC values from the time of birth (zero day) or 18 h after first colostrum intake (first day) or even after prolonged colostrum feeding for 30 days of age (table 5b).

4.2.3.5 Erythrocytic indices

The erythrocytic indices as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) of control calves of group I (fed colostrum and then milk as per standard feeding regime of the farms) and experimental animals of group II (fed colostrum for 30 days of age) are presented in tables 5c, 5d, 5d1 and fig.5.

Table 5. Normal haematological and biochemical parameters of the neonatal calves

Parameters	0 h	18 h	Day 7
a. *Haematological values			
Hb (g/dl)	13.6	10.1	11.15
RBC count ($x10^6/\mu l$)	8.5	9.3	9.5
WBC count (x/μl)	8870	9060	9910
VPRC (%)	45.15	38.93	36.10
b. *Serum protein			
Total serum protein (g/dl)	4.6	5.9	6.82
Albumin (g/dl)	3.3	2.9	3.49
Globulin (g/dl)	1.3	3.0	3.33
A:G ratio	0.93	0.88	1.04
c. **Serum lipid profile			
Total lipid (mg/dl)	140.56	211.82	350.44
Cholesterol (mg/dl)	29	44.3	92.34
Triglycerides (mg/dl)	3	35	34
NEFA (μmol/l)	540.44	389.12	400.04
d. **Serum biochemical parameters			
BGL (mg/dl)	108.24	120.34	122.45
BUN (mg/dl)	9.8	10.8	14.2
Creatinine (mg/dl)	1.1	0.79	0.72
Bilirubin (mg/dl)	0.2	1.2	1.34

Source: * Sridhar *et al.* (1988)

**Kurz and Willett (1991)

Table 5a. Effect of continued feeding of colostrum and milk on the haematological parameters of neonatal crossbred calves for a period of 30 days, Mean ± SD (n=6 per group)

Age in days	Haemoglobin (g/dl)		Total erythrocyte count (millions/µl)		Total leucocyte count (x/µl)		Volume of packed red blood corpuscles (%)	
	Group I	Group II	Group I	Group II	Group I	Group II	Group I	Group II
0	13.87± 2.51[a]	14.13± 1.16[a]	8.95± 2.42[a]	10.05± 2.59[a]	5117.00± 660.80[a]	5225.00± 1190.84[a]	42.33± 5.75[a]	38.03± 2.58[a]
1	12.57± 3.62[a]	13.15± 1.48[a]	9.13± 1.48[a]	10.71± 2.64[a]	5167.00± 801.04[a]	6092.00± 1011.15[a]	37.50± 4.23[a]	36.85± 5.62[a]
6	11.870± 2.93[a]	12.10± 2.52[a]	9.45± 2.59[a]	9.79± 1.06[a]	5500.00± 593.30[a]	6625.00± 1721.55[a]	34.53± 7.52[a]	35.72± 4.93[a]
12	11.82± 3.06[a]	11.31 1.83[a]	9.78± 2.05[a]	9.81± 0.81[a]	5533.33± 1531.88[a]	7458.33± 1156.90[b]	37.17± 4.71[a]	34.83± 1.94[a]
18	11.68± 3.76[a]	11.03± 2.82[a]	9.55± 0.90[a]	10.52± 2.47[a]	6033.33± 488.54[a]	7950.00± 151.66[b]	39.83± 3.49[a]	32.63± 4.84[b]
24	11.54± 3.68[a]	10.78± 3.35[a]	8.97± 0.92[a]	10.65± 1.52[b]	6121.67± 880.69[a]	8383.33± 1746.33[b]	34.85± 2.21[a]	31.82± 5.33[a]
30	11.07± 2.53[a]	10.37± 0.98[a]	8.78± 2.01[a]	11.40± 1.00[b]	6275.00± 1270.33[a]	8750.00± 1295.76[b]	32.52± 4.21[a]	32.35± 5.90[a]

Mean ± standard deviation (between groups) in rows bearing different superscripts for each parameter differ significantly ($p<0.05$)

Table 5b. Effect of the first colostrum intake and continued feeding of colostrum and milk on the haematological parameters of neonatal crossbred calves for a period of 30 days, Mean ± SD (n=6 per group)

Sampling period	Haemoglobin (g/dl)		Total erythrocyte count (millions/µl)		Total leucocyte count (x/µl)		Volume of packed red blood cells (%)	
	Group I	Group II	Group I	Group II	Group I	Group II	Group I	Group II
Before the start of the experiment (0 d)	13.87± 2.51[a]	14.13± 1.16[a]	8.95± 2.42[a]	10.05± 2.59[a]	5117.00± 660.80[a]	5225.00± 1190.84[a]	42.33 5.75[a]	38.03± 2.58[a]
18 h after the start of the experiment (1d)	12.57± 3.62[a]	13.15± 1.48[b]	9.13± 1.48[a]	10.71± 2.64[a]	5167.00± 801.04[b]	6092.00± 1011.15[a]	37.50 4.23[b]	36.85± 5.62[a]
At the end of the experiment (30 d)	11.07 ± 2.53[a]	10.37 ± 0.98[b]	8.78± 2.01[a]	11.40 ± 1.00[a]	6275.00 ± 1270.33[a]	8750.00 ± 1295.76[b]	32.52 ± 4.21[b]	32.35 ± 5.90[a]

Means ± standard deviation (between periods) in columns bearing different superscripts for each parameter differ significantly ($p<0.05$)

Table 5b1. Effect of continued feeding of colostrum and milk on the haematological parameters of neonatal crossbred calves for a period of 30 days, Mean ± SD (n=6 per group)

Age in days	Haemoglobin (g/dl)		Total erythrocyte count (millions/µl)		Total leucocyte count (x/µl)		Volume of packed red blood corpuscles (%)	
	Group I	Group II	Group I	Group II	Group I	Group II	Group I	Group II
0	13.87± 2.51[abcdef]	14.13± 1.16[ab]	8.95± 2.42[aghijk]	10.05± 2.59[bgklmn]	5117.00± 660.80[abc]	5225.00± 1190.84[a]	42.33± 5.75[abc]	38.03± 2.58[abcd]
1	12.57± 3.62[aghijk]	13.15± 1.48[cdef]	9.13± 1.48[chlpqr]	10.71± 2.64[ejmprt]	5167± 801.04[de]	6092± 1011.15[bcd]	37.50± 4.23[dghij]	36.85± 5.62[efghi]
6	11.870± 2.93[bglmno]	12.10± 2.52[acghij]	9.45± 2.59[dimpst]	9.79± 1.06[abcdef]	5500.00± 593.30[adfghi]	6625.00± 1721.55[abe]	34.53± 7.52[cfilnp]	35.72± 4.93[aejkl]
12	11.82± 3.06[chlpqr]	11.31 1.83[gklm]	9.78± 2.05[fortu]	9.81± 0.81[aghijk]	5533.33± 1531.88[befjkl]	7458.33± 1156.90[cfgh]	37.17± 4.71[begklm]	34.83± 1.94[bfjmno]
18	11.68± 3.76[dimpst]	11.03± 2.82[dhkno]	9.55± 0.90[ejknqsu]	10.52± 2.47[chkopq]	6033.33± 488.54[gjmn]	7950.00± 151.66[egij]	39.83± 3.49[adef]	32.63± 4.84[cgkmpq]
24	11.54± 3.68[ejnqsu]	10.78± 3.35[beilnp]	8.97± 0.92[bglmno]	10.65± 1.52[dieors]	6121.67± 880.69[hkmo]	8383.33± 1746.33[dgik]	34.85± 2.21[hkno]	31.82± 5.33[dioqr]
30	11.07± 2.53[fkortu]	10.37± 0.98[fjmop]	8.78± 2.01[abcdef]	11.40± 1.00[fknqst]	6275.00± 1270.33[cilno]	8750.00± 1295.76[hjk]	32.52± 4.21[jmop]	32.35± 5.90[hlnpr]

Means ± standard deviation (between periods) in columns bearing same superscripts for each parameter are homogenous

Fig.3. Effect of continued feeding of colostrum and milk on haemolglobin content, total erythrocyte (RBC) count and volume of packed red blood corpuscles (VPRC) of neonatal crossbred calves for a period of 30 days

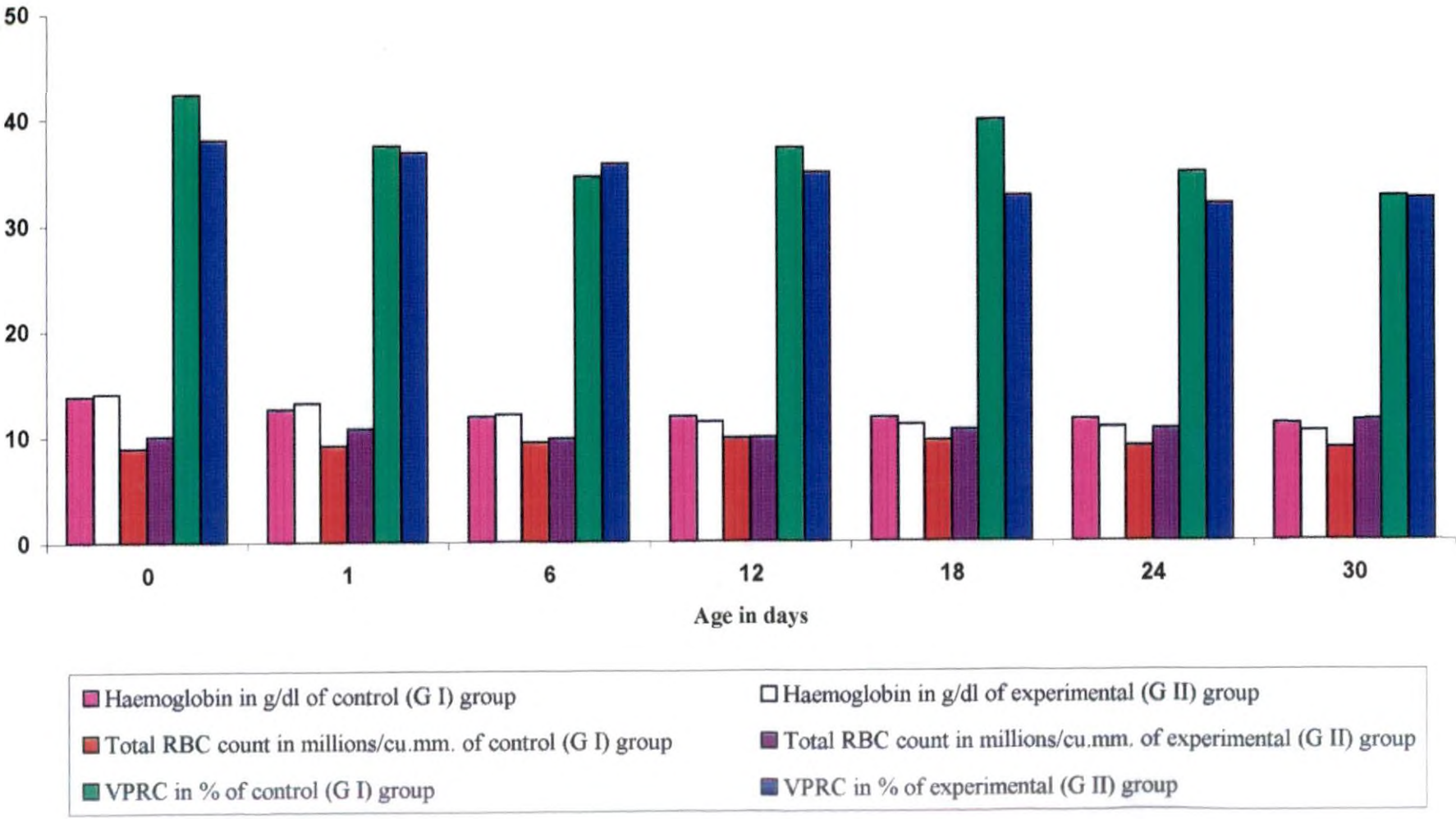
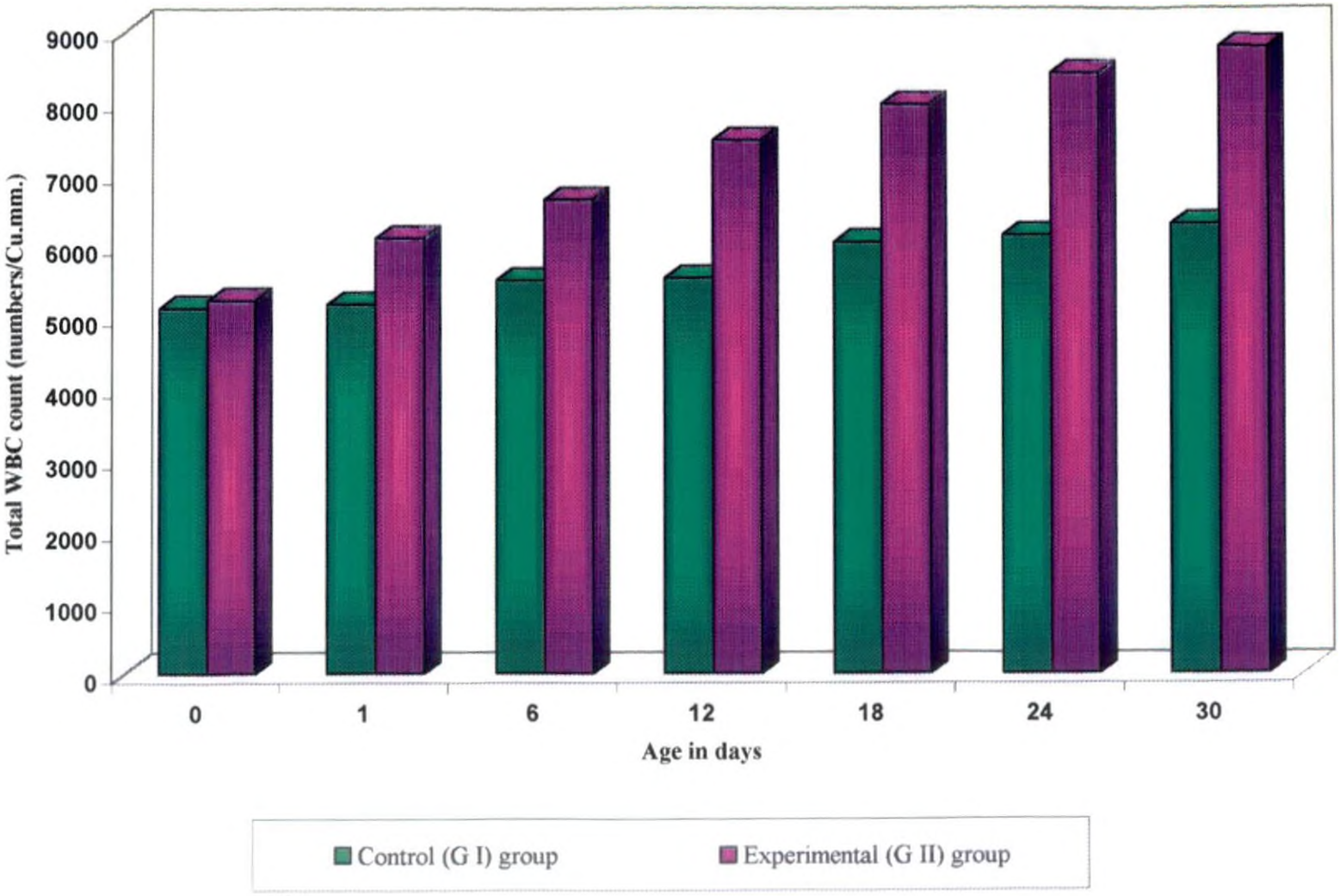


Fig.4. Effect of continued feeding of colostrum and milk on total leucocyte (WBC) count of neonatal crossbred calves for a period of 30 days



4.2.3.5.1 Mean corpuscular volume (MCV)

Mean corpuscular volume (MCV) in the control animals of group I which were fed with colostrum and then milk as per standard feeding regime of farms showed a fluctuating trend which declined from the peak value (51.68 ± 21.65 fl) recorded soon after birth (zero day) to the lowest (37.20 ± 4.78 fl) recorded value on sixth day of age and thereafter fluctuated with a marginal increase and then decrease till 30th day of the experimental period. The calves of group II, also showed a steady declining trend with the highest value (40.09 ± 10.91 fl) immediately after birth and the lowest value (28.54 ± 5.58 fl) on 30th day of age (table 5c and fig.5).

As the MCV values of the two groups were compared, it was noticed that the values of control calves of group I on 18th (41.94 ± 4.70 fl), 24th (39.12 ± 4.10 fl) and 30th day of age (38.38 ± 9.04 fl) were significantly ($P<0.05$) higher than the corresponding values of group II. The corresponding values of experimental animals of group II on 18th, 24th and 30th days of age were 32.38 ± 7.95 fl, 29.92 ± 3.00 fl and 28.54 ± 5.58 fl respectively (table 5c).

Control animals of group I failed to show any significant difference in the MCV values, 18 h after birth (first day), or after prolonged colostrum feeding (30th day) from the value obtained soon after birth (zero day). The MCV values of experimental calves of group II, recorded at the end of the experimental period of 30 days of age was significantly ($P<0.05$) lower (28.54 ± 5.58 fl) than the value recorded (40.09 ± 10.91 fl) immediately after birth (zero day) *vide* table 5d.

4.2.3.5.2 Mean corpuscular haemoglobin (MCH)

In the control animals of group I, the MCH values showed a fluctuating trend with the highest value (16.34 ± 4.53 pg) recorded soon after birth (zero day) which was reduced to the lowest level (12.13 ± 3.44 pg) on 18th day of age. In the experimental calves of group II also MCH values showed a reducing trend with the highest value (14.78 ± 3.61 pg) recorded immediately after birth (zero day) and lowest value (9.14 ± 1.05 pg) on the 30th day of age (table 5c and fig.5).

While analyzing the values of these two groups, it was observed that the value (12.90 ± 3.32 pg) recorded on 30th day of age of group I calves was significantly ($P<0.05$) higher than the corresponding value (9.14 ± 1.05 pg) recorded for calves of group II at the end of the experiment (30th day of age) *vide* table 5c.

Control animals of group I failed to show any significant difference in the MCH values, 18 h after birth (first day), or at the end of the experiment (30th day of age) from the value observed soon after birth (zero day). In the experimental calves of group II, the values recorded (14.78 ± 3.61 pg) immediately after birth (zero day) and value (13.13 ± 4.27 pg) recorded 18 h after birth (first day) were significantly ($P<0.05$) higher than the value (9.14 ± 1.05 pg) recorded at the end of the experimental period (30th day of age) *vide* table 5d.

