CHEMICAL CONSTITUENTS AFFECTING OSMOLALITY OF MILK IN CROSSBRED CATTLE

BY

SIMON DANIEL

THESIS

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DECLARATION

I hereby declare that this thesis entitled "CHEMICAL CONSTITUENTS AFFECTING OSMOLALITY OF MILK IN CROSSBRED CATTLE" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship, or other similar title, of any other University or Society.

SIMON DANIEL

Mannuthy,
31-7-1981.

CERTIFICATE

Certified that this thesis, entitled "CHEMICAL CONSTITUENTS AFFECTING OSMOLALITY OF MILK IN CROSSBRED CATTLE" is a record of research work done independently by Sri. Simon Daniel under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship, or associateship to him.

Dr. K. Pavithran,
Associate Professor,
Department of Dairy Science.

Mannuthy,
31-7-1981.

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I dedicate this thesis to my beloved parents.

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C O N T E N T S

INTRODUCTION

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Milk secretion is defined as the synthesis of milk by the cells of alveolar epithelium of mammary gland and the passage of this milk from the cytoplasm of the cells into the alveolar lumen. The constituents of milk are produced in two ways. One group of compounds, which includes milk fat, most of the protein compounds and lactose is synthesized in the epithelial cells from blood precursors and then released into the alveolar lumen. The remaining milk constituents pass from the blood and move across the epithelial cells or between them into the alveolar lumina without alteration by the cells. This group of the milk constituents includes minerals, of which sodium, potassium and chloride are important because of their osmotic activity in the milk. Lactose synthesized in the mammary epithelial cells is also responsible for the osmolality of milk and constitutes one third of the osmotic pressure of normal milk.

Milk is in osmotic equilibrium with the blood flowing through the udder throughout the period that milk remains in the udder and not just during its formation. Changes in milk composition occur as water moves in and out of the udder in response to changes in the osmotic pressure of blood. In addition to the primary secretion of water in milk, a blood plasma transudate which is rich in sodium and chloride contributes to the fluid of milk. This type of secretion is considered to be important in the secretion of milk that is associated with advancing lactation, advancing age and bacterial infection of the udder. The milk secreted during these conditions have increased contents of sodium and chloride and decreased contents of lactose and potassium. An alternate explanation for the variation in the sodium and chloride content of milk is that the milk secreted by the cells is richer in sodium and chloride than that obtained from gland at milking. As the fluid moves from alveoli through the ducts into the cistern of the gland, the sodium and chloride ions are reabsorbed. This resorption process is against the concentration gradient. A reduction in blood supplied to the ducts or an interference with the resorption process would increase the sodium and chloride contents of the milk (Schmidt, 1971). However, this quantitative variation in these constituents occuring due to disease conditions of the udder, is to maintain the osmotic pressure.

In the present study an attempt has been made to ascertain the secretory pattern of lactose, sodium, potassium and chloride in milk which control the osmotic pressure, in crossbred cattle. The information on the variation in these constituents in disease conditions of the udder is helpful to assess the damages caused by the disease. This comparative data can also be used to locate the origin of these substances in milk and to assess the secretory function of the udder.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Milk is the lacteal secretion, practically free from colostrum, obtained by complete milking of one or more helathy cows with an average gross chemical composition of 87% water, 3.9% fat, 4.9% lactose, 3.5% protein and 0.7% ash (Webb and Johnson, 1965). The composition of milk of various species has been discussed by Jenness and Solan (1970). They have also pointed out the factors affecting the variation of the different components of milk.

Lactose content of milk.

A detailed review on the secretion of water and water soluble constituents in the milk have been given by Rook and Wheelock (1967). The interrelationship of lactose wit different water soluble components and their importance in osmotic regulation have also been discussed. Comberg et a (1967) have estimated the lactose content of cows milk as 4.91 ± 0.006%. They have also found the heritability of lactose content in the milk as 0.29.

Lactose content of cow, buffalo and goat milk were found to be 4.891 ± 1.004, 5.126 ± 0.320 and 4.397 ± 0.280 respectively (Barakat et al. 1969).

Linzell and Peaker (1971b) have studied the permeability of mammary ducts to lactose and found that the mammary ducts were impermeable to lactose so that the milk eventhough stored for long time in the ducts would not change in composition.

Ali (1973) had estimated the amount of lactose in milk from large number of samples and made a study on the correlation between lactose and chloride contents. The lactose content was estimated to be 4.97 g/100 ml, chloride content as 0.07 g/100 ml and chloride-lactose number as 1.54.

Sook and Line (1961) have conducted a study to find out the effect of plane of energy nutrition on the constituents of milk secreted. They could not find any significant increase in the content of lactose by increasing the plane of nutrition. The effect of stage of lactation on lactose content of milk was studied by Kniga and Tenditnik (1968). They have got a lactose content ranging from 4.57 to 5.13%. The variations occuring in the course of lactation were small and no relationship was established between lactose content and lactation stage.

The decline in lactose content of milk during the winter was found to be associated with udder infection and an advancement in average stage of lactation (Dawson et al. 1974). Srivastava et al. (1978) have made their studies in lactose and mineral contents of milk of crossbred cattle.

They could not find any significant variation in lactose content due to genetic variation in cattle.

Traore and Schulz (1979) have found low lactose content in mastitis milk and discussed the relevance of lactose in the diagnosis of pathological conditions of udder to measure the changes in the udder brought about by disease.

Chloride content of milk.

The exchange of chloride ions across the mammary gland epithelium in the goat has been studied by Knuteson (1964) by infusing polyethylene glycol containing isotope chlorine (^{36}Cl) into the mammary gland. The milk was collected at different intervals and found that the chloride was absorbed at less faster rate than sodium and potassium from the secretion. The intracellular concentration of chloride in lactating mammary gland has been calculated (in guinea pig) from the analyses of fresh tissue and measurement of extra-cellular space with sucrose (^{14}C) and milk content with lactose (^{14}C) (Linzell and Peaker, 1971a). The intracellular concentration of chloride was calculated as 66m eq l^{-1} intra-cellular water. The calculated intracellular concentration of chloride was higher than milk but the ratios between the sodium, potassium and chloride ions were all identical. Comparison of intracellular concentration and the membrane potential between the cells and the milk suggested that the chloride ions were actively held in the cells unlike the sodium ion and potassium ion which are passively distributed across the apical membrane. The authors had also postulated the existence of another inward facing "chloride pump" in addition to the sodium pump.