4.2.3.5.3 Mean corpuscular hemoglobin concentration (MCHC)

Mean corpuscular hemoglobin concentration (MCHC) values of the control animals of group I, showed a fluctuating trend with the peak value (33.90 ± 10.79 g/dl) recorded soon after birth (zero day) and the value was reduced to the lowest level (28.37 ± 7.58 g/dl) on 12th day of age, which was further increased till the end of the experimental period. A similar fluctuating trend was recorded in experimental calves of group II, with the peak value (37.32 ± 4.14 g/dl) recorded soon after birth (zero day) and lowest value (32.22 ± 5.06 g/dl) on 12th day of age (table 5c and fig.5).

On comparing the MCHC values between the calves of group I and II, it was observed that the values did not show any significant variation (table 5c).

Control animals of group I and experimental calves of group II failed to show any significant differences in the MCHC values, 18 h after birth (first day), or at the end of the experimental period of 30 days of age from value observed soon after birth (zero day) *vide* table 5d.

4.2.4 Biochemical parameters

4.2.4.1 Serum protein profile

Effect of first colostrum intake and continued feeding of colostrum (Group II) and milk (Group I) on the concentrations of total serum protein, albumin, globulin and albumin : globulin (A : G) ratio of neonatal crossbred calves for a period of 30 days are shown in tables 6a, 6b, 6c and fig.6.

Table 5c. Effect of continued feeding of colostrum and milk on erythrocytic indices of neonatal crossbred calves for a period of 30 days, Mean ± SD (n=6 per group)

Age in days	Mean corpuscular volume – MCV (fl)		Mean corpuscular haemoglobin – MCH (pg)		Mean corpuscular haemoglobin concentration – MCHC (g/dl)	
	Group I	Group II	Group I	Group II	Group I	Group II
0	51.68 ±21.65[a]	40.09 ±10.91[a]	16.34 ±4.53[a]	14.78 ±3.61[a]	33.90 ±10.79[a]	37.32 ±4.14[a]
1	42.12 ±9.00[a]	37.51 ±17.12[a]	14.03 ±4.62[a]	13.13 ±4.27[a]	33.83 ±10.78[a]	36.74 ±9.13[a]
6	37.20 ±4.78[a]	36.84 ±6.09[a]	13.09 ±4.07[a]	12.65 ±3.70[a]	34.78 ±7.58[a]	34.27 ±8.32[a]
12	40.00 ±11.79[a]	35.63 ±2.00[a]	12.35 ±3.27[a]	11.56 ±1.75[a]	28.37 ±7.58[a]	32.22 ±5.06[a]
18	41.94 ±4.70[a]	32.38 ±7.95[b]	12.13 ±3.44[a]	11.26 ±4.56[a]	29.21 ±8.38[a]	34.53 ±9.36[a]
24	39.12 ±4.10[a]	29.92 ±3.00[b]	12.64 ±3.11[a]	10.58 ±4.53[a]	31.47 ±12.84[a]	36.49 ±16.71[a]
30	38.38 ±9.04[a]	28.54 ±5.58[b]	12.90 ±3.32[a]	9.14 ±1.05[b]	34.66 ±9.34[a]	32.78 ±5.59[a]

Means ± standard deviation (between groups) in rows bearing different superscripts for each parameter differ significantly ($p<0.05$)

Table 5d. Effect of first colostrum intake and continued feeding of colostrum and milk on the erythocytic indices of neonatal crossbred calves for a period of 30 days, Mean ± SD (n=6 per group)

Sampling periods	Mean corpuscular volume - MCV (fl)		Mean corpuscular haemoglobin - MCH (pg)		Mean corpuscular haemoglobin concentration - MCHC (g/dl)	
	Group I	Group II	Group I	Group II	Group I	Group II
Before the start of the experiment (0 d)	51.68 ±21.65^{a}	40.09 ±10.91^{a}	16.34 ±4.53^{a}	14.78 ±3.61^{a}	33.90 ±10.79^{a}	37.32 ±4.14^{a}
18 h after the s tart of the experiment (1 d)	42.12 ±9.00^{a}	37.51 ±17.12^{ac}	14.03 ±4.62^{a}	13.13 ±4.27^{a}	33.83 ±10.78^{a}	36.74 ±9.13^{a}
At the end of the experiment (30 d)	38.38 ±9.04^{a}	28.54 ±5.58^{bc}	12.90 ±3.32^{a}	9.14 ±1.05^{b}	34.66 ±9.34^{a}	32.78 ±5.59^{a}

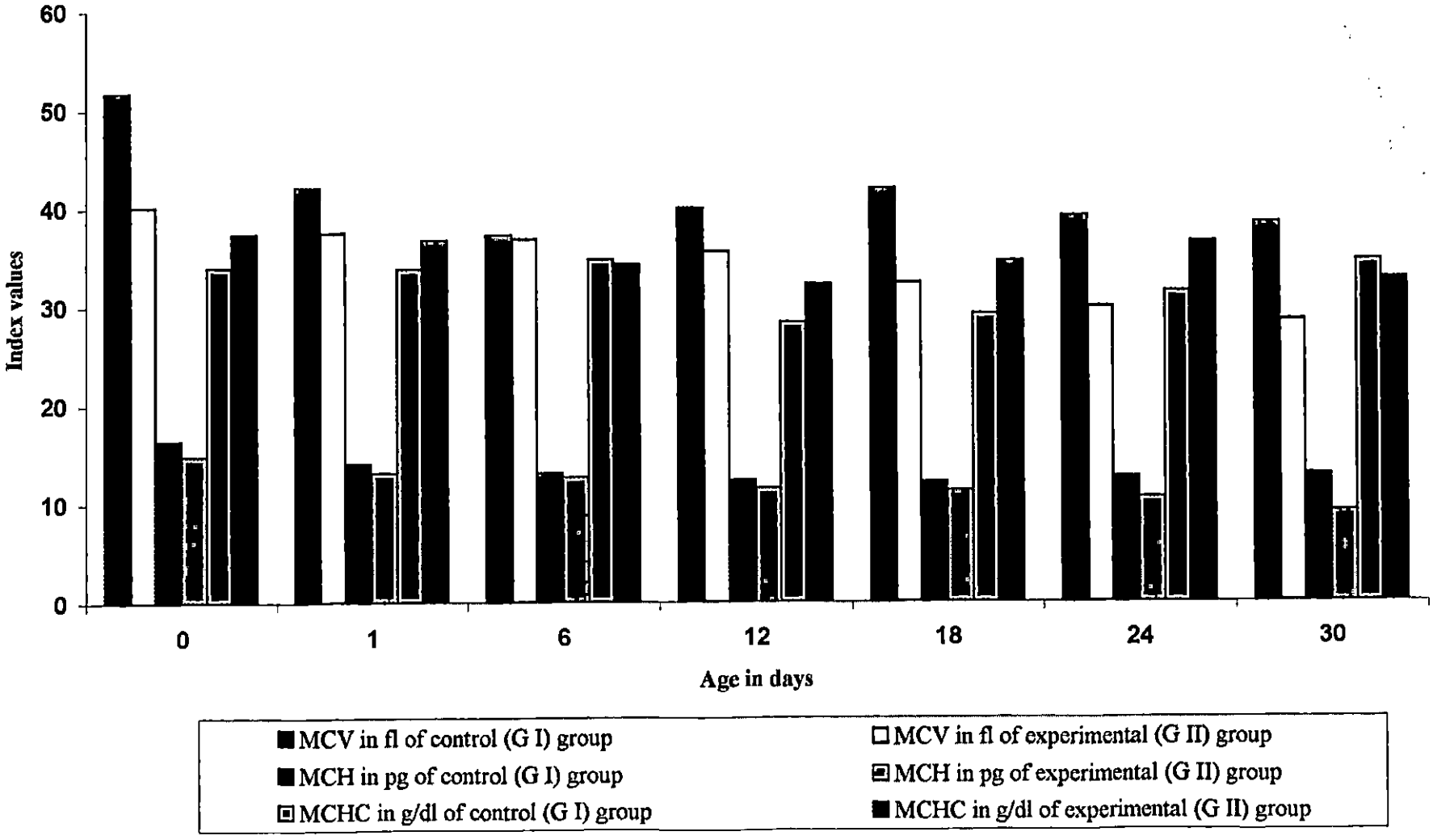
Mean ± standard deviation (between periods) in columns bearing different superscripts for each parameter differ significantly ($p<0.05$)

Table 5d1. Effect of continued feeding of colostrum and milk on erythrocytic indices of neonatal crossbred calves for a period of 30 days, Mean ± SD (n=6 per group)

Age in days	Mean corpuscular volume – MCV (fl)		Mean corpuscular haemoglobin – MCH (pg)		Mean corpuscular haemoglobin concentration – MCHC (g/dl)	
	Group I	Group II	Group I	Group II	Group I	Group II
0	51.68 ± 21.65^{abcdef}	40.09 ± 10.91^{abcde}	16.34 ± 4.53^{abcdef}	14.78 ± 3.61^{abcde}	33.90 ± 10.79^{glmno}	37.32 ± 4.14^{abcdef}
1	42.12 ± 9.00^{agjokl}	37.51 ± 17.12^{afghij}	14.03 ± 4.62^{aghijk}	13.13 ± 4.27^{afghi}	33.83 ± 10.78^{chlpqr}	36.74 ± 9.13^{aghij}
6	37.20 ± 4.78^{afkorta}	36.84 ± 6.09^{bfklmn}	13.09 ± 4.07^{bglmno}	12.65 ± 3.70^{bfjklm}	34.78 ± 7.58^{abcdef}	34.27 ± 8.32^{dimpst}
12	40.00 ± 11.79^{chlpqr}	35.63 ± 2.00^{egkopq}	12.35 ± 3.27^{ejnqsu}	11.56 ± 1.75^{cgjnop}	28.37 ± 7.58^{fkortu}	32.22 ± 5.06^{fkortu}
18	41.94 ± 4.70^{bglmno}	32.38 ± 7.95^{dhlors}	12.13 ± 3.44^{fkortu}	11.26 ± 4.56^{dhknqr}	29.21 ± 8.38^{ejnqsu}	34.53 ± 9.36^{fkortu}
24	39.12 ± 4.10^{dimpst}	29.92 ± 3.00^{eimprt}	12.64 ± 3.11^{dimpst}	10.58 ± 4.53^{eiloqs}	31.47 ± 12.84^{dimpst}	36.49 ± 16.71^{bglmno}
30	38.38 ± 9.04^{ejnqsu}	28.54 ± 5.58^{jnqst}	12.90 ± 3.32^{chlpqr}	9.14 ± 1.05^{mprs}	34.66 ± 9.34^{aghij}	32.78 ± 5.59^{ejnqsu}

Means ± standard deviation (between periods) in columns bearing same superscripts for each parameter are homogenous

Fig.5.Effect of continued feeding of colostrum and milk on erythrocytic indices viz., MCV, MCH and MCHC of neonatal crossbred calves for a period of 30 days



4.2.4.1.1 Total protein

Total serum protein concentration of the calves of control group I, which were fed with colostrum and then milk as per standard feeding regime of farms had a fluctuating trend with the lowest value (5.11 ± 0.92 g/dl) recorded soon after birth (zero day) and the highest value (6.40 ± 0.86 g/dl) recorded on 30th day of age. The serum protein concentration of the calves of experimental group II (which were fed colostrum for 30 days of age) recorded the lowest value (4.69 ± 0.35 g/dl) immediately after birth (zero day) and a peak level (6.79 ± 1.00 g/dl) on 30th day of age with a fluctuating trend (table 6a and fig. 6).

There was a significant difference ($P<0.05$) in the total protein concentration of the two groups on 18th day of age. The values for calves of group I (5.65 ± 0.25 g/dl) was lower than group II (6.70 ± 0.24 g/dl) *vide* table 6a.

Serum concentration of protein of the control animals of group I recorded soon after birth (zero day) was significantly ($P<0.05$) lower (5.11±0.92 g/dl) than the value obtained (6.16 ± 0.37 g/dl) 18 h after birth (first day). The experimental animals of group II showed significant ($P<0.05$) difference in the concentration of serum protein (6.36±0.85 g/dl) recorded 18 h after birth (first day of age) as well as the value (6.79 ± 1.00 g/dl) obtained at the end of the experimental period (30th day of age), when compared with the value (4.69±0.35 g/dl) recorded immediately after birth (zero day) *vide* table 6b.

4.2.4.1.2 Albumin

Effect of first colostrum intake and continued feeding of colostrum (Group II) and milk (Group I) on the serum albumin concentration are shown in tables 6a, 6b, 6c and fig.6.

In the calves of control group I which were fed with colostrum and then milk as per standard feeding regime, serum albumin concentration increased steadily from the lowest value (2.97±0.26 g/dl) recorded soon after birth (zero day of age) to a peak value (3.92±0.55 g/dl) on 30th day of age. In the experimental calves of group II, which were under prolonged colostrum feeding for 30 days of age, the value increased from 3.15±0.34 g/dl recorded immediately after birth (zero day) to the peak value (4.32±0.76 g/dl) on 30th day of age (table 6a and fig.6). Experimental animals had higher values than the control animals at all stages of the experimental period.

While analyzing the values from zero to 30 days of age of the two groups, it was observed that the value (3.33±0.31 g/dl) obtained on 12th day of age for calves of group I and group II (3.80±0.13 g/dl) showed a significant ($P<0.05$) variation with a higher value for experimental animals (table 6a).

Group I animals showed a significant ($P<0.05$) variation in the serum albumin concentration, when the value (3.92±0.55 g/dl) obtained on 30th day of age was compared with the value (2.97±0.26 g/dl) recorded soon after birth, as well as the value (2.75±0.38 g/dl) observed 18 h after birth (first day). The calves of group II showed significant ($P<0.05$) difference when value

(4.32±0.76 g/dl) recorded on 30th day was compared with the value (3.15±0.34 g/dl) recorded immediately after birth and the value (2.89±0.60 g/dl) recorded 18 h after birth (first day) *vide* table 6b.

4.2.4.1.3 Globulin

Serum globulin concentration in the control animals of group I, which were fed colostrum and then milk as per standard feeding regime, increased from the lowest value (2.15±1.02 g/dl) recorded soon after birth (zero day) and reached peak value (3.41±0.40 g/dl) 18 h after birth (first day of age) and the value reduced further by the end of the 30th day of age. In the experimental calves of group II, the peak value was recorded (3.49±0.50 g/dl) 18 h after birth (first day) and lowest value being recorded (1.54±0.57 g/dl) soon after birth (zero day) *vide* table 6a and fig.6.

On comparing the serum globulin concentration between the two groups, it was observed that there was no significant variation between the two groups (table 6a).

In the animals of group I, the serum globulin concentration (3.41±0.40 g/dl) recorded 18 h after birth (first day) was significantly ($P<0.05$) higher than the value (2.15±1.02 g/dl) recorded immediately after birth (zero day) and the value (2.48±0.61 g/dl) observed at the end of the experiment (30th day of age). The animals of group II had a significantly ($P<0.05$) higher concentration of globulin at 18 h after birth (3.49 ± 0.50 g/dl) than the value observed (1.54 ± 0.57 g.dl) soon after birth (zero day) *vide* table 6b.

4.2.4.1.4 Albumin : globulin (A:G) ratio

Effect of first colostrum intake and continued feeding of colostrum (Group II) and milk (Group I) on the A:G ratio of neonatal calves for a period of 30 days are presented in the tables 6a, 6b and 6c..

In the control animals of group I, which were fed colostrum and then milk as per standard feeding regime, A:G ratio declined from the peak value (1.81 ± 1.15) observed soon after birth (zero day) to the lowest value (0.82 ± 0.18) recorded 18 h after birth (first day of age) and increased thereafter till the end of the experiment. In the experimental calves of group II which were fed colostrum for 30 days of age the value reduced from the highest recorded value (2.37 ± 1.13) recorded immediately after birth (zero day) to the lowest value (0.83 ± 0.19) observed 18 h after birth (first day) *vide* table 6a.

While analyzing the values from zero to 30 days of age, it was observed that there was no significant variation between the two groups (table 6a).

In the calves of control group I, the value (1.65±0.41) recorded at the end of the trial (30^{th} day of age) was significantly ($P<0.05$) higher than the value (0.82±0.18) obtained 18 h after birth (first day). Whereas in group II animals, the value (2.37±1.13) recorded soon after birth (zero day) and the value (1.97±0.82) recorded at the end of the trial (30^{th} day of age) were significantly ($P<0.05$) higher than the value (0.83±0.19) recorded 18 h after birth (first day of age) *vide* table 6b.