The permeability of mammary ducts to chloride ions was studied by Linzell and Peaker (1971b) by using radioactive isotopes. Non lactating mammary ducts were found to be more permeable to chloride than the lactating ones. In their works they pointed out that the mammary ducts were impermeable to the chloride ion and explained the reason of maintenance of the composition of milk even if stored for many hours in the mammary gland.

Similar studies have been carried out by Linzell and Peaker (1974) to reveal the changes in the composition of colostrum in relation to the permeability of mammary epithelium at about the time of parturition in the goat. The reason of high chloride content of colostrum was attributed to the greater rate of passage of chloride into the milk from blood in late pregnancy than in lactation. Peaker (1977) has suggested a paracellular route for the movement of chloride ion and lactose.

Tapernoux and Magat (1967) have estimated the amount of chloride in the milk as 1.71 g/l. They could not find any significant difference with breed, feed or date in respect to the chloride content of the milk. Lactose-chloride ratios of different milk have been worked out by Barakat et al. (1969). The lactose-chloride ratio for cow buffalo and goat milk were estimated to be 47.5 ± 8.7, 78.6 ± 8.4 and 26.0 ± 2.6 respectively. The mean chloride contents were 0.1055 ± 0.015, 0.0657 ± 0.006 and 0.1717 ± 0.019% respectively for cow, buffalo and goat milk. The mean chloride was lower in buffalo milk than in cow milk.

The importance of chloride ion in osmotic regulation has been pointed out by Rook and Wheelock (1967) and Jenness and Solan (1970). The chloride content and chlori lactose number have been estimated by Ali (1973) for cow milk, goat milk and vendors milk as 0.07 g/100 ml and 1.54, 0.09 g/100 ml and 1.93 and 0.10 g/100 ml and 2.57 respectively.

The chloride content of colostrum on the first day wa 0.257% and 0.062% on the fourth day and fluctuated widely upto 10 days (Sebela et al. 1976). The chloride content of buffalo milk was estimated by ultracentrifugation process (Sindhu and Roy, 1976). After centrifuging the sk milk at 105000 x g for 30 minutes at 20°C in a Beckman model L centrifuge, the serum contained 98-100% of the chloride.

Sonkar *et al.* (1976) have studied the chloride and lactose contents of milk of Sahiwal cows and Murrah buffaloes over a whole lactation. Average Koestler number was estimated to be 2.16 (range - 1.55 to 3.11) and 2.18 (range - 1.32 to 2.81) respectively for cows and buffaloes. The value increased in both species as the lactation advanced and it varied significantly in early and late lactation periods. They have also estimated the average lactose-chloride number in the milk of cow and buffalo over a whole lactation respectively as 6.52 (range - 3.74 to 8.61) and 6.52 (range - 3.98 to 8.95). This number als increased with the advancement of lactation.

Ganguli (1979) estimated the amount of chloride in co milk and buffalo milk. The author also compared the chlor contents of blood plasma (350 mg/100 ml) and milk plasma (100 mg/100 ml). The chloride content of cows milk was estimated to be 1.697 g/l and buffalo milk as 0.052 g/l.

The variation in the chloride content of milk during successive stages of milk removal from mammary gland of co was studied by Mackenzie and Lascelles (1965) with oxytocin injection. The results showed an increase in concentration of chloride in whey upto 29%.

The changes in the yield and composition of mammary gland secretion in first 16 days of dry period have been investigated in six cows by Wheelock et al. (1967). The chloride content was decreased in the first half and within eight days after drying off the concentration of chloride was similar to those of blood.

The variation in chloride content due to season has been studied by Semjan et al. (1974). They have analysed the milk for chloride in the beginning of pasture feeding, beginning of stall feeding and at the end of stall feeding and got the values as 89.0 ± 1.2 mg/100 ml, 102 ± 1.4 mg/100 ml and 107 ± 1.2 mg/100 ml respectively. The stall feeding has increased the chloride content of the cows milk.

Ocampo et al. (1970) have studied the seasonal variation of chloride in cow milk. Average levels of chloride in autumn, winter, spring and summer respectively were 1.93, 1.92, 1.78 and 1.75 g/l. None of the differences in seasonal averages was statistically significant.

There were no detectable changes in chloride content of milk in association with chloride content of drinking water (Mussenden et al. 1977). The freezing point of milk also has not changed.

The electrical conductivity has been correlated with the chloride content of milk in the investigations carried out by Dosel et al. (1977). Electrical conductivity of milk was studied in 450 fresh milk samples. The minimum and maximum conductivity values obtained (results x 10^{-4} ohms) were 28.44 and 52.90 corresponding to 0.017 and 0.140% chlorides. The average values were 38.41 and 0.067% respectively.

Sodium content of milk.

Knutsson (1964) studied in detail the exchange of sodium ions in the mammary gland by using isotope sodium (^{24}Na). Exchange was demonstrated both after intravenous injection and introduction into the gland through teats. For isotope sodium the half time after infusion to pass through the teat was about 30 minutes or less and even shorter after intravenous injection. The resorption of sodium was significantly greater than that of chloride.

The transport of sodium in the formation of milk has been studied by Zako et al. (1969). The free flowing milk obtained after oxytocin injection and final udder massage has got similar sodium concentrations. But the milk removed at every fifteen minutes by oxytocin injection showed a four fold increase in sodium concentration and decrease in potassium and lactose contents. When sodium chloride solution was introduced into the mammary gland immediately after evacuation, the samples after four to four and half hours of infusion showed a decrease in sodium. The introduction of potassium chloride or glucose in similar way was followed by an increase in concentration of sodium. It was suggested that the liquid entering the alveoli has Na^+ and K^+ concentrations similar to that of plasma or intercellular fluid. The gland cells reabsorbed the sodium and secreted potassium and lactose in an osmotic equivalent with that of the reabsorbed sodium.