Table 6a. Effect of continued feeding of colostrum and milk on serum protein profile of neonatal crossbred calves for a period of 30 days, Mean ± SD (n=6 per group)

Age in days	Total protein (g/dl)		Albumin (g/dl)		Globulin (g/dl)		A:G ratio	
	Group I	Group II	Group I	Group II	Group I	Group II	Group I	Group II
0	5.11± 0.92[a]	4.69± 0.35[a]	2.97± 0.26[a]	3.15± 0.34[a]	2.15± 1.02[a]	1.54± 0.57[a]	1.81± 1.15[a]	2.37± 1.13[a]
1	6.16± 0.37[a]	6.36± 0.85[a]	2.75± 0.38[a]	2.89± 0.60[a]	3.41± 0.40[a]	3.49± 0.50[a]	0.82± 0.18[a]	0.83± 0.19[a]
6	6.19± 0.91[a]	6.64± 0.35[a]	3.18± 0.31[a]	3.31± 0.47[a]	3.01± 0.74[a]	3.34± 0.58[a]	1.11± 0.25[a]	1.04± 0.33[a]
12	6.12± 0.42[a]	6.60± 0.67[a]	3.33± 0.31[a]	3.80± 0.13[b]	2.79± 0.53[a]	2.81± 0.75[a]	1.24± 0.30[a]	1.45± 0.45[a]
18	5.65± 0.25[a]	6.70± 0.24[b]	3.41± 0.33[a]	3.81± 0.64[a]	2.24± 0.35[a]	2.89± 0.82[a]	1.58± 0.38[a]	1.47± 0.65[a]
24	6.22± 0.91[a]	6.69± 0.42[a]	3.46± 0.54[a]	3.95± 0.45[a]	2.76± 0.79[a]	2.74± 0.62[a]	1.38± 0.60[a]	1.52± 0.46[a]
30	6.40± 0.86[a]	6.79± 1.00[a]	3.92± 0.55[a]	4.32± 0.76[a]	2.48± 0.61[a]	2.47± 0.83[a]	1.65± 0.41[a]	1.97± 0.82[a]

Mean ± standard deviation (between groups) in rows bearing different superscripts for each parameter differ significantly ($p<0.05$)

Table 6b. Effect of the first colostrum intake and continued feeding of colostrum and milk on serum protein profile of neonatal crossbred calves for a period of 30 days, Mean ± SD (n=6 per group)

Sampling periods	Total protein (g/dl)		Albumin (g/dl)		Globulin (g/dl)		A:G ratio)	
	Group I	Group II	Group I	Group II	Group I	Group II	Group I	Group II
Before the start of the experiment (0 d)	5.11± 0.92^{a}	4.69± 0.35^{a}	2.97± 0.26^{a}	3.15± 0.34^{a}	2.15± 1.02^{a}	1.54± 0.57^{a}	1.81± 1.15^{a}	2.37± 1.13^{a}
18 h after the start of the experiment (1 d)	6.16± 0.37^{b}	6.36± 0.85^{b}	2.75± 0.38^{a}	2.89± 0.60^{a}	3.41± 0.40^{b}	3.49± 0.50^{b}	0.82± 0.18^{ab}	0.83± 0.19^{b}
At the end of the experiment (30 d)	6.40 ± 0.86^{ab}	6.79 ± 1.00^{b}	3.92 ± 0.55^{b}	4.32 ± 0.76^{b}	2.48 ± 0.61^{a}	2.47 ± 0.83^{ab}	1.65 ± 0.41^{ac}	1.97 ± 0.82^{a}

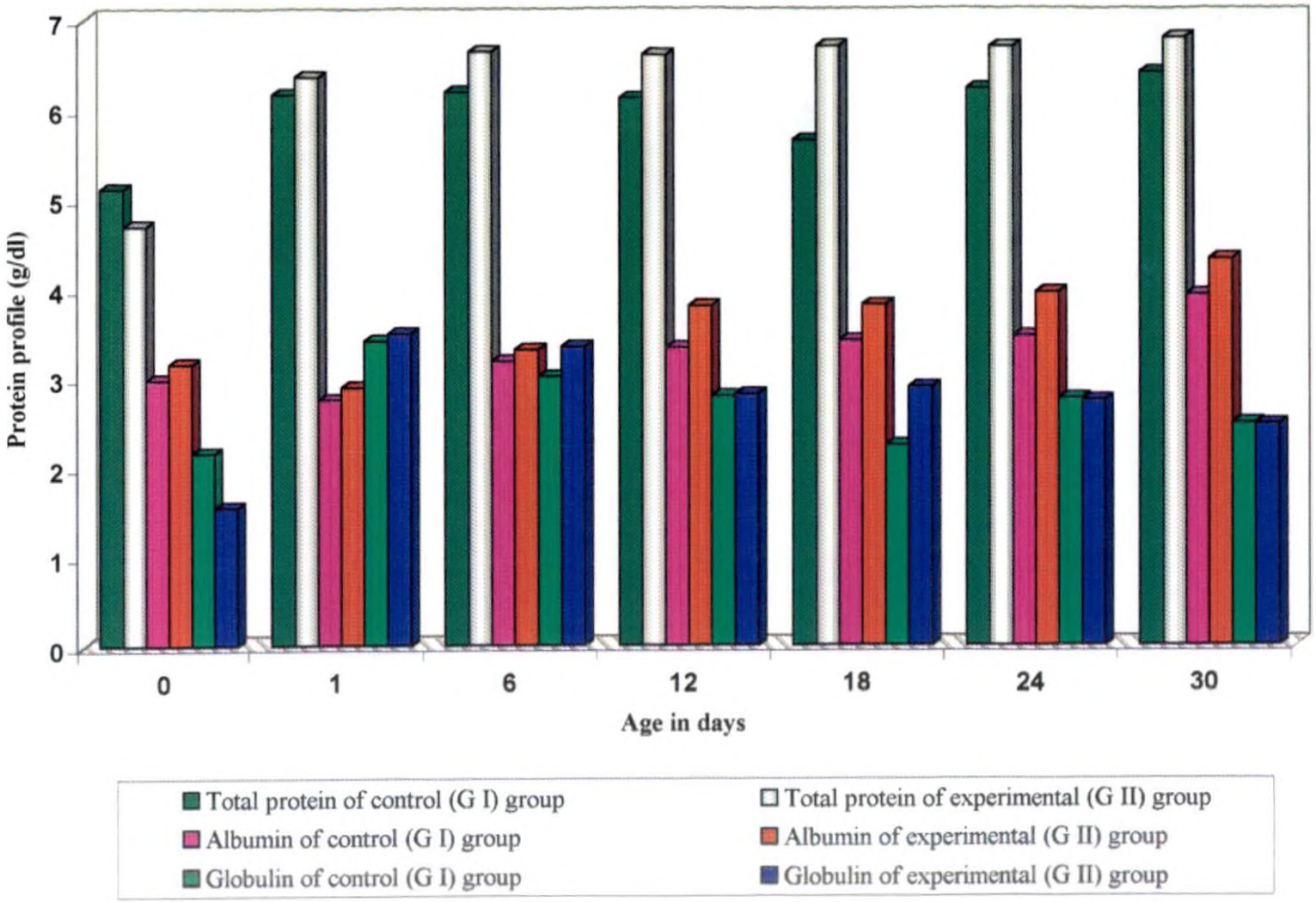
Mean ± standard deviation (between periods) in columns bearing different superscripts for each parameter differ significantly ($p<0.05$)

Table 6c. Effect of continued feeding of colostrum and milk on serum protein profile of neonatal crossbred calves for a period of 30 days,Mean ± SD (n=6 per group)

Age in days	Total protein (g/dl)		Albumin (g/dl)		Globulin (g/dl)		A:G ratio	
	Group I	Group II	Group I	Group II	Group I	Group II	Group I	Group II
0	5.11± 0.92^{abcd}	4.69± 0.35	2.97± 0.26^{adef}	3.15± 0.34^{ade}	2.15± 1.02^{abcde}	1.54± 0.57^{a}	1.81± 1.15^{abcdef}	2.37± 1.13^{abcd}
1	6.16± 0.37^{imno}	6.36± 0.85^{abcde}	2.75± 0.38^{abc}	2.89± 0.60^{abc}	3.41± 0.40^{oq}	3.49± 0.50^{fjno}	0.82± 0.18^{foq}	0.83± 0.19^{np}
6	6.19± 0.91^{bfjmpq}	6.64± 0.35^{bfjkl}	3.18± 0.31^{bdghi}	3.31± 0.47^{bdfgh}	3.01± 0.74^{ailnpq}	3.34± 0.58^{eilmo}	1.11± 0.25^{ejnpq}	1.04± 0.33^{hkmop}
12	6.12± 0.42^{eijkl}	6.60± 0.67^{afghi}	3.33± 0.31^{gjkl}	3.80± 0.13^{fijk}	2.79± 0.53^{bhkmp}	2.81± 0.75^{dhkmn}	1.24± 0.30^{dilmp}	1.45± 0.45^{dgjlo}
18	5.65± 0.25^{aefgh}	6.70± 0.24^{dhkmo}	3.41± 0.33^{ehjmn}	3.81± 0.64^{cegilm}	2.24± 0.35^{cfghi}	2.89± 0.82^{bcdef}	1.58± 0.38^{bgkl}	1.47± 0.65^{cfilmn}
24	6.22± 0.91^{cgknpr}	6.69± 0.42^{cgjmn}	3.46± 0.54^{efikmo}	3.95± 0.45^{jln}	2.76± 0.79^{dgjmno}	2.74± 0.62^{cgkl}	1.38± 0.60^{chkmno}	1.52± 0.46^{beijk}
30	6.40± 0.86^{dhloqr}	6.79± 1.00^{eilno}	3.92± 0.55^{lno}	4.32± 0.76^{hkmn}	2.48± 0.61^{efjkl}	2.47± 0.83^{abghij}	1.65± 0.41^{aghij}	1.97± 0.82^{aefgh}

Means ± standard deviation (between periods) in columns bearing same superscripts for each parameter are homogenous

Fig.6. Effect of continued feeding of colostrum and milk on serum protein profile of neonatal crossbred calves for a period of 30 days



4.2.4.1.5 Fractionation of serum protein by Agarose gel electrophoresis

The electrophoretic separation of serum proteins of calves of control (Group I) and experimental (Group II) groups on zero day, first day (18 h), sixth day, 12th day, 18th day, 24th day and 30th day of age are given in Plates 1, 2 and 3. The lanes of the electrophoretic separation of serum proteins of calves of both groups follow their respective trend as in biochemical evaluation of serum proteins.

4.2.4.2 Serum lipid profile

Effect of first colostrum intake and continued feeding of colostrum (Group II) and milk (Group I) on concentration of total lipids, cholesterol, triglycerides and nonesterified fatty acids (NEFA) of neonatal cross bred calves for a period of 30 days are shown in the tables 7a, 7b, 7c, fig 7 and 8.

4.2.4.2.1 Total lipids

In the animals of control group I, which were fed with colostrum and then milk as per standard feeding regime of farms, the serum concentration of total lipids increased steadily from the lowest value (134.70±28.42 mg/dl) recorded soon after birth (zero day) and reached the peak value (394.19±50.34 mg/dl) on 30th day of age. In the experimental animals of group II, which were fed colostrum for 30 days of age same trend was followed with lowest value(145.70±38.47 mg/dl) recorded soon after birth and the peak value (483.67±76.51 mg/dl) obtained on 30th day of age (table 7a and fig 7).

Plate 1 Electrophoretic separation of serum proteins of animals of control group (G I) and experimental group (G II) on days 0, 1 and 30

Lane 1 - on 1 d

Lane 2 – on 0 d

Lane 3 – on 30 d (control group)

Lane 4 – on 30 d (experimental group)

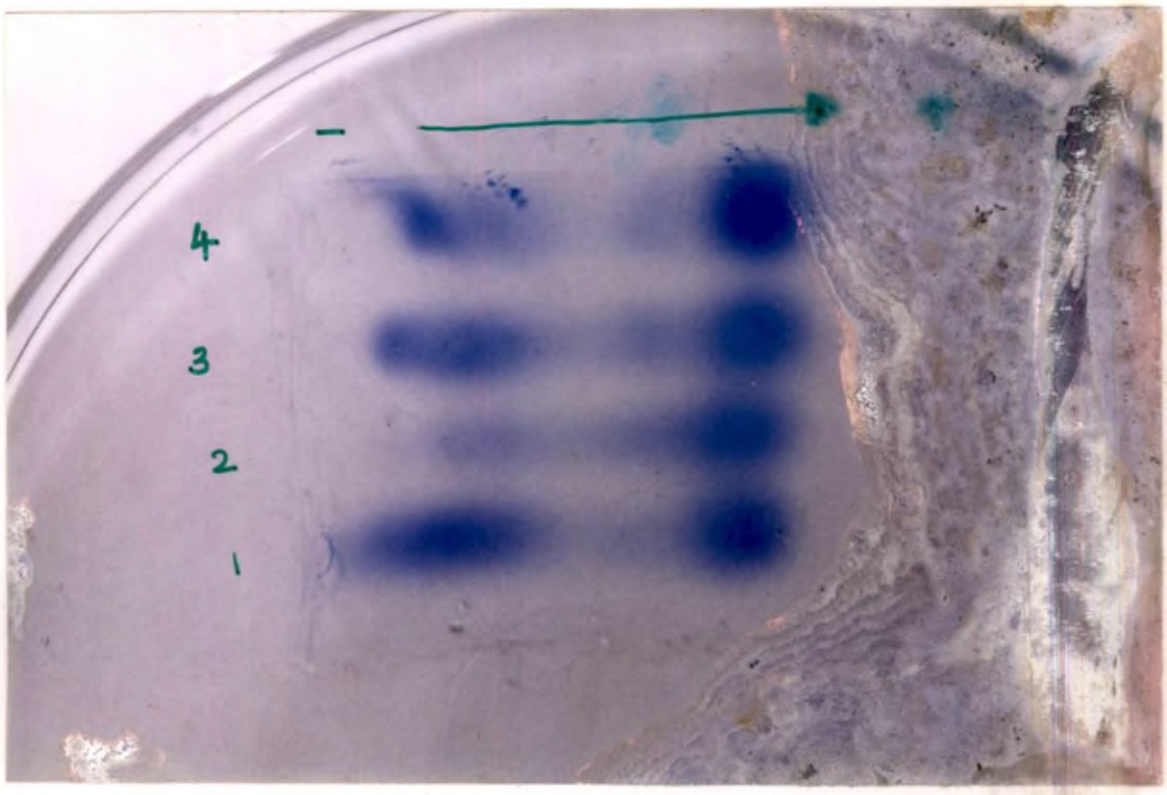

4
3
2
1

Plate 2 Electrophoretic fractionation of serum proteins of calves of control group (G I) on days 6, 12, 18 and 24

Lane 1 - on 6 d

Lane 2 – on 12 d

Lane 3 – on 18 d

Lane 4 – on 24 d

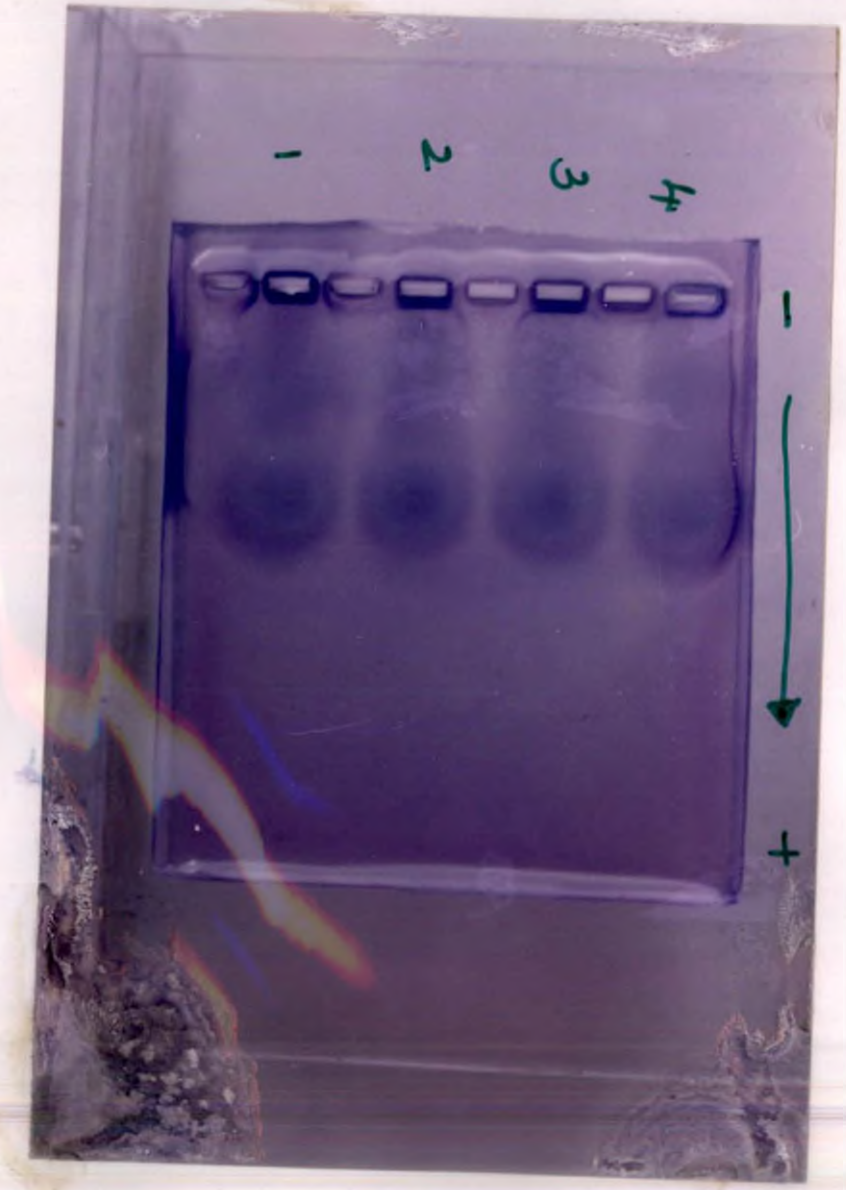

1
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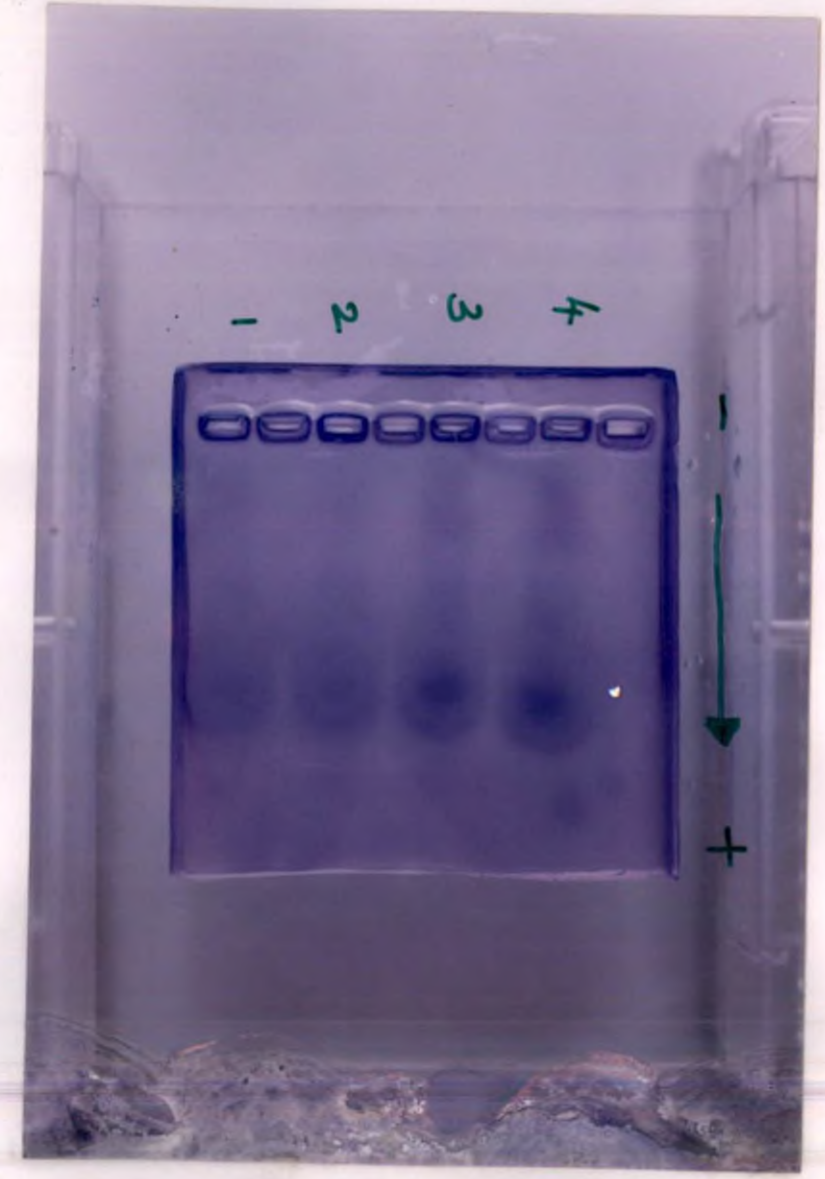
Plate 3 Electrophoretic separation of serum proteins of animals of experimental group (G II) on days 6, 12, 18 and 24

Lane 1 - on 6 d

Lane 2 – on 12 d

Lane 3 – on 18 d

Lane 4 – on 24 d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On comparing serum concentration of total lipids between the two groups, it was observed that the values for experimental group on the sixth day, 12th day, 18th day, 24th day and 30th day of age were significantly ($P<0.05$) higher than their corresponding values of the control group (table 7a).