Comberg et al. (1967) have estimated the sodium content of milk as 43.6 ± 0.374 and its heritability as 0.30. Similar studies have been carried out by Kant et al. (1971) who estimated the sodium content of cow milk as 56.8 mg%.

In the review presented by Jenness and Solan (1970), the importance of sodium as a solute contributing to osmotic activity has been revealed. Various factors like lactation periods, age of the animal and feeding have also been discussed to affect the sodium content of the milk.

The method of transport of major ions present in the milk across the mammary gland epithelium has been derived by Linzell and Peaker (1971b). They have noticed that the non lactating mammary ducts were more permeable to sodium ions than lactating. Linzell and Peaker (1971a) have estimated the intracellular concentration of sodium by the analysis of fresh tissue as 42 m eq l^{-1} intracellular water. The concentration of sodium was higher than in milk but the ratios between chloride, potassium and sodium were almost identical to that of milk.

The sodium content of colostrum was found to be high due to high rate of passage of sodium from blood into the mammary secretion in the later pregnancy (Linzell and Peaker, 1974).

Sobels et al. (1976) estimated the sodium content of colostrum and milk for 16 days after calving. On the first day sodium content was 0.034% and increased steadily to 0.47% on the fourth day of calving. Similar studies were carried out by Vinovrski et al. (1976) and presented a mean value for sodium as 92.0 mg/100 ml in the first three days and 83.9 mg/100 ml in the next six days. The average sodium-potassium ratio was 0.385.

Sodium content of milk and blood plasma were compared by Ganguli (1979). The sodium content was estimated to be 50 mg/100 ml in cows milk and 330 mg/100 ml in blood plasma. The buffalo milk contained 64 mg/100 ml of sodium.

Variation of sodium content in milk was reported by different authors. Sodium content of milk showed an increase with advancing of age (Vanschoubroek et al. 1964). The reduction in variability after correction for age was 18.5%. Wheelock et al. (1966) have studied the effect of different intervals on milk secretion and the rate of secretion of sodium was found to be decreased curvi-linearly with the duration of the milking interval.

The fluid removed from the udder during the dry period was having the similar sodium content that of extracellular fluid (Smith et al. 1967 and Wheelock et al. 1967).

Semjan et al. (1974) estimated the sodium content of milk during the four periods namely the beginning and end of the pasture feeding and beginning and end of stall feeding as 46.3 ± 0.608, 38.3 ± 0.640, 36.9 ± 0.494 and 37.8 ± 0.524 mg/100 ml respectively. Ocampo et al.(1976) have studied the variation of sodium content in the milk during different seasons. They have recorded the sodium content of milk as a range (0.40 to 0.78 g/l). Average levels in autumn, winter, spring and summer respectively were 0.520, 0.512, 0.512 and 0.516 g/l and reported that none of the differences were significant. The sodium content in the milk was found to be significantly higher after two months of drought (Luquest et al. 1977).

Mussenden et al. (1977) studied the effect of sodium concentration in drinking water on sodium content and freezing point of milk. Associated changes of sodium could be noticed, but no changes in freezing point reported. Sodium content was estimated in fresh milk samples and recorded as 0.35 g/l (range - 0.20 to 0.85) by Pinto et al. (1978). They have studied the seasonal variation of sodium in cow milk and found high amount of sodium in autumn than in winter. Similar studies have been carried out by Juarez et al. (1979) and stated that the sodium varied significantly by season and ranged from 0.033% (February) to 0.049% (May).

Potassium content of milk.

Permeability of mammary epithelium to potassium have been studied by Knutsson (1964) in goat by introducing isotope potassium (^{42}K) intravenously or intramammarily. Milk was collected at different time intervals and observed that potassium could be absorbed at a slightly faster rate than chloride. Mackenzie and Lascelles (1965) have confirmed in their studies in ewes that a net movement of ions could take place across the mammary epithelium, where sodium, potassium and chloride were secreted into the solution in which the concentrations were lower than in the milk and absorbed when the concentrations were higher. The free flowing milk after an injection of oxytocin and milk obtained after the final udder massage had similar potassium concentrations (Zaks *et al.* 1965). It was also suggested that the liquid entering the alveoli has a potassium concentration similar to that of blood plasma or intracellular fluid. The gland would secrete potassium and lactose and reabsorb sodium so as to maintai the osmotic pressure.

Permeability studies carried out by Linzell and Peake (1971a) by using radioactive isotope showed that the conce tration of potassium in the intracellular fluid was higher (115 m eq l^{-1} intracellular fluid) than in milk and that t potassium ions were passively distributed across the apica membrane. Another study carried out by the same authors (1971b) revealed the impermeability of mammary ducts to potassium.

Comberg *et al.* (1967) estimated the potassium content of cows milk as 162.0 ± 0.472. The genetic dependence of potassium content of milk was calculated in terms of heritability as 0.43.

Wheelock *et al.* (1967) studied the changes of potassium content in dry period and recorded a potassium content in mammary secretion similar to that of blood. Kani *et al.* (1971) have estimated the potassium content of milk as 144.2 mg.%.

The potassium content of colostrum has been estimated by Sobela *et al.* (1976) and noted that potassium content was less than that in normal milk (0.139%) on the first day and increased to fluctuate about normal values ranging from 0.14 to 0.16% for the rest of the time. Similar studies carried out by Vinovrski *et al.* (1976) showed a potassium content of 149.0 mg/100 ml in the first three days and 140.5 mg/100 ml in the next six days. The potassium content has changed to the normal milk value between five and seven days after parturition.

Ganguli (1979) studied the mineral composition of cow and buffalo milk and estimated the potassium content as 1.766 g and 1.927 g per litre respectively.

Variation in potassium content of milk due to advancing of age has been revealed by Vanschoubrock *et al.* (1964). The potassium content was found to be high at five to eight years of age and thereafter decreased to a minimum.

Whether pasture feeding has got any effect on potassium content of milk ?. Samjan *et al.* (1974) studied this aspect in cattle. They have estimated the potassium content of milk in the four periods namely beginning of pasture feeding, end of pasture feeding, beginning of stall feeding and end of stall feeding as 133.9 ± 1.202, 142 ± 1.566, 135.5 ± 1.5 and 145.1 ± 1.256 mg/100 ml respectively. It was concluded that the potassium in milk did not vary significantly due to the change of animal from pasture to stall feeding.

Ocampo *et al.* (1976) estimated the amount of potassium in cow milk and studied the seasonal variation of potassium content in milk. They have observed that the seasonal differences of potassium content of milk were not statistically significant. Similar studies have been carried out by Pinto *et al.* (1976). The potassium content was ranging from 1.382 to 2.36 g/l and the variation was less during different seasons. Juarez *et al.* (1979) also could not find any seasonal variation in respect of the potassium content of milk. They have estimated the potassium content of milk as 0.152 ± 0.019%.

Induction of lactation.

Smith and Schanbacher (1973) injected 17-beta oestradiol and progesterone at a rate of 0.1 mg and 0.25 mg/kg body weight respectively for seven days into nine cows and one heifer. Half the daily dose was given subcutaneously at twelve hour intervals. Lactation was commenced 11 to 21 days after the first injection and the peak yield was attained in 30 to 50 days. They observed that the animals in fifth to seventh day post-oestrus responded better than the animals in mid cycle and with functioning corpusluteum Similar studies have been carried out by Erb *et al.* (1976) and Chakriyarat *et al.* (1978).

Joseph and Pavithran (1979 a & b) induced infertile cows by using steroid hormones and studied the effect of different length of treatment periods. They have also analysed the induced milk for various constituents like fat, protein, lactose, ash and chloride and could not find any difference in composition from that of post-partum mil

Mastitis.

Mastitis has been found to produce compositional chan and also depression in milk yield. This may be due to the impairment of function of the mammary epithelial cells.

Jenness and Patton (1959) showed an increase of chlor content in milk of mastitis affected quarters above 0.14 o 0.15% and have explained the possibility of using the estimation of chloride content as a tool for mastitis stud Udder infection also lowered the lactose content of milk. Chloride-lactose number (100 x %chloride/% lactose) propos by Koestler could be used to express the reduction in lactose and increase in chloride contents of milk. The chloride-lactose number generally ranged from 1.5 to 3.0 for normal milk. But increased markedly in mastitis cases

Wheelock *et al.* (1966) noticed a decrease in the concentration of lactose and potassium in milk from the infected quarter. The sodium, chloride and non casein protein in milk showed increase in concentration.

Walsh and Neave (1968) reported that the lactose content of milk from infected quarter, contained only less lactose (0.08% less) than normal milk. Reduction of lactose was significantly greater when infection was due t *Streptococci* (0.154%) than *Staphylococci* (0.105%).

The importance of chloride content of cow milk in diagnosing disturbances of udder has been pointed out by Konrad *et al.* (1969). The average chloride concentration was 152.2 mg% for quarters suffering from chronic mastitis with positive bacteriological test, 137.1 mg% for mastitis with negative bacteriological test and 115.5 mg% for irrit quarters. The mastitis caused an increase in the chloride content from a normal of 100 mg%.

Ingr *et al.* (1973) suggested critical values for chlo (0.15%), chloride-lactose number (3.3), sodium content (0.

and potassium sodium ratio (2.0) in the diagnosis of sub-clinical mastitis of individual udder quarters.

The blood derived whey proteins, sodium and chloride were shown to be increased (Newstead, 1973) with a decreas in potassium and lactose contents in the milk affected gland. Similar observations have been made by Grigoryon et al. (1978) and Oshima (1979).

Mielke (1975) studied the behaviour of osmotically active substances in the milk of intact and pathologically affected quarters and showed an increase in chloride and decrease in lactose contents. Sreekumar et al. (1975) recorded the lactose content of milk as 3.696%, 3.460% and 4.798% in subclinical mastitis, clinical mastitis and normal milk respectively. The chloride content were also found to vary due to mastitis and estimated as 0.195%, 0.190% and 0.136% in subclinical mastitis, in clinical mastitis and in normal milk respectively.

Oshima (1977) and Traore et al. (1979) described the use of electrical conductivity to study the pathological changes of the udder depending on the compositional change produced by infection. The change in the electrical condu tance has been related to the changes in sodium, potassium and chloride contents of milk in disease condition.

Kaseda *et al.* (1977) showed an increase in sodium and decrease in potassium in low acid milk. It was considered that those cows that form milk with a high content of sodium and a low content of potassium might have an abnormality of the udder tissue. Kaseda and Kubozono (1978) estimated the sodium and potassium contents of milk of affected cows and showed that the cows which secreted milk with low potassium-sodium ratio seemed to be suffering from mastitis. They have estimated the ratio of potassium and sodium in mastitis milk as 2.31.

Inter relationships between lactose, potassium, sodium and chloride.

Direct rectilinear relationships between sodium and chloride as well as inverse rectilinear relationships between lactose and chloride, lactose and sodium, potassium and sodium as well as chloride and potassium values for milk have been established in different investigations.

Praphulla and Ananthakrishnan (1970) studied the inter relation between the osmotically active components. There was a strong positive correlation between the sodium and chloride contents of cow and buffalo milk. The correlation between potassium and chloride was also positive for both cow and buffalo milk, though not very highly significant. The correlation between chloride and lactose was negative for both milk, whereas a highly significant relation between sodium and potassium contents of cows milk was established. No such positive correlation between sodium and potassium was noticed in the case of buffalo milk. The correlation between milk yield and yields of sodium, potassium and chloride and lactose were all positive and highly significant statistically.

Relationships between lactose, sodium and potassium were derived by Cachev (1972) for the milk of different species. The author could notice low concentrations of sodium and potassium when the lactose concentration was high in many cases. Similar relationships have been worked out by Luck et al. (1974) and Peaker and Taylor (1975). In the similar manner Kureljuic and Vujicic (1977) studied the correlations between mineral constituents and worked out the correlation coefficient between ash and sodium as 0.502 and ash and potassium as 0.418.

In the late pregnancy the concentration of chloride was inversely correlated with concentrations of lactose and positively correlated with concentration of sodium (Linzell and Peaker, 1974). The chloride content of colostrum was higher due to the high rate of passage of chloride into milk from blood in late pregnancy.