Serum concentrations of the total lipids of control animals of group I recorded soon after birth (zero day) as 134.70±28.42 mg/dl, 18 h after birth as first day (199.75±29.80 mg/dl) and the value at the end of the experiment (30th day of age) as 394.19±50.34 mg/dl had significant ($P<0.05$) variations. The same trend was observed in the experimental calves of group II. The value (145.70±38.47 mg/dl) recorded immediately after birth (zero day) showed an increase at 18 h after birth i.e., at the first day of age (200.81±31.24 mg/dl) and at the end of the experiment (483.67±76.51 mg/dl) *vide* table 7b.

4.2.4.2.2 Cholesterol

The serum cholesterol concentration of neonatal calves of group I and II for a period of 30 days are depicted in the tables 7a, 7b, 7c and fig 7.

In the control animals of group I, which were fed with colostrum and then milk as per standard feeding regime of farms, serum cholesterol concentration started to increase from the lowest value of 29.86±9.23 mg/dl immediately after birth (zero day) and reached a peak value (91.97±34.34 mg/dl) observed on 24th day of age, which was then further reduced by the end of the experiment. In the experimental calves of group II, which were fed colostrum for 30 days of age, the lowest value was (27.64±2.46 mg/dl) recorded

soon after birth (zero day) and peak value (112.09±4.20 mg/dl) on 30th day of age (table 7a and fig. 7).

While analyzing the values from zero to 30 days of age of the two groups, it was observed that the value recorded on 18th day (71.14±23.76 mg/dl) and 30th day of age (67.73±23.58 mg/dl) of calves of group I were significantly ($P<0.05$) lower than the corresponding values of calves of group II. The values of calves of group II on 18th and 30th day of age were 108.17±13.72 mg/dl and 112.09±4.20 mg/dl respectively (table 7a).

In the control animals of group I, the serum concentration of cholesterol (67.73±23.58 mg/dl) recorded at the end of the experimental (30th day of age) was significantly ($P<0.05$) higher than the value (29.86±9.23 mg/dl) recorded soon after birth as well as the value (36.45±8.36 mg/dl) recorded 18 h after birth (first day). In the experimental animals of group II, all the three values such as the concentration (27.64±2.46 mg/dl) obtained soon after birth (zero day), value recorded (39.62±5.46 mg/dl) 18 h after birth (first day) and the value observed (112.09±4.20 mg/dl) after prolonged colostrum intake (30th day of age) varied significantly ($P<0.05$) *vide* table 7b.

4.2.4.2.3 Triglyceride (TG)

Serum triglyceride concentration in the control animals of group I, which were fed with colostrum and then milk as per standard feeding regime of farms increased steadily from the lowest recorded value (19.15±4.55 mg/dl) obtained soon after birth (zero day) and reached a peak value (24.83±11.38

mg/dl) on 30th day of age. A similar trend was observed in experimental calves of group II which had the lowest value (15.90±1.64 mg/dl) immediately after birth (zero day) and peak value (33.33±8.07 mg/dl) obtained on 30th day of age (table 7a and fig.7).

On comparing serum triglyceride concentration between these two groups; it was observed that none of the pair of values showed any significant variation (table 7a).

In the control animals of group I none of the three values of lipid profile showed any significant variation between them. The experimental calves of group II showed a significantly ($P<0.05$) increased concentration of triglyceride (33.33±8.07 mg/dl) at the end of the trial (30th day of age) when compared to the value (15.90±1.64 mg/dl) recorded soon after birth (zero day) and the value (19.24±9.90 mg/dl) observed 18 h after birth (first day) *vide* table 7b.

4.2.4.2.4 Non-esterified fatty acids (NEFA)

Effect of first colostrum intake and continued feeding of colostrum (Group II) and milk (Group I) on serum NEFA concentration of neonatal calves for a period of 30 days are shown in the tables 7a, 7b, 7c and fig.8.

In the control animals of group I, which were fed with colostrum and then milk as per standard feeding regime of farms, serum NEFA concentration showed a fluctuating trend which declined from the peak value (551.57±101.28 μmol/l) recorded soon after birth (zero day) to the lowest value (376.90±59.61 μmol/l) recorded on 24th day of age and a moderate increase by the end of the

experiment. In the experimental animals of group II, which were fed colostrum for 30 days of age a similar fluctuating trend was observed with the peak value (579.60±102.78 μmol/l) recorded soon after birth (zero day) and the lowest value (414.50±80.16 μmol/l) recorded on sixth day of age (table 7a and fig. 8).

While analyzing the serum NEFA concentration from zero to 30 days of age in the two groups, it was observed that none of the values between the two groups of corresponding periods showed any significant variation (table 7a).

In the calves of control group I, the concentration of NEFA at the end of the experiment (30^{th} day of age) was significantly ($P<0.05$) lower (382.03±51.37 μmol/l) than the value (551.57±101.28 μmol/l) recorded soon after birth (zero day). The experimental animals of group II failed to show any significant variation between the three periods. (table 7b).

4.2.4.3 Blood glucose level (BGL)

Effect of first colostrum intake and continued feeding of colostrum (Group II) and milk (Group I) on the blood glucose level of neonatal crossbred calves for a period of 30 days of age are presented in the tables 8a, 8b, 8c and fig. 9.

In the control animals of group I, BGL increased steadily from the lowest value (102.00 ±15.99 mg/dl) observed immediately after birth (zero day) and reached a peak value (137.33±38.35 mg/dl) on 30^{th} day of age. The experimental calves of group II which were continuously fed colostrum for 30 days of age also followed a similar tendency with the least value (111.17±15.48

Table 7a. Effect of continued feeding of colostrum and milk on serum lipid profile of neonatal crossbred calves for a period of 30 days, Mean ± SD (n=6 per group)

Age in days	Total lipids (mg/dl)		Cholesterol (mg/dl)		Triglycerides (mg/dl)		Non-esterified fatty acids NEFA (µmol/l)	
	Group I	Group II	Group I	Group II	Group I	Group II	Group I	Group II
0	134.70± 28.42[a]	145.70± 38.47[a]	29.86± 9.23[a]	27.64± 2.46[a]	19.15± 4.55[a]	15.90± 1.64[a]	551.57± 101.28[a]	579.60± 102.78[a]
1	199.75± 29.80[a]	200.81± 31.24[a]	36.45± 8.36[a]	39.62± 5.46[a]	21.00± 8.00[a]	19.24± 9.90[a]	410.47± 66.09[a]	419.66± 88.52[a]
6	289.29± 7.57[a]	383.38± 35.26[b]	87.31± 60.02[a]	86.93± 23.03[a]	19.51± 3.61[a]	22.34± 6.69[a]	427.03± 70.21[a]	414.50± 80.16[a]
12	312.00± 49.87[a]	389.31± 45.76[b]	78.40± 18.80[a]	84.41± 23.91[a]	20.68± 3.69[a]	23.74± 1.42[a]	391.58± 55.41[a]	431.38± 79.94[a]
18	318.62± 168.42[a]	441.65± 59.15[b]	71.14± 23.76[a]	108.17± 13.72[b]	24.48± 11.78[a]	25.05± 6.90[a]	405.44± 101.20[a]	444.33± 92.50[a]
24	336.82± 41.50[a]	445.62± 83.88[b]	91.97± 34.34[a]	88.65± 6.21[a]	24.55± 2.04[a]	29.07± 8.92[a]	376.90± 59.61[a]	424.86± 157.22[a]
30	394.19± 50.34[a]	483.67± 76.51[b]	67.73± 23.58[a]	112.09± 4.20[b]	24.83± 11.38[a]	33.33± 8.07[a]	382.03± 51.37[a]	474.44± 122.71[a]

Mean ± standard deviation (between groups) in rows bearing different superscripts for each parameter differ significantly ($p<0.05$)

Table 7b. Effect of the first colostrum intake and continued feeding of colostrum and milk on serum lipid profile of neonatal crossbred calves for a period of 30 days, Mean ± SD (n=6 per group)

Sampling periods	Total lipids (mg/dl)		Cholesterol (mg/dl)		Triglycerides (mg/dl)		Non-esterified fatty acids NEFA (µmol/l)	
	Group I	Group II	Group I	Group II	Group I	Group II	Group I	Group II
Before the start of the experiment (0 d)	134.70± 28.42^{a}	145.70± 38.47^{a}	29.86± 9.23^{a}	27.64± 2.46^{a}	19.15± 4.55^{a}	15.90± 1.64^{a}	551.57± 101.28^{a}	579.60± 102.78^{a}
18 h after the start of the experiment (1 d)	199.75± 29.80^{b}	200.81± 31.24^{b}	36.45± 8.36^{a}	39.62± 5.46^{b}	21.00± 8.00^{a}	19.24± 9.90^{a}	410.47± 66.09^{ab}	419.66± 88.52^{a}
At the end of the experiment (30 d)	394.19± 50.34^{c}	483.67± 76.51^{c}	67.73± 23.58^{b}	112.09± 4.20^{c}	24.83± 11.38^{a}	33.33± 8.07^{b}	382.03± 51.37^{b}	474.44± 122.71^{a}

Mean ± standard deviation (between periods) in columns bearing different superscripts for each parameter differ significantly ($p<0.05$)

Table 7c. Effect of continued feeding of colostrum and milk on serum lipid profile of neonatal crossbred calves for a period of 30 days, Mean ± SD (n=6 per group)

Age in days	Total lipids (mg/dl)		Cholesterol (mg/dl)		Triglycerides (mg/dl)		Non-esterified fatty acids NEFA (µmol/l)	
	Group I	Group II	Group I	Group II	Group I	Group II	Group I	Group II
0	134.70± 28.42	145.70± 38.47	29.86± 9.23^{a}	27.64± 2.46	19.15± 4.55^{abcde}	15.90± 1.64^{ab}	551.57± 101.28^{abc}	579.60± 102.78^{abcde}
1	199.75± 29.80^{a}	200.81± 31.24	36.45± 8.36^{a}	39.62± 5.46	21.00± 8.00^{cgjnop}	19.24± 9.90^{acdef}	410.47± 66.09^{bdijkl}	419.66± 88.52^{eimprt}
6	289.29± 7.57^{bc}	383.38± 35.26^{abc}	87.31± 60.02^{dgik}	86.93± 23.03^{ac}	19.51± 3.61^{afghi}	22.34± 6.69^{bcghi}	427.03± 70.21^{adefgh}	414.50± 80.16^{jnqst}
12	312.00± 49.87^{bde}	389.31± 45.76^{ad}	78.40± 18.80^{cfij}	84.41± 23.91^{ab}	20.68± 3.69^{bfjklm}	23.74± 1.42^{dgjk}	391.58± 55.41^{fjmpq}	431.38± 79.94^{cgkopq}
18	318.62± 168.42^{acdfg}	441.65± 59.15^{bef}	71.14± 23.76^{bfgh}	108.17± 13.72^{d}	24.48± 11.78^{dhknqr}	25.05± 6.90^{ehjlm}	405.44± 101.20^{ceimno}	444.33± 92.50^{bfklmn}
24	336.82± 41.50^{ef}	445.62± 83.88^{ideg}	91.97± 34.34^{ehjk}	88.65± 6.21^{bc}	24.55± 2.04^{loqs}	29.07± 8.92^{fikln}	376.90± 59.61^{hloqr}	424.86± 157.22^{dhlors}
30	394.19± 50.34^{g}	483.67± 76.51^{fg}	67.73± 23.58^{bcde}	112.09± 4.20^{d}	24.83± 11.38^{eimprs}	33.33± 8.07^{mn}	382.03± 51.37^{gknpr}	474.44± 122.71^{afghij}

Means ± standard deviation (between periods) in columns bearing same superscripts for each parameter are homogenous

Fig.7. Effect of continued feeding of colostrum and milk on serum lipid profile of neonatal crossbred calves for a period of 30 days

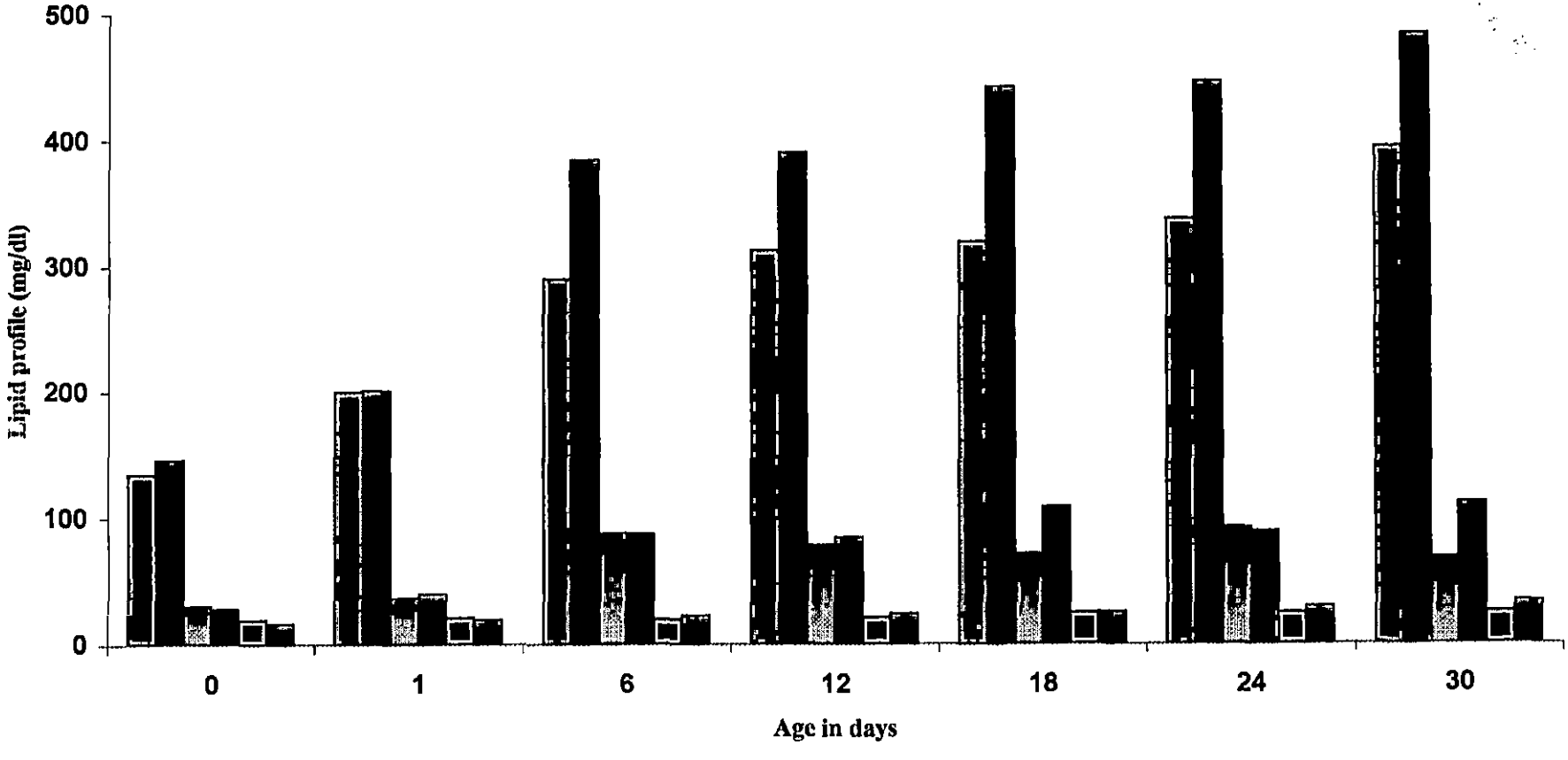
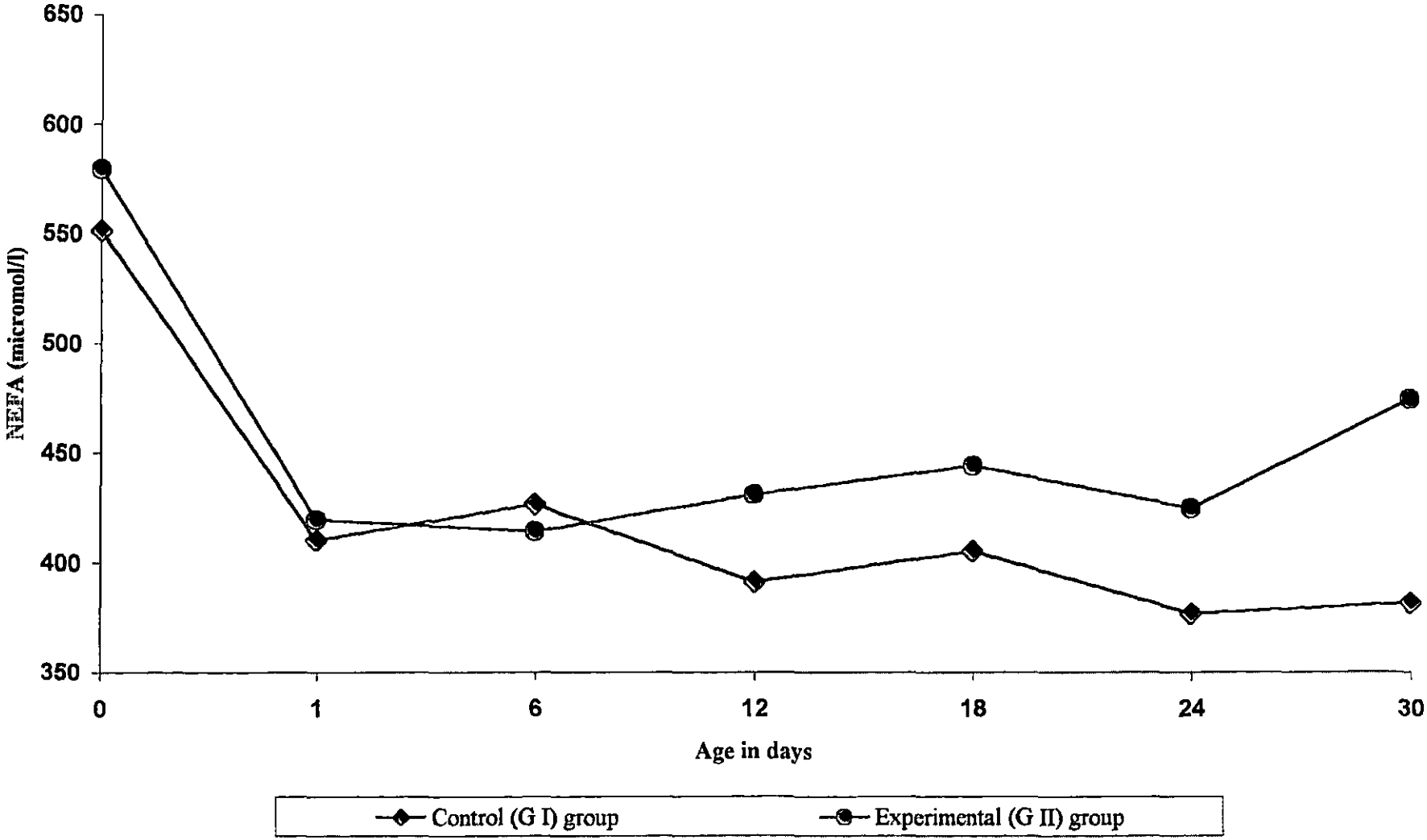


Fig.8. Effect of continued feeding of colostrum and milk on non-esterified fatty acids (NEFA) of neonatal crossbred calves for a period of 30 days



mg/dl) recorded soon after birth (zero day), and the peak value (140.67±24.15mg/dl) on 24th day of age with a slight decrease on 30th day of age (table 8a and fig. 9).