Oshima et al. (1974a) noticed a linear relationship between sodium chloride concentration and both specific conductivity and hydrogen ion concentration of quarter milk. Oshima et al. (1974b) studied the inter relationships between the increase in sodium and chloride contents and the changes in other constituents in abnormal quarter milk. No relationship could be established between the increase in sodium and chloride content and variation in fat percentage in abnormal milk. The protein content was found to be increased slightly and the SNF content decreased with increasing sodium and chloride. It was concluded that in abnormal quarters, a body fluid containing sodium and chloride concentration similar to those in blood plasma could enter into milk while at the same time there was a suppression in normal secretory activity of the gland contents in the milk.

The importance of relationships between the concentrations of lactose and the major ions, potassium, sodium and chloride in the secretory mechanism of ions in relation to water secretion has been studied by Peaker (1977). Positive correlations were established between protein and lactose ($r = 0.413$), sodium and potassium ($r = 0.313$), sodium and chloride ($r = 0.718$) and potassium and chloride ($r = 0.631$).

Negative correlations were found between electrolyte concentration and the contents of lactose and protein except the correlation between sodium and protein which was not significant. It was stated that the electrolyte concentration could influence SNF content of milk due to osmotic balance in the milk.

Oshima *et al.* (1980) have also analysed the milk of cows administered thyroxin in order to examine whether the inverse relationship existed between the electrolyte concentration when composition of the milk changes experimentally. The electrolytes expressed as sodium + potassium + chloride decreased in an inverse manner to lactose, reached a minimum at the end of thyroxin treatment period and increased thereafter.

MATERIALS AND METHODS

MATERIALS AND METHODS

Collection of samples

Samples of milk were collected for analyses from randomly selected crossbred cows of University Livestock Farm, Mannuthy. Depending on the stage of lactation these animals were arranged into three groups.

In the first group of six cows, the animals were in the advanced stage of pregnancy. The samples of mammary secretion were collected one day prior to the day of expected parturition and continued upto 60th day of lactation at fixed intervals of 1, 3, 7, 15, 30, 45 and 60 days.

The collection of milk samples were started in the second group of six cows from 140th day of lactation and continued to 200th day at an interval of ten days.

In the similar manner, the milk samples were collected from the third group of eight animals commencing on 250th day of lactation and continued upto 330 days.

In all these cases afternoon milk samples were collected from individual animals after complete milking following the standard method of collection of samples described in Indian Standards IS. 1479 part I (1960).

Two infertile crossbred cows were induced to lactate with the injections of oestradiol di propionate (ovocyclin CIBA) and Progesterone (Progestin, ORGANON) at a daily dos of 0.1 mg and 0.25 mg/kg body weight respectively for seve days. The animals were milked after about 14 days of completion of injection when the udder growth was apparent complete. Composite samples of all the quarters were collected for analyses.

All the crossbred cows of the University Livestock Fa were screened for mastitis with California Mastitis Test reagent. Four animals which showed positive reaction were selected and samples of milk collected from infected quart of the udder of each animal. The collection of milk sampl was started from the subclinical stage of mastitis till th clinical manifestations were exhibited.

Analyses of Milk Samples

All the milk samples were analysed for lactose, chlor sodium and potassium. Lactose and chloride were estimated just after the collection and sodium and potassium on the following day after storing the samples under refrigeratio

<u>Estimation of lactose content of milk samples.</u>

Lactose was estimated according to the Micromethod, t modified procedure of Folin and Wu Method (Oser, 1965).

The protein free milk filtrate obtained after the treatment with sodium tungstate (10%) and sulphuric acid (2/3 N) was allowed to react with alkaline copper solution in aspecial tube, to prevent reoxidation. The cuprous oxide formed was treated with phosphomolybdic acid solutio to form a blue colour and then compared with that of a standard. The photometric readings of the standard and unknown were utilised for the calculation using the following formula:-

$$\frac{\text{optical density of unknown}}{\text{optical density of standard}} \times 0.6 \times \frac{100}{0.01} \times \frac{1}{1000}$$

= % lactose.

Estimation of chloride content of milk samples.

The chloride content was estimated by the direct titration method as described by Pearson (1970).

Special silver nitrate solution (0.0291 N) was added in excess to react with chloride present in milk and then the free silver nitrate was found out by titration with potassium thiocyanate of equivalent strength. The chlorid was calculated as follows:-

$$\frac{\text{volume of silver nitrate used} \times 0.01 \times 58.46}{35.46}$$

= % chloride.

where,

58.46 = Molecular wt. of sodium chloride.

35.46 = Molecular wt. of chloride.

Determination of sodium and potassium contents of milk.

Flame photometric method as described by Oser (1965) was employed for the estimation of sodium and potassium contents of milk samples.

Diluted (1 in 20 for sodium and 1 in 100 for potassiu fat free milk was introduced in the form of a fine continuous spray into a nonluminous gas flame of flame photomet (Systronics, Type 121) and the emitted light characteristi of the ion was isolated by sodium or potassium filters and allowed to fall on a photoelectric cell. The galvanometer reading corresponding to the intensity of emitted light wa recorded for different samples. The concentrations of sodium and potassium in the samples were found out from a pre-plotted calibration curve established by analysing a series of combined standard solutions containing 2 to 40 p of sodium and 1 to 20 ppm of potassium.

RESULTS

RESULTS

Lactose content of milk.

The colostrum, starting from one day prior to calving and milk samples from the cows were analysed for lactose content and the mean value and standard error were calculated. The data is given in the Table 1. The analyses of samples of mammary secretion carried out one day prior to parturition showed a low content of lactose (1.14 ± 0.31%). The colostrum samples drawn on the first day of calving had a mean lactose content of 2.72 ± 0.37%. The lactose showed a tendency to increase and attained the normal level by seventh day of lactation. From there it remained almost static in the early phase of lactation (Fig. 1). The mean percentage of lactose in the colostral period was 3.53 and in the early phase of lactation it was 4.56.

The lactose content of milk remained unchanged in the middle phase of lactation and averaged 4.54 ± 0.03% (Table 2).