While analyzing the values from zero to 30 days of age, it was observed that there was no significant difference in the blood glucose level of the two groups at any of the period studied (table 8a).

Animals of group I and II failed to show any significant difference in the blood glucose levels recorded soon after birth (zero day), 18 h after birth (first day) or at the end of the experimental period (30th day of age) *vide* table 8b.

4.2.4.4 Serum urea nitrogen (BUN)

Serum urea nitrogen concentration of the control animals of group I, which were fed with colostrum and then milk as per standard feeding regime of farms, steadily increased from the lowest value (9.58±1.41 mg/dl) recorded immediately after birth and reached the peak value (15.09±2.81 mg/dl) on 30th day of age. Same trend was observed in experimental calves of group II which were fed colostrum continuously for 30 days of age, with the lowest recorded value (9.64±0.78 mg/dl) soon after birth (zero day) and the highest value (17.05±3.11 mg/dl) on 30th day of age (table 8a and fig. 10).

On comparing serum urea nitrogen levels between the calves of two groups, it was observed that none of the values showed any significant variation (table 8a).

In the calves of control group I, the serum concentration of urea nitrogen (15.09±2.81 mg/dl) at the end of the experiment (30th day of age) was significantly (P<0.05) higher than the value (9.58±1.41 mg/dl) recorded soon after birth (zero day) as well as the concentration (10.21±0.93 mg/dl) recorded 18 h after birth (first day). In the experimental calves of group II, the value (9.64±0.78 mg/dl) recorded immediately after birth (zero day) was significantly (P<0.05) lower than the value (10.43±3.08 mg/dl) recorded 18 h after birth (first day) and the value (17.05±3.11 mg/dl) obtained at the end of the experimental period (table 8b).

4.2.4.5 Serum creatinine

The serum creatinine concentration of neonatal calves of group I and II for a period of 30 days are depicted in the tables 8a, 8b, 8c and fig. 10.

Serum creatinine concentration in control animals of group I followed a fluctuating trend with the highest value recorded (0.89±0.13 mg/dl) soon after birth (zero day) and lowest value on sixth day of age (0.59±0.03 mg/dl). In the experimental calves of group II, which were fed colostrum continuously for 30 days of age, a similar fluctuating trend was observed with the highest value recorded (1.02±0.39 mg/dl) immediately after birth (zero day) and the lowest value (0.56±0.22 mg/dl) on sixth day of age (table 8a and fig. 10).

While analyzing the values from zero to 30 days of age of the two groups, it was observed that the values obtained for calves of group I on 24th day (0.70±0.08 mg/dl) and 30th day of age (0.62±0.03 mg/dl) were significantly

($P<0.05$) lower than the corresponding values of calves of group II. The values for calves of experimental group on 24th and 30th days of age were 0.94±0.19 mg/dl and 0.88±0.24 mg/dl respectively (table 8a).

The serum concentration of creatinine recorded at the end of the trial(30th day of age) for calves of group I was significantly ($P<0.05$) lowest (0.62±0.03 mg/dl) among the value (0.89±0.13 mg/dl) recorded soon after birth (zero day) and the value (0.88±0.08 mg/dl) of 18 h after birth (first day of age). However the experimental animals of group II failed to show any significant variation in these periods (table 8b).

4.2.4.6 Serum bilirubin

Serum bilirubin concentration in the control animals of group I which were fed with colostrum and then milk as per standard feeding regime of farms showed a fluctuating trend which started from the lowest value (0.52±0.05 mg/dl) recorded soon after birth and increased to reach the highest value (1.02±0.48 mg/dl) on sixth day of age. In the experimental animals of group II which were fed colostrum for 30 days of age a similar fluctuating trend was observed with the peak value (0.89±0.43 mg/dl) at 18 h after birth (first day of age) and the lowest value (0.47±0.07 mg/dl) on 18th day of age (table 8a and fig. 10).

While analyzing the values of the two groups from zero to 30 days of age of it was observed that there were no significant variations between the two groups (table 8a).

Table 8a. Effect of continued feeding of colostrum and milk on blood glucose level and serum biochemical parameters of neonatal crossbred calves for a period of 30 days, Mean ± SD (n=6 per group)

Age in Days	Blood glucose (mg/dl)		Urea nitrogen (mg/dl)		Creatinine (mg/dl)		Bilirubin (mg/dl)	
	Group I	Group II	Group I	Group II	Group I	Group II	Group I	Group II
0	102.00± 15.99[a]	111.17± 15.48[a]	9.58± 1.41[a]	9.64± 0.78[a]	0.89± 0.13[a]	1.02± 0.39[a]	0.52± 0.05[a]	0.63± 0.43[a]
1	119.17± 11.70[a]	118.83± 16.77[a]	10.21± 0.93[a]	10.43± 3.08[a]	0.88± 0.08[a]	0.81± 0.10[a]	0.93± 0.81[a]	0.89± 0.43[a]
6	119.00± 4.86[a]	123.50± 26.21[a]	10.74± 2.09[a]	13.98± 2.93[a]	0.59± 0.03[a]	0.56± 0.22[a]	1.02± 0.48[a]	0.88 ± 0.33[a]
12	114.83± 35.81[a]	129.00± 18.99[a]	11.51± 4.25[a]	14.26± 2.62[a]	0.79± 0.13[a]	0.83± 0.12[a]	0.53± 0.15[a]	0.63 ± 0.22[a]
18	115.33± 36.35[a]	136.67± 22.27[a]	11.74± 2.03[a]	14.68± 2.81[a]	0.80± 0.13[a]	0.81± 0.12[a]	0.85± 0.43[a]	0.47 ± 0.07[a]
24	125.33± 23.84[a]	140.67± 24.15[a]	13.70± 3.81[a]	16.78± 2.13[a]	0.70± 0.08[a]	0.94± 0.19[b]	0.92± 0.36[a]	0.69± 0.18[a]
30	137.33± 38.35[a]	140.50± 23.24[a]	15.09± 2.81[a]	17.05± 3.11[a]	0.62± 0.03[a]	0.88± 0.24[b]	0.87± 0.35[a]	0.84± 0.37[a]

Means ± standard deviation (between groups) in rows bearing different superscripts for each parameter differ significantly ($p<0.05$)

Table 8b. Effect of the first colostrum intake and continued feeding of colostrum and milk on blood glucose levels and on serum biochemical parameters of neonatal crossbred calves for a period of 30 days Mean ± SD (n=6 per group)

Sampling periods	Blood glucose level (g/dl)		Urea nitrogen (mg/dl)		Creatinine (mg/dl)		Bilirubin (mg/dl)	
	Group I	Group II	Group I	Group II	Group I	Group II	Group I	Group II
Before the start of the experiment (0 d)	102.00± 15.99[a]	111.17± 15.48[a]	9.58± 1.41[a]	9.64± 0.78[a]	0.89± 0.13[a]	1.02± 0.39[a]	0.52± 0.05[a]	0.63± 0.43[a]
18 h after the start of the experiment (1 d)	119.17± 11.70[a]	118.83± 16.77[a]	10.21± 0.93[a]	10.43± 3.08[b]	0.88± 0.08[a]	0.81± 0.10[a]	0.93± 0.81[a]	0.89± 0.43[a]
At the end of the experiment (30 d)	137.33± 38.35[a]	140.50± 23.24[a]	15.09± 2.81[b]	17.05± 3.11[b]	0.62± 0.03[b]	0.88± 0.24[a]	0.87± 0.35[a]	0.84± 0.37[a]

Mean ± standard deviation (between periods) in columns bearing different superscripts for each parameter differ significantly ($p<0.05$)

Table 8c. Effect of continued feeding of colostrum and milk on blood glucose level and serum biochemical parameters of neonatal crossbred calves for a period of 30 days Mean ± SD (n=6 per group)

Age in Days	Blood glucose (mg/dl)		Urea nitrogen (mg/dl)		Creatinine (mg/dl)		Bilirubin (mg/dl)	
	Group I	Group II	Group I	Group II	Group I	Group II	Group I	Group II
0	102.00± 15.99[abcde]	111.17± 15.48[abcde]	9.58± 1.41[abcde]	9.64± 0.78	0.89± 0.13[abcdef]	1.02± 0.39[abcdef]	0.52± 0.05[abcd]	0.63± 0.43[bcghij]
1	119.17± 11.70[chlors]	118.83± 16.77[afghij]	10.21± 0.93[afgh]	10.43± 3.08[abcd]	0.88± 0.08[agh]	0.81± 0.10[ejmop]	0.93± 0.81[dhloqs]	0.89± 0.43[fjmop]
6	119.00± 4.86[glcopq]	123.50± 26.21[bfklmn]	10.74± 2.09[bfijk]	13.98± 2.93[aefgh]	0.59± 0.03[fm]	0.56± 0.22[fq]	1.02± 0.48[imprs]	0.88 ± 0.33[ilnp]
12	114.83± 35.81[afghij]	129.00± 18.99[cgkopq]	11.51± 4.25[cgilmn]	14.26± 2.62[beijk]	0.79± 0.13[chik]	0.83± 0.12[chkno]	0.53± 0.15[aefghi]	0.63 ± 0.22[acdef]
18	115.33± 36.35[bfklmn]	136.67± 22.27[dhlors]	11.74± 2.03[dhijlo]	14.68± 2.81[cfilm]	0.80± 0.13[bgij]	0.81± 0.12[dilnpq]	0.85± 0.43[bcjklm]	0.47 ± 0.07[ab]
24	125.33± 23.84[dimprt]	140.67± 24.15[imprt]	13.70± 3.81[ekmop]	16.78± 2.13[gjln]	0.70± 0.08[djkl]	0.94± 0.19[aghij]	0.92± 0.36[gkhqr]	0.69± 0.18[dgklm]
30	137.33± 38.35[ejnqst]	140.50± 23.24[ejnqst]	15.09± 2.81[np]	17.05± 3.11[dhkmn]	0.62± 0.03[clm]	0.88± 0.24[bgklm]	0.87± 0.35[cfjnop]	0.84± 0.37[ehkno]

Means ± standard deviation (between periods) in columns bearing same superscripts for each parameter are homogenous

Fig.9. Effect of continued feeding of colostrum and milk on blood glucose level (BGL) and on serum insulin concentration of neonatal crossbred calves for a period of 30 days

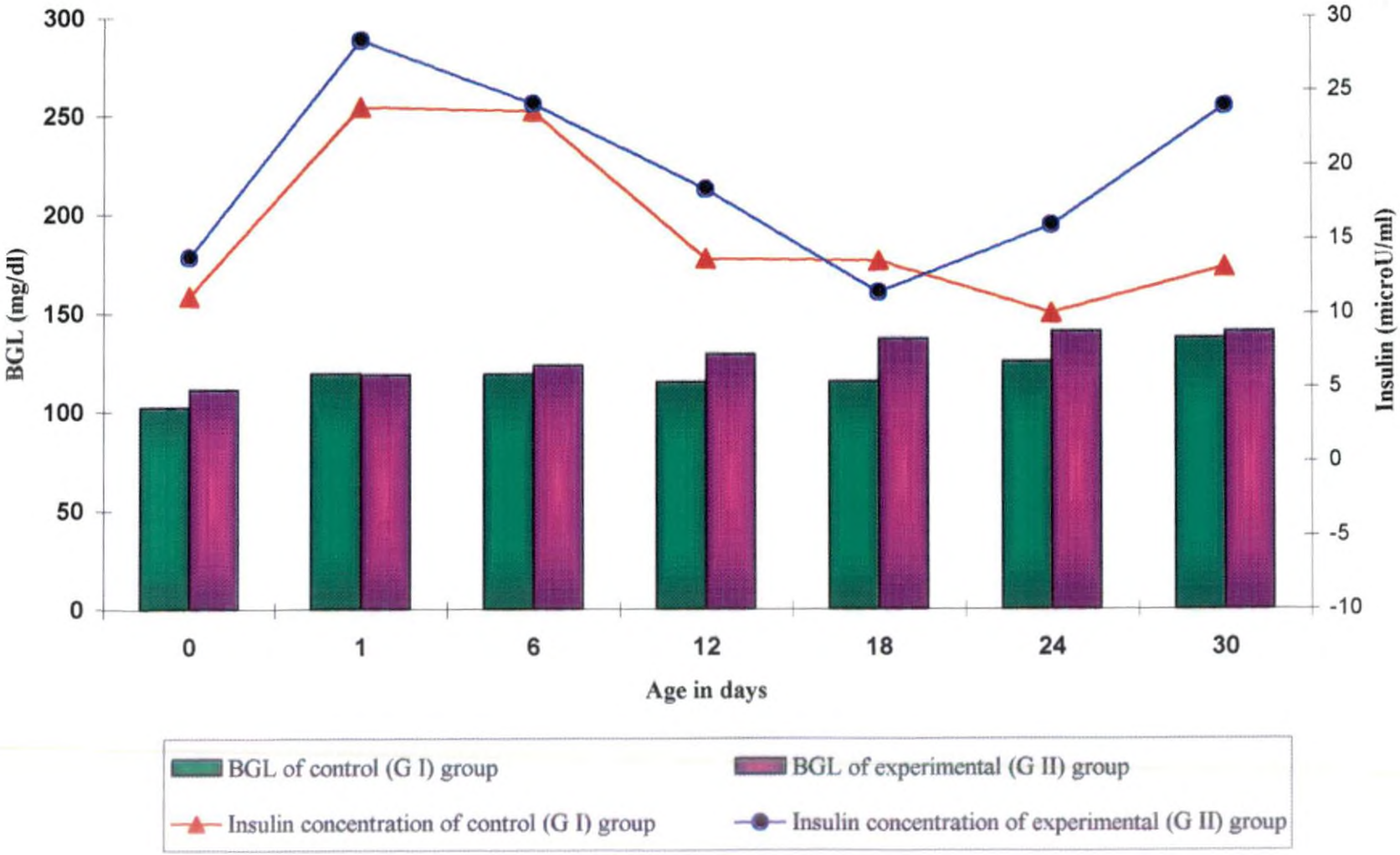
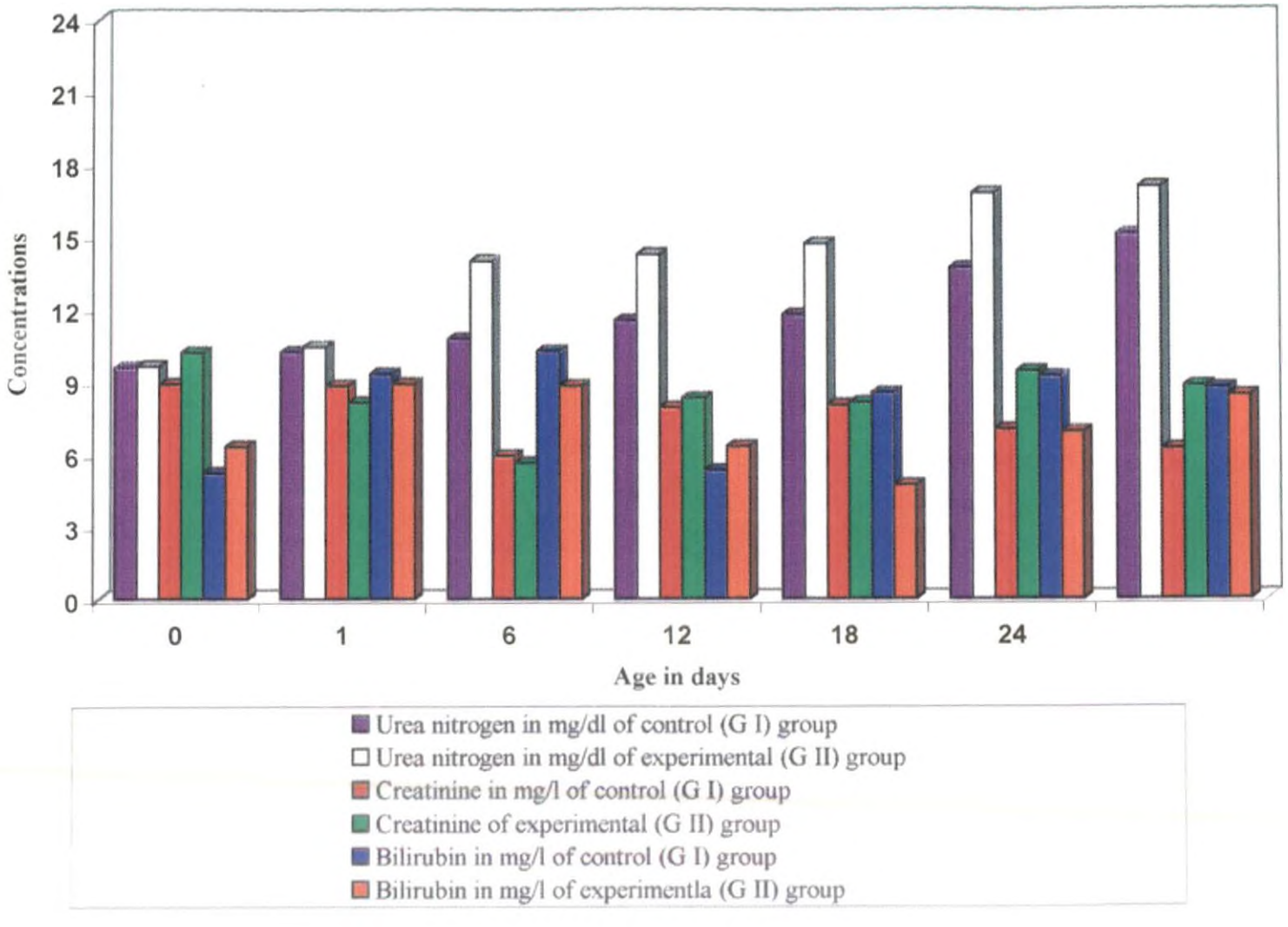


Fig.10. Effect of continued feeding of colostrum and milk on serum urea nitrogen, creatninine and bilirubin of neonatal crossbred calves for a period of 30 days



Group I and II animals failed to show any significant differences in the serum bilirubin concentration, at 18 h after birth (first day) or at the end of the experiment (30th day of age) from the value obtained immediately after birth (zero day) *vide* table 8b.

4.2.5 Serum hormonal profile

Effect of first colostrum intake and continued feeding of colostrum (Group II) and milk (Group I) on serum concentration of thyroxine (T_4), triiodothyronine (T_4), T_4: T_3 ratio and insulin of neonatal crossbred calves for a period of 30 days are shown in the tables 9a, 9b, 9c, fig.9 and 11.

4.2.5.1 Thyroxine (T_4)

Serum thyroxine (T_4) concentration of the control animals of group I which were fed colostrum and then milk as per standard feeding regime of farms declined steadily from the highest value (69.12 ± 9.65 ng/ml) recorded 18 h after birth (first day) to the lowest value (40.37±3.01 ng/ml) on 24th day of age. The experimental calves of group II, which were under prolonged colostrum feeding for 30 days of age, also followed a similar trend with the peak value recorded (71.79±10.68 ng/ml) 18 h after birth (first day) and the lowest value (40.24±2.19 ng/ml) on 24th day of age (table 9a and fig. 11).

On comparing the serum thyroxine (T_4) concentrations of the two groups, it was observed that there was no significant variation between them (table 9a).

In the control animals of group I, the serum concentration of T_4 (41.27 ± 3.37 ng/ml) obtained on 30th day of age was significantly ($P<0.05$) lower than the value (64.79 ± 19.66 ng/ml) recorded immediately after birth (zero day) as well as the value (69.12 ± 9.65 ng/ml) observed 18 h after birth (first day). The experimental calves of group II also showed significant ($P<0.05$) variations in the concentrations of T_4 recorded (43.09 ± 1.75 ng/ml) on 30th day of age when compared with the value (69.09 ± 9.99 ng/ml) observed immediately after birth (zero day) and the value (71.79 ± 10.68 ng/ml) at 18 h after birth (first day of age) *vide* table 9b.

4.2.5.2 Triiodothyronine (T_3)

The serum T_3 concentrations of neonatal calves of both group I and II for a period of 30 days are presented in the tables 9a, 9b,9c and fig.11.

Serum T_3 concentration in the control animals of group I which were fed colostrum and then milk as per standard feeding regime of farms showed a fluctuating trend with the peak value (2.58 ± 0.18 ng/ml) recorded soon after birth and the value declined to the lowest level (0.99 ± 0.17 ng/ml) on 12th day of age, which further increased slightly till the end of the experiment. The experimental calves of group II which were fed colostrum for 30 days of age, showed a similar fluctuating trend with the highest value (2.30 ± 0.11 ng/ml) recorded immediately after birth (zero day) and the lowest value (1.18 ± 0.26 ng/ml) on 18th day of age (table 9a and fig. 11).

While analyzing the values from zero to 30 days of age of the two groups, it was observed that the values obtained on 12th day (0.99 ± 0.17 ng/ml) and 30th day of age (1.22 ± 0.22 ng/ml) for the calves of group I were significantly (P<0.05) lower than the corresponding values of group II calves. The values of group II calves on 12th day and 30th day of age were 1.54 ± 0.20 ng/ml and 1.54 ± 0.21 ng/ml respectively (table 9a).

Group I and group II animals showed a significant (P<0.05) variation in the serum T_3 concentration when the value (1.22 ± 0.22 ng/ml) of the group I animals recorded on 30th day of age was compared with the value (2.58 ± 0.18 ng/ml) recorded soon after birth (zero day) and the value (2.47 ± 0.21 ng/ml) obtained 18 h after birth (first day). Group II animals showed significant (P<0.05) variations when the concentration (1.54 ± 0.21 ng/ml) recorded on 30th day of age was compared with the value (2.30 ± 0.11 ng/ml) recorded immediately after birth (zero day) and the concentration (2.48 ± 0.20 ng/ml) 18 h after birth (first day) *vide* table 9b.

4.2.5.3 T_4: T_3 ratio

Effect of first colostrum intake and continued feeding of colostrum (Group II) and milk (Group I) on serum T_4: T_3 ratio of neonatal calves for a period of 30 days are shown in the tables 9a, 9b and 9c.

In the control animals of group I, which were fed colostrum and then milk as per standard feeding regime of farms, T_4: T_3 ratio showed a fluctuating trend starting from the lowest value (23.95 ± 3.93) recorded 18h after birth

First day) which increased to the peak value (47.07 ± 8.35) recorded on 12th day of age and further declined by the end of the experiment. The experimental calves of group II which were fed colostrum for 30 days of age, also showed a fluctuating trend with a peak value (37.67 ± 7.79) obtained on 18th day of age and lowest value (28.29 ± 2.90) recorded on 30th day of age (table 9a).

On comparing T_4: T_3 ratio between the two groups, there was significant ($P<0.05$) variation between the values obtained only on 12th day of age for calves of group I (47.07 ± 8.35) and group II (29.40 ± 3.67) *vide* table 9a.

In the group I calves, value of T_4: T_3 ratio (35.09 ± 8.94) recorded at the end of the experiment (30th day of age) was significantly ($P<0.05$) higher than the value (23.95 ± 3.93) obtained 18 h after birth (first day of age). Whereas, in the group II animals none of the three values showed any significant variation (table 9b).

4.2.5.4 Insulin

Effect of first colostrum intake and continued feeding of colostrum (Group II) and milk (Group I) on serum insulin concentration of neonatal calves for a period of 30 days are presented the tables 9a, 9b, 9c and fig. 9.

Serum insulin concentration in the control animals of group I, which were fed with colostrum and then milk as per standard feeding regime, showed a fluctuating trend with the highest value (24.01 ± 3.50 μU/ml) recorded 18 h after birth (first day) and the lowest value (10.06 ± 1.88 μU/ml) on 24th day of age. Experimental calves of group II, which were fed colostrum for 30 days of

age, also showed a similar fluctuating trend with the lowest value recorded (13.79 ± 3.77 μU/ml) soon after birth and the peak value (28.51 ± 2.90 μU/ml) obtained 18 h after birth (first day) which further got reduced and then increased to a value of 24.04 ± 2.84 μU/ml by the end of the experiment (table 9a and fig. 9).

While analyzing the values from zero to 30 days of age of the two groups, it was observed that the values obtained on 12th day (13.73 ± 3.80 μU/ml), 24th day (10.06 ± 1.88 μU/ml) and 30th day of age (13.20 ± 2.59 μU/ml) of calves of group I were significantly ($P<0.05$) lower than the corresponding values of group II calves on 12th, 24th and 30th days of age. The corresponding values were 18.43 ± 2.90 μU/ml, 15.98 ± 2.43 μU/ml and 24.04 ± 2.84 μU/ml respectively (table 9a).

In the control animals of group I, the serum concentration of insulin (24.01 ± 3.50 μU/ml) obtained 18 h after birth (first day) was significantly ($P<0.05$) higher than the value (11.17 ± 2.89 μU/ml) recorded soon after birth (zero day) and the value (13.20 ± 2.59 μU/ml) recorded at the end of the experiment (30th day of age). In the experimental calves of group II the concentration (13.79 ± 3.77 μU/ml) recorded soon after birth (zero day) was significantly ($P<0.05$) lower than the value (28.51±2.90 μU/ml) recorded 18 h after birth (first day) and the value (24.03 ± 2.84 μU/ml) on 30th day of age (table 9b).

Table 9a. Effect of continued feeding of colostrum and milk on serum hormonal parameters of neonatal crossbred calves for a period of 30 days, Mean ± SD (n=6 per group)

Age in Days	Thyroxine (T_4) (ng/ml)		Triiodothyronine(T_3) (ng/ml)		T_4:T_3 ratio		Insulin (µU/ml)	
	Group I	Group II	Group I	Group II	Group I	Group II	Group I	Group II
0	64.79± 19.66[a]	69.09± 9.99[a]	2.58± 0.18[a]	2.30± 0.11[a]	25.04± 7.86[a]	30.21± 5.19[a]	11.17± 2.89[a]	13.79± 3.77[a]
1	69.12± 9.65[a]	71.79± 10.68[a]	2.47± 0.21[a]	2.48± 0.20[a]	23.95± 3.93[a]	29.20± 5.20[a]	24.01± 3.50[a]	28.51± 2.90[a]
6	55.84± 11.64[a]	49.60± 2.28[a]	1.52± 0.17[a]	1.62± 0.23[a]	37.52± 10.51[a]	31.06 ± 4.48[a]	23.65± 2.73[a]	24.17± 4.24[a]
12	45.60± 3.44[a]	44.67± 2.51[a]	0.99± 0.17[a]	1.54± 0.20[b]	47.07± 8.35[a]	29.40± 3.67[b]	13.73± 3.80[a]	18.43± 2.90[b]
18	44.45± 2.17[a]	41.31± 2.91[a]	1.11± 0.23[a]	1.18± 0.26[a]	41.35± 8.13[a]	37.67± 7.79[a]	13.60± 4.26[a]	11.44± 1.89[a]
24	40.37± 3.01[a]	40.24± 2.19[a]	1.16± 0.41[a]	1.24± 0.21[a]	39.49± 17.18[a]	33.01± 4.65[a]	10.06± 1.88[a]	15.98± 2.43[b]
30	41.27± 3.37[a]	43.09± 1.75[a]	1.22± 0.22[a]	1.54± 0.21[b]	35.09± 8.94[a]	28.29± 2.90[a]	13.20± 2.59[a]	24.04± 2.84[b]

Means ± standard deviation (between groups) in rows bearing different superscripts for each parameter differ significantly ($p<0.05$)

Table 9b. Effect of the first colostrum intake and continued feeding of colostrum and milk on serum hormonal parameters of neonatal crossbred calves for a period of 30 days mean ± SD (n=6 per group)

Sampling periods	Thyroxine (T_4) (ng/ml)		Triiodothyronine (T_3) (ng/ml)		T_4:T_3 ratio		Insulin (µU/ml)	
	Group I	Group II	Group I	Group II	Group I	Group II	Group I	Group II
Before the start of the experiment (0 d)	64.79± 19.66^{a}	69.09± 9.99^{a}	2.58± 0.18^{a}	2.30± 0.11^{a}	25.04± 7.86^{a}	30.21± 5.19^{a}	11.17± 2.89^{a}	13.79± 3.77^{a}
18 h after the start of the experiment (1 d)	69.12± 9.65^{a}	71.79± 10.68^{a}	2.47± 0.21^{a}	2.48± 0.20^{a}	23.95± 3.93^{ab}	29.20± 5.20^{a}	24.01± 3.50^{b}	28.51± 2.90^{b}
At the end of the experiment (30 d)	41.27± 3.37^{b}	43.09± 1.75^{b}	1.22± 0.22^{b}	1.54± 0.21^{b}	35.09± 8.94^{ac}	28.29± 2.90^{a}	13.20± 2.59^{a}	24.03± 2.84^{b}

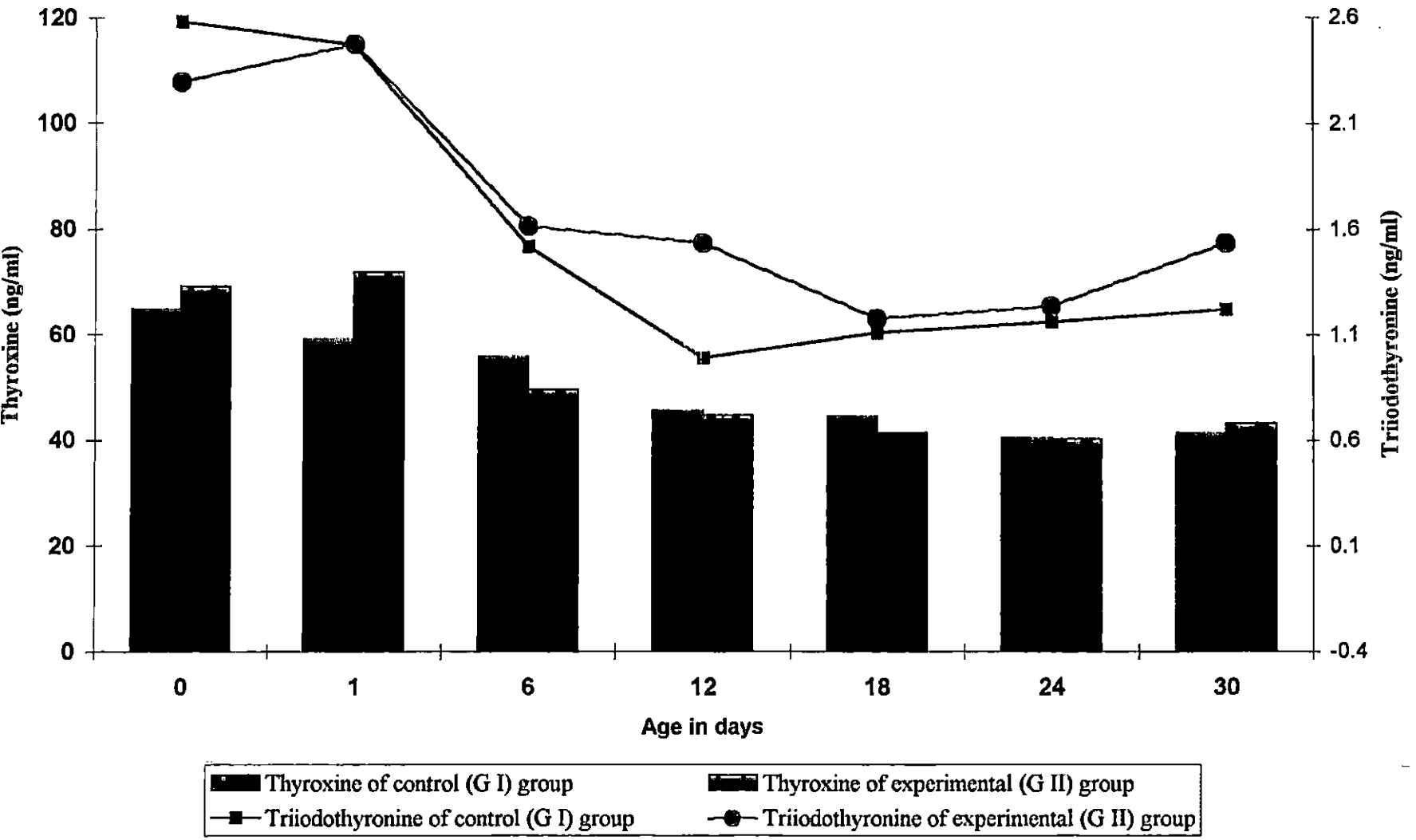
Mean ± standard deviation (between periods) in columns bearing different superscripts for each parameter differ significantly ($p<0.05$)

Table 9c. Effect of continued feeding of colostrum and milk on serum hormonal parameters of neonatal crossbred calves for a period of 30 days Mean ± SD (n=6 per group)

Age in Days	Thyroxine (T_4) (ng/ml)		Triiodothyronine(T_3) (ng/ml)		T_4:T_3 ratio		Insulin (µU/ml)	
	Group I	Group II	Group I	Group II	Group I	Group II	Group I	Group II
0	64.79± 19.66^{acdefg}	69.09± 9.99^{a}	2.58± 0.18^{a}	2.30± 0.11^{ab}	25.04± 7.86^{acd}	30.21± 5.19^{cfjno}	11.17± 2.89^{adef}	13.79± 3.77^{ab}
1	69.12± 9.65^{ab}	71.79± 10.68^{a}	2.47± 0.21^{a}	2.48± 0.20^{a}	23.95± 3.93^{ab}	29.20± 5.20^{aefghi}	24.01± 3.50^{j}	28.51± 2.90^{f}
6	55.84± 11.64^{bch}	49.60± 2.28	1.52± 0.17^{b}	1.62± 0.23^{bcd}	37.52± 10.51^{ehij}	31.06 ± 4.48^{bgknpr}	23.65± 2.73^{j}	24.17± 4.24^{bef}
12	45.60± 3.44^{di}	44.67± 2.51	0.99± 0.17^{egh}	1.54± 0.20^{gh}	47.07± 8.35^{jlm}	29.40± 3.67^{bejklm}	13.73± 3.80^{cfhi}	18.43± 2.90^{c}
18	44.45± 2.17^{ehijk}	41.31± 2.91^{b}	1.11± 0.23^{dfh}	1.18± 0.26^{fh}	41.35± 8.13^{gikm}	37.67± 7.79^{imqr}	13.60± 4.26^{begi}	11.44± 1.89^{a}
24	40.37± 3.01^{gkl}	40.24± 2.19^{b}	1.16± 0.41^{bcfg}	1.24± 0.21^{defg}	39.49± 17.18^{bdfhkl}	33.01± 4.65^{hlopr}	10.06± 1.88^{abc}	15.98± 2.43^{bcd}
30	41.27± 3.37^{fjl}	43.09± 1.75	1.22± 0.22^{cde}	1.54± 0.21^{ce}	35.09± 8.94^{cefg}	28.29± 2.90^{abcd}	13.20± 2.59^{dgh}	24.04± 2.84^{e}

Means ± standard deviation (between periods) in columns bearing same superscripts for each parameter are homogenous

Fig.11. Effect of continued feeding of colostrum and milk on serum thyroxine and triiodothyronine concentrations of neonatal crossbred calves for a period of 30 days



Discussion

5. DISCUSSION

5.1 Colstrum and milk

5.1.1 Effect of ultra-violet irradiation on total viable count of pooled colostrum

Mean value of total viable count of pooled colostrum samples after ultra-violet (UV) irradiation was significantly ($P<0.05$) lower than the count recorded before UV irradiation (table 1 & fig.1) and this was in consonance with earlier recordings of Chumachenko (1977), Sarkin (1977) and Caserio *et al.* (1978) where UV treatment greatly improved the keeping quality of colostrum and milk by destroying most of the bacterial flora including Coliforms, Staphylococci, Enterococci besides controlling yeast and mould growth.