The mean lactose content in the late phase of lactation was 4.33 ± 0.04%. A decrease in the percentage of lactose was observed after 300 days of lactation (Table 3).

The lactose content of milk from induced lactation also showed trends, except on the first day when it amounted to 0.61% (Table 25).

Mastitis lowered the lactose content of the milk. The average lactose level in milk in subclinical stage was 2.64%. Clinical mastitis had low lactose content than milk collected in the subclinical mastitis (Table 19).

Chloride content of milk.

The chloride content of mammary secretion drawn one day prior to calving was 0.234 ± 0.003% (Table 4). A slight reduction in the chloride content was noticed in the colostrum drawn on the first day of calving (0.227 ± 0.008%). Afterwards there was a steady decline in the chloride content. The chloride content of milk after seventh day of lactation was similar to that of mid-lactation values.

The chloride content remained almost steady in the middle phase of lactation and amounted to 0.141 ± 0.002% (Table 5).

A slight increase in the chloride was noticed in the beginning of late phase of lactation and it reached upto 0.191 ± 0.003% in the 330th day (Table 6).

The average chloride content in the milk collected from infected udder was 0.223% (Table 20). Not much difference in chloride content was noticed between the subclinical and clinical stage of mastitis.

The milk from induced lactation had a chloride content of 0.273% on the first day and was higher than the normal colostral values (Table 26). On the seventh day it was only slightly above the post-partum lactation values. But it remained on the higher side itself, around 0.14% even on 150th day.

Sodium content of milk.

Sodium content of mammary secretion collected one day prior to parturition was 1380 ± 197.59 ppm. The colostrum contained 857.5 ± 87.73 ppm of sodium. The sodium content decreased after the colostrum period and the average value for the rest of the early phase was 546 ± 15.66 ppm (Table 7).

The mean sodium content of milk in the middle phase of lactation was 547.86 ± 10.12 ppm (Table 8).

An increase in the sodium content was obtained at the beginning of the late phase of lactation and amounted to 562.6 ± 12.78 ppm at the 230th day (Table 9). The mean sodium content of milk in the late phase was 626.86 ± 7.60 ppm. The content of sodium at 330th day of lactation was

690 ± 21.60 ppm. This value was higher than the normal mid-lactation values. The sodium content of milk was found to be increased from mid-lactation values to the late lactation (Fig. 2).

The milk collected from lactation induced animals had higher sodium content (1650 ppm) in the first day than normal post-partum colostrum (Table 27). The mid-lactation values of sodium also were higher than the normal and amounted to 620 ppm.

Milk samples collected from mastitis affected quarters showed higher sodium contents. Average sodium content was 1023.33 ppm in mastitis milk samples (Table 22).

Potassium content of milk.

The samples of mammary secretion collected one day prior to lactation contained 640 ± 69.64 ppm potassium (Table 10). Steady increase of potassium content was noticed from first day of calving (Fig. 2). The mean potassium content of the colostral period was 1120.83 ± 47.46 and during the rest of the phase was 1325 ± 14.92 ppm.

The milk samples of middle phase of lactation had a mean potassium content of 1367.86 ± 6.10 ppm (Table 11). The potassium content of milk collected in the middle phase of lactation was almost constant throughout the phase (Fig.2).

The mean value for potassium in the late phase of lactation was 1290.36 ± 6.86 ppm. This value was lower than the mid-lactation values of potassium (Table 12).

The potassium content of milk of lactation induced animals showed similar trends as that normal post-partum milk (Table 28).

Infection of the udder decreased the potassium content of milk considerably and amounted to an average of 1147.92 ppm (Table 23).

Inter relationships between the components.

The inter relations between lactose, chloride, sodium and potassium are graphically represented in Figures 1 and 2. Inverse relationships existed between lactose and chloride content of milk. The correlation between lactose and chloride contents of milk was negative in all the three phases; r = -0.76 for early, r = -0.34 for middle and r = -0.43 for late phases of lactation (Table 29).

The chloride-lactose ratio (Koestler number) was 20.53 in the mammary secretion drawn one day prior to parturition. In the colostrum period the Koestler number was 6.36 (Table 13). The Koestler number decreased steadily to arrive at 3.06 on the fifteenth day of lactation. The average chloride-lactose ratio in the mid-lactation was

3.09 (Table 14) and was increased in the late phase (Table
The average ratio in the late phase of lactation was 3.87.
Mastitis increased the ratio from a normal of 3.06 to 9.04
(Table 21).

The potassium-sodium ratio of milk were claculated f
early, middle and late phases of lactation as 2.44, 2.49 a
2.06 respectively. The ratio was 1.36 in the colostrum
(Tables 16, 17 and 18). Mastitis decreased the potassium-sodium ratio below an average value of 1.12 (Table 24).

Correlations between lactose and sodium were negative for early, middle and late phases of lactation and were, r = -0.85, r = -0.30 and r = -0.27 respectively (Table 29)

Positive correlations were established between lactos and potassium for early (r = 0.86), middle (r = 0.16) and late (r = 0.26) phases of lactation.

Strong positive correlations were obtained between sodium and chloride in all the three phases of lactation (r = 0.77 for early, r = 0.46 for middle and r = 0.03 for late). But the correlations between sodium and potassium were negative in the three phases namely early (r = -0.83) middle (r = -0.44) and late (r = -0.14) phases of lactatio

TABLES

Table 1. Lactose content of milk in the early phase of lactation (in percentage).

Day	Crossbred cow No.						Mean
	520	662	902	408	541	559	
-1	1.00	-	0.63	2.30	1.00	0.75	1.14 ± 0.31
1	3.25	2.75	2.18	2.44	1.56	4.14	2.72 ± 0.37
3	4.34	4.35	4.13	4.50	4.31	4.40	4.34 ± 0.03
7	4.40	4.58	4.40	4.58	4.44	4.90	4.53 ± 0.06
15	4.51	4.60	4.55	4.30	4.38	4.40	4.57 ± 0.08
30	4.60	4.36	4.60	4.52	4.60	4.50	4.53 ± 0.04
45	4.58	4.55	4.50	4.50	4.71	4.50	4.56 ± 0.03
60	4.67	4.55	4.89	4.58	4.70	4.60	4.67 ± 0.05

Mean lactose in colostral period : 3.53 ± 0.30%.