5.1.2 Crude protein content of colostrum and milk

There was a significant ($P<0.05$) decrease in the crude protein content of colostrum from first milking (14.23 ± 1.25 g%) to the fifth milking (5.15 ± 0.36 g%) *vide* table 2. The protein content of the pooled colostrum (samples collected soon after parturition, first as well as second day) showed an intermediate value of 9.51 ± 1.44 g%, while protein content of whole milk was found to be much lower (2.63 ± 0.28 g%) *vide* table 1. These observations are in agreement with the findings of Larson and Kendall (1957), Singh *et al.* (1993) and Hadorn *et al.* (1997) who recorded that total protein content was highest in the first milking

(colostrum) and decreased as days of lactation was advanced. Geene (1984) was also of the same opinion and according to him the reduction of protein content of colostrum in cows was because of the rapid fall in total immunoglobulins within 24 h after parturition.

5.2 Experimental animals

5.2.1 Health status

The calves used in the study were apparently healthy and there were no significant differences between the two groups in various health parameters recorded. Clinical parameters of all the animals of both control and experimental groups (table 3) were within the normal range explaining that the calves were apparently healthy. According to Rauprich *et al.* (2000), the normal range for the three clinical parameters are as follows: 30-45/min for respiratory rate, 90-110/min for heart rate and 100-110°F for rectal temperature respectively. The observations were in consonance with the findings of Hadorn *et al.* (1997) and Vermorel *et al.* (1998).

5.2.2 Weekly body weight

In the present study it was recorded that the calves of experimental group (Group II) gained higher weekly body weight gain and thereby an overall weight

gain at the end of experimental period when compared to control calves of group I (table 4). The observations are in consonance with the reports of Kuhne *et al.* (2000) who explained that the increase in body weight gain in colostrum fed calves was due to high intake of energy and protein. Earlier in 1970, Owen *et al.* also reported that when colostrum was fed continuously for 21 days of age, calves showed 60 per cent improved body weight gain at three weeks, 40 per cent at six weeks and 25 per cent at twelve weeks of age with an improved starter intake. Calves which received colostrum with high immunoglobulin content in this study maintained heavier weight as observed by Nocek *et al.* (1984).

In the growing animals, intake of increased amount of dietary proteins over and above the requirement is likely to result in an increased growth rate and thereby, an increased body weight gain. In animals of group II the continued feeding of protein rich colostrum led to an increased availability of amino acids by intestinal absorption. The elevated systemic availability of amino acids in animals of group II might have resulted in better growth rate via an increased concentration of certain anabolic hormones, viz., insulin and thyroid hormones observed in the study and growth hormone, in bringing about comparatively more body mass than the control animals of group I which were fed with whole milk for a period of 30 days.

5.2.3 Haematological parameters

Evaluation of various haematological parameters depicts an overall picture of health status of an animal and hence any deviation from the normal physiological status will be reflected from the assessment. Haematological dynamics are well selected depending upon the physiological needs of animal, which vary with the different stages of growth of the animal.

The haematological parameters such as the haemoglobin (Hb) content and volume of packed red blood corpuscles (VPRC) of calves of group I and group II showed a declining trend in the present study (table 5a). These observations are in consonance with the findings of Sridhar *et al.* (1988) and Rauprich *et al.* (2000) who opined that it would be probably a consequence of haemodilution after liquid food intake whether it is milk or colostrum. Total leucocyte (WBC) count on the other hand, showed progressively an increasing trend in both the groups after the first colostrum intake which is more pronounced in the experimental calves under continued colostrum feeding than the control animals (tables 5a, 5b and 5b1). These observation corroborate well with the findings of Jain (1986) who had reported a significantly increasing trend of total leucocyte (WBC) count upto one week of age in calves. Total erythrocyte (RBC) count was almost constant throughout the experimental period in both the groups, although experimental calves recorded a slight increase in the RBC count by continued colostrum

feeding. Elevated level of total solids, especially proteins in the colostrum would have resulted in an increased RBC count in the experimental calves. .

In the present investigation, the erythrocytic indices viz., mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) of both group I (control) and group II (experimental) animals revealed a fluctuating trend (table 5c). The erythrocytic indices are helpful in the diagnosis of various anaemias, with MCV expressing the average red blood cell size, MCH revealing the average weight of haemoglobin present in erythrocytes and MCHC indicating the average percentage of erythrocytic volume that the haemoglobin occupies.

Changes in the erythrocytic indices between, as well as within the animals of group I & II were non-significant, reflecting the non-significant changes observed in the three haematological parameters as Hb concentration, total RBC count and volume of packed red blood cells (VPRC) *vide* (tables 5c, 5d and 5d1).

5.2.4 Biochemical parameters

5.2.4.1 Serum protein profile

5.2.4.1.1 Total proteins

In the present investigation, concentration of total serum proteins was found to be increased after first colostrum intake as well as after 30 days of age than the values observed at birth (tables 6a, 6b and 6c). The increase of total serum protein concentration was more for experimental calves which received

continued colostrum feeding than the control animals. The electrophoretic pattern of serum proteins fractionation of the calves of group I and II agreed with the results of biochemical evaluation of total serum proteins. Significant ($P<0.05$) increase in protein concentration was recorded in experimental calves than control calves on 18^{th} d of experiment (table 6a).

Lents *et al.* (1998) concluded that the plasma protein concentration of calves increased from zero to one day of life (5.8 vs 7.2 g% for day 0 and day 1 respectively) which was associated with first nursing and that plasma proteins increased significantly after ingestion of colostrum. It can be concluded that overall protein profile of experimental calves was increased, due to the continued intake of protein rich colostrum.

Estimation of serum total protein concentration is one of the reliable indirect method for evaluating the humoral immune status of calves since there exist significant correlation among serum concentration of total protein, immunoglobulin-G (IgG) and the risk of neonatal diseases. The significantly ($P<0.05$) higher serum total protein content observed in calves fed colostrum rich in total protein in the critical first 24 h of life due to higher intestinal absorption rate of immunogloulin-G. Calves under continued feeding of colostrum acquired enteric immunity too. Higher protein intake in experimental calves by the virtue of continuous feeding of colostrum with a higher content of protein, over and above the requirement of growing calves, would have shifted the nitrogen balance

of the body to positive side resulting in a significantly higher proportion of protein fractions in the blood. These observations agreed with the findings of Knowles *et al.* (2000) and Zanker *et al.* (2000) who believed that higher serum total protein concentration could be due to the enhanced intestinal absorption of immunoglobulins of colostrum indicating an overall improved protein status of the calves. Proteins form the major solid portion of dissolved substances in the plasma and form the basic structural components of the body. They constitute the enzyme present in the body and also act as a secondary source of energy. The other functions include distribution of water, buffering, transport of various components, defence and coagulation of blood in the body.

5.2.4.1.2 Albumin

In the present study serum albumin concentration decreased after first colostrum intake but was found to be elevated significantly ($P<0.05$) by the end of the experiment in both groups of calves and the increase was prominent in experimental group of calves (tables 6a, 6b and 6c). These observations agreed closely with the reports made by Sridhar *et al.* (1988) who observed an increase in concentration of total serum protein and globulin with a relative reduction in albumin level immediately after birth. The increase in albumin concentration thereafter was more in the experimental calves compared to control group. These findings corroborate well with the observations made by Hadorn *et al.* (1997) who

described that increase in albumin concentration was due to the enhanced hepatic synthesis of albumin which was dependent on the colostrum supply.

Albumin which is synthesized in the liver constitutes a major part of the total proteins in the body, and they form the major portion of the dissolved substances in the plasma. Functions of albumin include distribution of extracellular fluid, regulation of osmotic pressure, and as a transport agent for a wide variety of substances such as hormones, lipids, vitamins etc.

There appears a direct correlation between albumin turn-over and body size (Kaneko, *et al.* 1997). The continuous increase in body size of the calves of both groups in the present experiment would have relied more on albumin content for the increased osmolarity as well as transportation related functions and the increased demand for amino acids turn-over might have been effectively met with increased synthesis following an elevated protein supply to the animals.

5.2.4.1.3 Globulin

Serum globulin concentration in the present study increased significantly ($P<0.05$) after the first colostrum intake; but thereafter decreased from the initial peak in both groups almost at the same rate till the end of the experiment (tables 6a, 6b and 6c). These observations are in consonance with the findings of Morin *et al.* (1997) and Hammon and Blum (1998) who reported that serum globulin concentration increased immediately after birth in colostrum fed calves as a

consequence of absorption of immunoglobulins as such in colostrum. Decrease of serum globulin concentration of calves thereafter to the end of the experiment could be due to rapid postpartum aging and maturation of intestinal epithelial cells which led to the reduction of pinocytotic activity and subsequent decrease of immunoglobulin absorption (Todd and Whyte, 1995). However, present finding disagreed with the observations of Sridhar *et al.* (1988) who reported that the globulin concentration increased with age of calves.

Colostrum is a rich source of immunoglobulins (gammaglobulins) when compared to whole milk and the immunoglobulins derived from colostrum is the most important factor in determining the immune status of the new born calf and hence the morbidity and mortality of the young calves (White and Andrews, 1986). Serum globulin fraction consists of 4 subunits namely α_1-globulins, α_2-globulins, β-globulins and γ-globulins (immunoglobulins). The former three are glycoproteins in nature and are synthesized by liver, while the latter is synthesized by plasma cells, in response to antigen stimulation. The immunoglobulins form a critical component with regard to calf nutrition and immune status. The amount of maternal antibody transferred from the colostrum of the dam to the blood serum of the new born calf is considered as a measurement of the calf's ability to withstand pathogenic challenge. The timing of first colostrum intake by calf is of paramount importance in that the bovine neonatal gut wall has the ability to absorb intact macromolecules for the first 24 h of life.

5.2.4.1.4 Albumin:Globulin (A:G) ratio

The albumin:globulin (A:G)ratio showed a fluctuating trend in both the groups during the experimental period of 30 days (table 6a). The albumin:globulin (A:G) ratio decreased after the first colostrum intake (table 6b) which could be due to the pinocytotic absorption of immunoglobulins from the colostrum in large amounts leading to a relative reduction in the serum albumin level. However, thereafter A:G ratio increased till the end of the experiment in both the groups, with the increase being more in experimental calves, which could be due to the enhanced hepatic synthesis of albumin because of continued colostrum intake.

5.2.4.2 Serum Lipid Profile

5.2.4.2.1 Total lipids

The lipid constituents estimated in the present investigation revealed an overall increasing trend (tables 7a and fig. 7). Significant ($P<0.05$) increase in the concentration of total lipids of animals of group I and group II was evident during the experimental period (table 7a). Serum total lipid concentration increased significantly ($P<0.05$) after first colostrum intake and even thereafter till the end of the experiment in both groups with the increase being more prominent in the experimental calves under continued colostrum feeding (tables 7a, 7b and 7c). These observations were in close agreement with the findings of Kuhne *et al.*

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(2000), who explained that the ingestion of greater amounts of first colostrum rich in fat content immediately after birth markedly improved fat absorption and lipid status of neonatal calves. Lipids in blood plasma of ruminants may arise from intestinal absorption of dietary lipids, mobilization of lipids from storage in adipose tissue, or synthetic processes. Most plasma lipids are present as chylomicrons and other density lipoproteins. In addition, non-esterified or free fatty acids are transported as a complex of fatty acid with albumin. Two of the most important functions of lipids are energy storage and formation of membrane structure. Dietary protein have been demonstrated to regulate the rate of lipogenesis and have also been shown to control the rate of fatty acid synthesis and related activities of a number of enzymes (Kaneko *et al.*, 1997).

The lipid anabolism observed in the present study may be correlated with the increasing body size (Kaneko *et al.*, 1997). The bioconversion of dietary proteins and other essential nutrients to lipids in the liver is seen when it is continually and excessively supplied with such substrates and so formed lipids can act as energy reserve. Growth in terms of increase in size and number of cellular components probably requires more membrane constituents, 40 per cent of which are essentially lipids in nature. Hence, an increased total lipid concentration in animals with increasing body size may be attributed to the elevated requirement of lipids for membrane constituents and as energy reserve.

Blum and Hammon (2000) speculated that bioactive components such as insulin like growth factor (IGF) and insulin in colostrum modified digestion and absorption of fatty acids, possibly by altering lipase activity or fatty acid binding proteins. According to Rauprich *et al.* (2000), higher serum lipid concentration in neonatal calves during their first week of life could be due to the higher fatty acid absorption rate of a fat rich diet, the colostrum.

5.2.4.2.2 Cholesterol

In the present investigation, the serum cholesterol concentration was elevated after the first colostrum intake and increased to a more significantly ($P<0.05$) higher level by the end of 30 days of age in both the groups; with the rise being more prominent in the experimental calves under continued colostrum feeding (tables 7a, 7b and 7c). Cholesterol is the main lipid component of blood, bile and brain tissues. It is also one of the most important steroids of the body and is a precursor of many steroid hormones. Two third of cholesterol present in the blood is esterified. Liver metabolizes the cholesterol and it is transported as lipoproteins in the blood stream.

The observations in present study agreed with the findings of Blum *et al.* (1997) who justified that the higher serum cholesterol concentration at one week of age in calves under intensive colostrum feeding might be due to the higher absorption rate of a diet (colostrum)rich in fat.

5.2.4.2.3 Triglycerides

In the present study the serum triglyceride concentration increased after first colostrum intake and even thereafter till the end of the trial in both groups of calves. The calves of experimental group II under prolonged colostrum feeding showed a more elevated concentration than the control animals (tables 7a, 7b and 7c). These findings are in consonance with the observations of Blum *et al.* (1997). Hammon and Blum (1998) who justified that an improved triglyceride concentration in calves fed colostrum on a high level could be due to a greater fat intake, digestion and absorption.

Triglycerides or triacylglycerols are simple lipids, they are formed in the liver by glycerol and fatty acids. They are transported by very low density lipoptroteins (VLDL) and low density lipoproteins (LDL) and constitute about 95 per cent of fat, stored as source of energy in the tissues and plasma. They are the most significant group of lipids from the stand point of energy metabolism in animals. Triglycerides may be synthesized from non-lipid sources, largely in the liver, adipose tissue and the lactating mammary gland.

The role of insulin in this arena cannot be ruled out. The enhanced amino acid absorption from colostral proteins would have increased glucose uptake and fatty acid reesterification with glycerol in adipose tissue, which in turn would have promoted adipose tissue to release lesser amounts of free fatty acids to the blood with subsequent increase in triacylglycerol formation.

5.2.4.2.4 Non-esterified fatty acids (NEFA)

Serum non-esterified fatty acids (NEFA) concentration of the calves of group I and II was reduced after the first colostrum intake and thereafter followed a fluctuating trend to the end of 30 days of age where the experimental calves showed an increase of values compared to the control group (tables 7a, 7b and 7c). The observation is in agreement with the reports of Lents *et al.* (1998) and Kuhne *et al.* (2000) who believed that calves had greater NEFA concentration at birth because of the mobilization of fat reserves for the energy demand, but later when the calves got nutrients in the form of colostrum or milk, the NEFA concentration was reduced and thereby lesser mobilization of fat for energy purposes.

Fatty acids are released to plasma from the triglycerides of adipose tissue mediated by hormone-sensitive lipase. The fatty acids then physically got bound to plasma albumin from lipoproteins and are transported to the heart, skeletal muscles, liver and other tissues for oxidation or conversion to other lipids. Since the turn-over rate of NEFA is extremely rapid (1-3 min.), their serum concentrations are normally low. Non esterified fatty acids (NEFA) got mobilized more when glucose and other energy substrates were less available as found before the first colostrum intake (Webb *et al.*, 1969).

5.2.4.3 Blood glucose level (BGL)

In the present study, the lowest BGL recorded in calves immediately after birth increased after the first colostrum intake and thereafter to reach the peak value by the end of the experiment in both the groups which were fed colostrum and milk till 30 days of age (table 8b). The observation closely agrees with the findings of Young *et al.* (1970) and Massip (1980) who justified that the transient rise in BGL immediately after birth was the result of adrenal response to the stressors of birth and the postnatal environment. It was also attributed to the increased activity of the sympathetic innervation to the liver, stimulating glycogenolysis, a direct cause for the increased BGL. Glucose is the major carbohydrate present in the blood and serves as a primary source of energy. Glucose is usually available from the ingested starch or sugar. The glucose concentration is normally maintained at constant level. Excessive glucose is stored as an inactive glycogen mainly in the liver, and little in the muscles (Swenson and Reece, 1996).

The blood glucose level was found to be higher for experimental calves under continued colostrum feeding for 30 days of age when compared to control animals which were fed colostrum and then milk under standard farm condition, for which different reasons could be attributed (table 8a). According to Grutter and Blum (1991b) and Hammon *et al.* (2000) the colostrum intake in high amounts and immediately after birth was shown to have prolonged positive

influence on the plasma glucose level during the first week of life in calves. Girard (1986) reported that increase in BGL could be due to the action of glucagon by gluconeogenesis especially in the neonates which was enhanced by colostrum intake. Tivey *et al.* (1994) also agreed with the present observation and stated that the high BGL was because of the stimulation of small intestinal lactase activity on ingested colostrum and the consequent lactose digestion and absorption as glucose and galactose.

5.2.4.4 Serum urea nitrogen (BUN)

The concentration of serum urea nitrogen was increased after the first colostrum intake and till the end of the experiment in both groups of calves with the rise being greater in experimental calves under continued colostrum feeding for 30 days of age (tables 8a, 8b and 8c). These observations are in agreement with the findings of Carlson and Muller (1976) who justified that the increase of serum urea nitrogen after first colostrum intake resulted in a higher protein degradation and amino acid deamination, probably as a consequence of the high intake of crude protein and amino acids that could not be channelised for protein synthesis.

If there is excess proteins that cannot be stored in the animal body, it should be broken down. Amino acids which form the components of proteins, break down to give ammonia which is toxic and so through a series of chemical reactions (urea cycle) urea is produced and released into the blood which is

filtered out in the kidney and excreted in the urine. According to Carlson and Muller (1976), the serum urea nitrogen concentration continued to increase with age, in calves fed with colostrum alone for 20 days of age. But the present finding disagreed with the observations of Kurz and Willett (1991) who reported a decrease in serum urea nitrogen with age, who believed that non nutritional factors such as insulin like growth factor (IGF) and insulin would have exerted an anabolic effect in the formation of tissue protein (growth) and thereby reduced serum urea concentration.

5.2.4.5 Creatinine

In the present study, the serum creatinine concentration reduced after the first colostrum intake and thereafter till the end of the experiment, with reduction being only slightly more in the control group of calves (tables 8a, 8b and 8c) which agreed with the reports of Kurz and Willett (1991) who explained that increased serum creatinine at birth had been associated with a decrease in glomerular filtration rate as the renal blood flow does not change much at birth but increases substantially during one week of life leading to an increased glomerular filtration of creatinine and hence a decrease in serum creatinine levels with the advancement of age.

Creatinine is derived from muscles as phosphocreatinine and is an important form of high energy phosphate. Creatinine

determination is having an advantage over the urea determination that it is not affected by a high protein diet (Kaneko *et al.*, 1997).

5.2.4.6 Bilirubin

In the present study total serum bilirubin concentration was increased after the first ingestion of colostrum from birth, and thereafter decreased showing a fluctuating trend in both the groups of calves (as control and experimental) with more or less same values (tables 8a, 8b and 8c). There were no significant differences between the two groups. The increase in bilirubin concentration could be probably due to heavy destruction of foetal haemoglobin (HbF) and replacement by adult haemoglobin (HbA) after birth leading to the elevated levels of one of the metabolic end products as bilirubin. According to Kurz and Willett (1991) in human neonates, intestinal absorption of unconjugated bilirubin contributed to hyperbilirubinemia and the decrease thereafter reflected the continuous increase in glomerular filtration rate with the advancement of age.

Bilirubin is formed by the break down of haemoglobin (aged or damaged erythrocyte) in the spleen, liver and bone marrow. Small amount of bilirubin circulates in the plasma, loosely bound to albumin, which is not water soluble. This is referred to as indirect or unconjugated bilirubin. In the liver, bilirubin is conjugated with glucuronic acid, which forms a soluble compound, referred as direct bilirubin.

5.2.5 Serum hormonal parameters

5.2.5.1 Thyroxine (T_4)

Serum concentration of T_4 was elevated on the first day of age when compared to the time/day of birth (zero day). However the values declined in both the groups of calves of group I and group II almost at the same rate after first day of birth till 30 days of age (tables 9a, 9b and 9c). These observations corroborate well with the recordings of Kahl and Bitman (1983) and Hammon and Blum (1998). The level of thyroxine in the blood represents the algebraic sum of thyroxine secretion and peripheral utilization. According to Nathanielsz (1969) the half life of thyroxine varied considerably between 1 and 6.5 days of age in the young calf. Kahl and Bitman (1983) reported a diurnal rhythm for plasma T_3 and T_4 concentration in dairy cattle.

The thyroid hormones viz., thyroxine (T_4) and triiodothyronine (T_3) control the metabolic processes, growth and differentiation, reproduction and lactation in all animals. Thyroid hormones stimulate the basic metabolic rate (BMR) via the metabolism of carbohydrates, lipids and proteins (Hoch, 1974). The actions are mediated by increasing the activities of specific enzymes that contribute to oxygen consumption. Of the two iodinated thyronines, thyroxine (T_4) was predominant in all the calves of group I and group II. According to Blum and Hammon (2000) concentrations of plasma thyroid hormones were not influenced either by feeding different amounts of colostrum or by delaying colostrum feeding or by fasting.

5.2.5.2 Triiodothyronine (T_3)

Serum T_3 concentration was higher at birth and after first colostrum intake, but thereafter decreased till the end of the experiment almost equally in both the groups of calves with slightly higher values observed for the experimental calves with a sudden drop of concentration from first day to sixth day (tables 9a, 9b and 9c). The observation was in consonance with the findings of Nathanielsz (1969) and Kahl and Bitman (1983).

Most of the circulating T_3 is derived from peripheral deiodination of thyroxine. The *in vivo* potency of T_3 is about three times that of thyroxine. A fluctuating yet a reducing trend in T_3 concentration was exhibited by the animals of both groups during the experimental period (table 9a). The metabolic requirement for oxygen consumption by growing calves would have warranted an increased synthesis and release of T_3 from thyroid gland, as in the case of thyroxine. Blum and Hammon (2000) observed that the concentrations of plasma thyroid hormones were not influenced either by feeding different amounts of colostrum or by delaying colostrum feeding or by fasting.

5.2.5.3 T_4 : T_3 ratio

In the present investigation the T_4/T_3 ratio showed a fluctuating trend in both the groups (tables 9a, 9b and 9c). These observations had agreed closely with the findings of Kahl *et al.* (1977). The serum concentration of T_3 increased faster than T_4 both in males and females, so T_3 should be considered as the main

biologically active thyroid hormone and that T_4, the prohormone must be converted to T_3 before its activity is exerted (Kahl and Bitman, 1983).

5.2.5.4 Insulin

Serum insulin concentration was elevated drastically after the first colostrum intake from values recorded at birth, but thereafter got reduced till the end of the experiment in both groups with the reduction being lower in the experimental calves under continued colostrum feeding compared to control calves (tables 9a, 9b and 9c). These observations had corroborated well with the findings of Mears (1993) who believed that greater insulin response in colostrum fed calves could be a consequence of enhanced insulin secretion as a result of greater nutritional intake during the entire period. Insulin, a protein hormone with two-chain peptide molecule, have equally important effects on protein and fat metabolism as does with carbohydrate metabolism. Insulin increases the transport of amino acids into muscle, stimulates protein synthesis and inhibits protein catabolism. It stimulates hepatic synthesis of fatty acids and insulin is thus, considered as a hormone that promotes anabolism (Swenson and Reece, 1996). Guerino *et al.,* (1991) opined that increased insulin secretion would be utilized for protein anabolism and triglyceride synthesis. They also demonstrated a positive relationship between amino acid absorption and pancreatic insulin secretion. Elevated serum insulin concentration observed after birth in calves fed colostrum intensively, may be due to the accelerated pancreatic development (Kuhne *et al.*,

2000). According to Hadorn *et al.* (1997) factors such as feeding density, energy intake, protein intake and gastrointestinal hormones possibly contributed to modified insulin secretion in colostrum fed calves.

The basal concentration of circulating hormones in ruminants fluctuate due to a number of intrinsic factors such as episodic hormone release and diurnal rhythm. Other factors such as ambient temperature, diet and feed intake can also influence basal hormone concentration. Plasma concentration of a hormone is the net result of secretion into the circulatory system minus clearance from the blood (Guerino *et al.*, 1991). Evaluation of key hormones viz., thyroxine and triiodothyronine, during the growing period of animals in the present study revealed a decreasing trend whereas insulin concentration showed an increasing trend in calves of both groups as group I and group II.

Summary

6. SUMMARY

The present study was conducted in twelve numbers of neonatal crossbred calves of either sex of the University Livestock Farm (ULF), College of Veterinary and Animal Sciences, Mannuthy for a period of 30 days (zero to 30 days of age)

The calves were selected from those born as singles from cows of normal pregnancy and parturition.

Sufficient quantity of colostrum was collected under hygienic conditions from recently calved healthy cows for the first three days, pooled and stored in clean, dry and sterile plastic containers and preserved in deep freezer at –20°C until fed to the calves. Samples of pooled colostrum, colostrum collected from first milking to fifth milking and whole milk samples were evaluated for crude protein content by kjeldahl method. Microbial count of the pooled colostrum samples were evaluated before and after ultra violet irradiation for 30 minutes, before feeding the colostrum to calves.

The calves selected for the study were divided into two groups, Group I (control) and Group II (experimental) with six calves in each group. Group I calves were fed with fresh colostrum for three days and then with milk (one-tenth of body weight) as per standard feeding regime of farms, whereas group II calves were maintained continuously on colostrum feeding (one-tenth of body weight)

for 30 days of age. All the calves were provided 250 g of calf starter by 15th day of age and water was given *ad libitum.*

Body weight of all the calves were recorded at the time of birth (initial) and at weekly intervals during the entire period of study. Clinical health status (respiratory rate, heart rate and rectal temperature) of all the animals were monitored daily. Blood samples were collected with and without anticoagulant from all calves of both groups soon after birth (zero day), thereafter 18 h after birth (first day), sixth day, 12th day, 18th day, 24th day and on 30th day of age. The blood samples were collected with suitable anti coagulant (sodium fluoride heparin) and analyzed for blood glucose level (BGL) and haematological parameters like haemoglobin (Hb) content, total erythrocyte (RBC) and total leucocyte (WBC) count and volume of packed red blood corpuscles (VPRC) and erythrocytic indices were calculated. The serum that was separated from whole blood without anticoagulent was subjected for the estimation of biochemical parameters such as concentration of total protein, albumin, globulin, total lipids, cholesterol, triglycerides, non-esterified fatty acids (NEFA), urea nitrogen (BUN), creatinine and bilirubin. Hormonal parameters like thyroxine (T_4), triiodothyronine (T_3) and insulin were also estimated.

On analyzing the crude protein content of colostrum and milk samples, it was observed that the protein content of colostrum decreased with the post partum milkings and the lowest value was recorded for the whole milk collected on fifth day. Total viable count of pooled colostrum was found to be significantly reduced after ultraviolet irradiation for 30 minutes. Calves of

experimental group II recorded a higher weekly body weight gain. The clinical health status of all the calves were within the normal range throughout the experimental period.

Of the various haemotological parameters evaluated, haemoglobin concentration and volume of packed red blood cells exhibited a declining trend, probably due to the haemodilution after intake of colostrum and milk. The total erythrocyte count remained almost constant whereas WBC count showed a persistently increasing trend in calves of both groups. Serum concentration of total proteins pursued an increasing trend in both groups. Although serum albumin showed a reduction in concentration after first colostrum intake, due to the increased absorption of unaltered immunoglobulins from colostrum, albumin concentration showed a steady rise thereafter till the end of the experiment indicating the enhanced hepatic albumin synthesis. The serum globulin concentration increased significantly after first colostrum intake, but thereafter showed a marginal decline. The pattern of electrophoretic fractionation of serum protein components agreed closely with the above results in both control and experimental calves.

The lipid profile of calves of both groups indicated an increased requirement of nutrients as membrane constituents and as energy reserve for growth. Release of elevated concentration of insulin caused by increased availability of amino acids would have stimulated increased triglyceride synthesis. Initial peak at birth and subsequent reduction in concentration of non-esterified fatty acids may be due to the increased mobilization of fat

reserves for energy demands, but after receiving nutrients in the form of colostrum or milk, the concentration was lowered due to lesser mobilization of fat for energy purposes.

The elevated glucose level (BGL) observed in calves of both groups indicated the increased energy demands of growing period. The increased serum urea nitrogen would have resulted because of the higher protein degradation and amino acid degradation probably as a consequence of high intake of crude protein and the whole aminoacids might not have channalised for protein synthesis. Elevated serum creatinine levels at birth had been associated with a reduction in glomerular filtration rate at birth which in turn reduced as glomerular filtration rate of creatinine was increased. The rise in bilirubin level during the experimental period in both the groups of calves could be due to heavy destruction of foetal haemoglobin for replacement by adult haemoglobin. The fluctuating trends of thyroid hormones concentration indicated that the plasma thyroid hormones concentration were not influenced by amount or time of feeding of colostrum or milk. The greater insulin response in colostrum fed calves could be a consequence of enhanced insulin secretion as a result of greater nutritional or protein intake.

It could be summarized that the availability of increased dietary protein to the neonatal calves in the form of colostrum will bring about protein anabolism with positive nitrogen balance and improved growth performance of neonatal calves. The excess colostrum in large dairy farms need not be wasted and could be properly preserved and fed to the calves as a protein rich nutrient.

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METABOLIC AND ENDOCRINE PROFILE OF CROSSBRED PRE-RUMINANT CALVES UNDER EXTENDED COLOSTRUM FEEDING

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ABSTRACT OF THE THESIS

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ABSTRACT

Colostrum is a highly fortified source of nutrients having seven times proteins, twice the total solids, higher content of vitamins, minerals and a very high immune value than normal milk. In most of the commercial dairy farms where day old weaning of calves is practiced, lion share of this potent calf protein supplement is practically wasted since colostrum is unmarketable for human consumption. In this circumstance, the present study was undertaken with the objective of evaluating the effects of enhanced feeding of preserved colostrum in neonatal cross-bred calves on the health status, growth, haematological, biochemical and hormonal parameters and to find any correlations exist among these factors during their first month of life.

Fresh colostrum was collected in hygienic conditions from recently calved healthy cows from the first six milkings and then pooled. The pooled colostrum was preserved in dry sterile bottles by deep freezing at $^{-}20°C$ until fed to calves. Crude protein content of colostrum and whole milk were estimated. Total viable count of pooled colostrum samples were recorded before and after ultra violet irradiation for 30 minutes. Twelve numbers of healthy neonatal crossbred calves of either sex of the Kerala Agricultural University Livestock Farm, College of Veterinary and Animal Sciences, Mannuthy were divided into two groups as Group I (control) and Group II (experimental) with six calves in each group. The calves of group I were fed with colostrum for three days and then milk (one-tenth of body weight) as

followed in the farm. The calves of group II were fed with colostrum for 30 days of age continuously at the rate of one-tenth of body weight. All calves were provided with drinking water *ad libitum* and calf starter (250 g/day/calf) from 15 days of age. The animals were maintained under standard management conditions.

Regular monitoring of clinical health status and individual weighing at weekly intervals from day zero (on the day of birth) to one month of all the calves were performed and recorded. Blood samples were collected from the calves of both groups soon after birth (zero day), thereafter 18 h after birth (first day), sixth day, twelfth day, eighteenth day, twenty fourth day and thirtieth day of age. The blood samples were analysed for blood glucose level and haematological parameters like haemoglobin content, total erythrocyte, total leukocyte count, volume of packed red blood cells (VPRC) and subsequently the erythrocyte indices were calculated. Estimation of concentration of serum total protein, albumin, globulin, total lipids, cholesterol, triglycerides, non-esterified fatty acids (NEFA), urea nitrogen (BUN), creatinine and bilirubin were conducted. Hormonal profile of serum thyroxine (T_4), triiodothyronine (T_3) and insulin were estimated.

Clinical parameters of both the groups of calves were within the normal range. Calves of group II recorded a higher weekly body weight gain. Of the various haemotological parameters evaluated, haemoglobin concentration and volume of packed red blood cells exhibited a declining trend, probably due to the haemodilution after intake of colostrum and milk. The total erythrocyte

count remained almost constant whereas WBC count showed a persistently increasing trend in calves of both groups. Serum concentrations of total protein and globulin also exhibited an ascending pattern in both the groups, which can be attributed to the enhanced absorption of unaltered immunoglobulins by pinocytosis, the property which is lost soon after the maturation of intestinal epithelial cells. Although serum albumin showed a reduction in concentration after first colostrum intake, due to the increased absorption of unaltered immunoglobulins from colostrum, albumin concentration showed a steady rise thereafter till the end of the experiment indicating the enhanced hepatic albumin synthesis. The electrophoretic separation of serum protein components of both groups of calves agreed closely with the biochemical estimation. A steady progressive increase was observed in serum concentrations of total lipids, cholesterol and triglycerides of calves of both the groups throughout the experimental period with the magnitude being more in case of experimental group. This might be explained by the increased fat content of colostrum, obviously due to an increased requirement of these components as membrane constituents and as energy reserves for the build up of body size and weight. Elevated insulin release stimulated by increased availability of amino acids would have favoured an increase in triglyceride synthesis. Fluctuating pattern in serum NEFA status of both the groups might signify reduced mobilisation of fat reserves for energy demands after birth.

Blood glucose level of calves of both the groups followed a continuous upstream trend, attributed to the increased energy demand for the enhanced

count remained almost constant whereas WBC count showed a persistently increasing trend in calves of both groups. Serum concentrations of total protein and globulin also exhibited an ascending pattern in both the groups, which can be attributed to the enhanced absorption of unaltered immunoglobulins by pinocytosis; the property which is lost soon after the maturation of intestinal epithelial cells. Although serum albumin showed a reduction in concentration after first colostrum intake, due to the increased absorption of unaltered immunoglobulins from colostrum, albumin concentration showed a steady rise thereafter till the end of the experiment indicating the enhanced hepatic albumin synthesis. The electrophoretic separation of serum protein components of both groups of calves agreed closely with the biochemical estimation. A steady progressive increase was observed in serum concentrations of total lipids, cholesterol and triglycerides of calves of both the groups throughout the experimental period with the magnitude being more in case of experimental group. This might be explained by the increased fat content of colostrum, obviously due to an increased requirement of these components as membrane constituents and as energy reserves for the build up of body size and weight. Elevated insulin release stimulated by increased availability of amino acids would have favoured an increase in triglyceride synthesis. Fluctuating pattern in serum NEFA status of both the groups might signify reduced mobilisation of fat reserves for energy demands after birth.

Blood glucose level of calves of both the groups followed a continuous upstream trend, attributed to the increased energy demand for the enhanced

growth. A progressive increment in BUN was more evident in experimental calves. This could be due to the higher protein degradation and subsequent amino acid deamination, probably as a consequence of the high intake of crude protein and amino acids that were not utilised for protein synthesis. The decreasing trend of serum creatinine levels in both the groups could be due to the decreased glomerular filtration rate (GFR) of serum creatinine at birth leading to a high serum level at birth, which was reduced later due to the elevated GFR of creatinine during the early days of life. Increased serum bilirubin soon after birth could probably be due to the increased destruction of foetal haemoglobin (Hb) for the replacement of adult haemoglobin after birth. The serum bilirubin levels diminished towards the last quarter of the experiment tenure. Serum thyroid hormones (T_3 and T_4) were not found to be influenced by time or amount or colostrum or milk fed to calves. There was a fluctuating trend of T_3 and T_4 ratios in both the groups. The increase in insulin concentration after birth in both the groups of calves could be a consequence of enhanced insulin secretion as a result of greater nutritional intake in the neonatal life.

The present investigation proved that increased dietary protein in neonatal cross-bred calves which were fed colostrum continuously for 30 days from birth brought about elevated protein anabolism in association with haematological, biochemical and hormonal changes. They were definitely having an advantage over calves fed colostrum for three days and then switched to milk as per standard feeding regime. Postnatal growth of ruminants is chiefly

influenced by metabolic hormones, the secretion of which being regulated by the circulating levels of critical amino acids. The observations of the present study revealed that nourishing the neonatal calves with protein rich colostrum for a prolonged period ensured increased availability of amino acids, especially the critical ones which can be exploited in enhancing the growth rate of the calves. Since, there is always an interest in maximising the utilisation of protein supplements, the most expensive ingredient in ruminant ration, enhanced feeding of preserved colostrum can be a promising method of improving the weight gain and health status of neonatal calves in farm conditions. Excess of colostrum that is usually wasted in large dairy farms, could be properly preserved and fed to the calves as protein rich nutrient.