Mean lactose in the rest of the phase : 4.56 ± 0.02%.

Table 2. Lactose content of milk in the middle phase of lactation (in percentage).

Day	Crossbred cow No.						Mean
	528	840	811	540	685A	009	
140	4.53	4.50	4.30	4.51	4.23	4.76	4.52 ± 0.07
150	4.55	4.52	4.45	4.52	4.25	5.11	4.57 ± 0.12
160	4.55	4.35	4.50	4.45	4.30	4.75	4.48 ± 0.07
170	4.50	4.45	4.60	4.50	4.40	4.60	4.51 ± 0.03
180	4.45	4.40	4.80	4.40	4.50	4.60	4.53 ± 0.06
190	4.55	4.42	4.75	4.30	4.58	4.70	4.58 ± 0.05
200	4.45	4.52	4.65	4.60	4.60	4.70	4.59 ± 0.04

Overall average : 4.54 ± 0.03.

Table 3. Lactose content of milk in the late phase of lactation (in percentage).

Day	Crossbred cow No. 689	687	24001	1922	T641	690	494	688	Mean
250	4.55	4.72	4.35	4.40	4.45	4.47	4.50	4.65	4.51 ± 0.05
260	3.72	4.43	3.72	3.90	4.15	4.40	4.44	4.20	4.12 ± 0.11
270	4.53	4.55	4.60	4.60	4.22	4.52	4.25	4.80	4.51 ± 0.07
280	4.50	4.52	4.35	4.55	4.56	4.60	4.52	4.40	4.46 ± 0.04
290	4.44	4.60	4.80	4.50	4.40	4.40	4.20	4.40	4.47 ± 0.07
300	4.80	4.60	4.35	4.60	4.50	3.98	4.80	4.20	4.48 ± 0.10
310	4.35	4.38	4.40	4.25	4.20	4.20	4.20	4.10	4.27 ± 0.04
320	4.30	4.25	--	4.10	4.10	4.20	4.40	4.00	4.19 ± 0.05
330	4.30	4.00	--	3.45	4.35	4.20	3.40	3.30	3.86 ± 0.17

Overall average : 4.33 ± 0.04.

Table 4. Chloride content of milk in the early phase of lactation (in percentage).

Day	Crossbred cow No. 520	662	992	408	541	659	Mean
-1	0.231	--	0.236	0.233	0.240	0.240	0.234 ± 0.003
1	0.190	0.233	0.236	0.236	0.239	0.231	0.227 ± 0.008
3	0.170	0.218	0.182	0.185	0.226	0.157	0.189 ± 0.011
7	0.146	0.202	0.142	0.189	0.226	0.141	0.175 ± 0.015
15	0.124	0.150	0.130	0.131	0.157	0.149	0.140 ± 0.006
30	0.135	0.140	0.130	0.131	0.148	0.140	0.137 ± 0.003
45	0.130	0.141	0.141	0.141	0.138	0.141	0.139 ± 0.002
60	0.140	0.140	0.163	0.140	0.136	0.130	0.142 ± 0.005

Mean chloride content in colostral period : 0.209 ± 0.009%.

Mean chloride content in the rest of the phase : 0.147 ± 0.004%.

Table 5. Chloride content of milk in the middle phase of lactation (in percentage).

Day	Crossbred cow No.						Mean
	528	840	811	540	685A	009	
140	0.130	0.155	0.135	0.132	0.130	0.132	0.139 ± 0.004
150	0.137	0.160	0.130	0.135	0.145	0.132	0.139 ± 0.005
160	0.137	0.160	0.166	0.138	0.141	0.125	0.143 ± 0.007
170	0.136	0.155	0.160	0.131	0.141	0.135	0.143 ± 0.005
180	0.138	0.148	0.149	0.149	0.140	0.132	0.143 ± 0.003
190	0.130	0.150	0.140	0.135	0.138	0.132	0.138 ± 0.003
200	0.134	0.150	0.145	0.135	0.135	0.131	0.138 ± 0.003

Overall average : 0.141 ± 0.002.

Table 6. Chloride content of milk in the late phase of lactation (in percentage).

Day	Crossbred cow No.								Mean
	689	687	24001	1922	T641	690	494	688	
250	.140	.143	.143	.165	.160	.148	.142	.142	.149 ±.003
260	.149	.158	.162	.165	.165	.155	.147	.147	.157 ±.002
270	.144	.149	.140	.165	.165	.165	.150	.150	.156 ±.004
280	.156	.149	.145	.165	.162	.185	.155	.155	.161 ±.004
290	.153	.157	.160	.169	.160	.165	.173	.173	.163 ±.002
300	.165	.156	.171	.155	.171	.168	.162	.162	.165 ±.002
310	.178	.165	.168	.186	.175	.170	.168	.188	.174 ±.003
320	.195	.181	--	.180	.173	.168	.195	.195	.182 ±.004
330	.199	.201	--	.190	.182	.192	.184	.184	.191 ±.003

Overall average : 0.165 ± 0.002.

Table 7. Sodium content of milk in the early phase of lactation (in parts per million).

Day	Crossbred sow No.						Mean
	520	662	992	408	541	C59	
-1	960	----	2080	1280	1300	1060	1380.00 ± 197.59
1	400	920	1160	1120	1500	950	1025.00 ± 151.56
3	640	900	740	600	800	560	706.67 ± 53.08
7	470	800	600	510	620	520	586.67 ± 48.56
15	460	600	500	500	560	520	523.33 ± 20.28
30	670	520	500	800	320	500	585.00 ± 50.45
45	440	500	520	520	580	500	510.00 ± 18.44
60	500	500	620	500	520	510	525.00 ± 19.28

Mean sodium content in the colostral period : 857.5 ± 87.73

Mean sodiumcontent in the rest of the phase : 546 ± 15.60 pp

Table 8. Sodium content of milk in the middle phase of lactation (in parts per million).

Day	Crossbred sow No.						Mean
	528	840	811	540	685A	009	
140	450	500	600	520	530	520	520.00 ± 19.83
150	480	520	620	510	560	520	551.67 ± 17.3
160	580	700	700	520	520	480	583.33 ± 39.1
170	480	580	730	500	500	530	548.33 ± 33.51
180	520	600	500	730	520	520	565.00 ± 35.94
190	520	520	500	600	380	500	533.33 ± 18.3
200	580	520	520	620	520	520	546.67 ± 17.64

Overall average : 547.86 ± 10.12.

Table 9. Sodium content of milk in the late phase of lactation (in parts per million).

Day	Crossbred cow No.								Mean
	689	687	24001	1922	T641	690	494	688	
250	580	600	540	520	600	520	540	600	562.50 ± 12.73
260	620	650	600	620	620	580	540	620	606.25 ± 11.79
270	520	560	600	800	620	600	540	640	610.00 ± 30.71
280	520	620	620	570	600	700	540	620	598.75 ± 19.80
290	580	600	620	580	600	680	620	700	622.50 ± 15.78
300	520	620	620	590	620	680	620	620	611.25 ± 15.7[illegible]
310	700	660	680	600	700	700	680	620	667.50 ± 13.59
320	680	720	----	620	750	720	700	630	688.57 ± 18.31
330	750	680	----	620	700	720	750	610	690.00 ± 21.60

Overall average : 626.86 ± 7.60.

Table 10. Potassium content of milk in the early phase of lactation (in parts per million).

Day	Crossbred cow No.						Mean
	520	662	992	408	541	C59	
-1	750	----	600	400	650	800	640.00 ± 69.64
1	1200	1000	1200	1000	700	1150	1041.66 ± 77.91
3	1050	1250	1300	1250	1150	1200	1193.33 ± 38.87
7	1200	1300	1200	1200	1350	1400	1283.33 ± 33.33
15	1300	1300	1250	1300	1300	1400	1308.33 ± 20.06
30	1300	1250	1250	1150	1350	1400	1283.33 ± 35.75
45	1250	1350	1350	1400	1250	1450	1341.06 ± 32.70
60	1350	1350	1450	1450	1400	1450	1408.33 ± 20.07

Mean potassium content in colostral period : 1120.83 ± 47.46.

Mean potassium in the rest of the period : 1325 ± 14.92.

Table 11. Potassium content of milk in the middle phase of lactation (in parts per million).

Day	Crossbred cow No.						Mean
	528	840	811	540	685A	009	
140	1400	1350	1350	1350	1350	1350	1358.33 ± 8.33
150	1350	1350	1350	1450	1350	1350	1366.67 ± 16.67
160	1350	1300	1300	1400	1350	1350	1341.67 ± 15.37
170	1400	1300	1300	1400	1400	1350	1358.33 ± 20.07
180	1400	1300	1400	1400	1400	1400	1383.33 ± 16.67
190	1400	1350	1400	1400	1350	1450	1391.67 ± 15.06
200	1400	1300	1400	1400	1350	1400	1375.00 ± 17.08

Overall average : 1367.86 ± 6.10.

Table 12. Potassium content of milk in the late phase of lactation (in parts per million).

Day	Crossbred cow No.								Mean
	689	687	24001	1922	T641	690	494	608	
250	1200	1150	1250	1300	1300	1350	1350	1300	1275.00±25.00
260	1100	950	1150	1200	1300	1300	1350	1300	1206.25±47.60
270	1300	1350	1250	1350	1400	1250	1350	1300	1325.00±23.13
280	1300	1350	1250	1350	1400	1250	1300	1350	1306.25±14.75
290	1300	1350	1250	1300	1300	1250	1250	1250	1281.25±13.15
300	1400	1300	1300	1450	1300	1200	1325	1300	1321.88±20.49
310	1400	1350	1300	1300	1300	1150	1300	1350	1306.25±25.76
320	1300	1300	--	1300	1300	1350	1300	1300	1314.29± 9.22
330	1300	1300	--	1300	1250	1300	1250	1250	1278.57±10.10

Overall average : 1290.36 ± 8.86.

Table 13. Content of chloride and lactose and chloride-lactose ratio in the early phase of lactation (average of six cows).

Day	Chloride %	Lactose %	Chloride-lactose ratio
-1	0.234	1.14	20.53
1	0.227	2.72	8.35
3	0.189	4.34	4.36
7	0.175	4.53	3.86
15	0.140	4.57	3.06
30	0.137	4.53	3.02
45	0.139	4.56	3.05
60	0.142	4.67	3.04

Average chloride-lactose ratio in colostral period : 6.36.

Average chloride-lactose ratio in the rest of the phase: 3.21.

Table 14. Content of chloride and lactose and chloride-lactose ratio in the middle phase of lactation (average of six cows).

Day	Chloride %	Lactose %	Chloride-lactose ratio
140	0.139	4.52	3.07
150	0.139	4.57	3.04
160	0.143	4.48	3.19
170	0.143	4.51	3.17
180	0.143	4.53	3.16
190	0.138	4.58	3.00
200	0.138	4.59	3.01

Overall average ratio : 3.09.

Table 15. Content of chloride and lactose and chloride-lactose ratio in the late phase of lactation (average of eight cows).

Day	Chloride %	Lactose %	Chloride-lactose ratio
250	0.149	4.51	3.30
260	0.157	4.12	3.80
270	0.156	4.51	3.46
280	0.167	4.46	3.59
290	0.163	4.47	3.65
300	0.165	4.48	3.68
310	0.174	4.27	4.08
320	0.182	4.19	4.34
330	0.191	3.86	4.95

Overall average ratio : 3.87.

Table 16. Contents of potassium and sodium and potassium-sodium ratio in the early phase of lactation (average of six cows).

Day	Potassium (ppm)	Sodium (ppm)	Potassium-sodium ratio
-1	640.00	1380.00	0.46
1	1041.66	1025.00	1.02
3	1196.33	706.27	1.69
7	1283.33	586.67	2.19
15	1308.33	523.33	2.50
30	1283.33	585.00	2.19
45	1341.66	510.00	2.63
60	1408.33	525.00	2.68

Average potassium-sodium ratio of colostral period : 1.36.

Table 17. Content of potassium and sodium and potassium-sodium ratio in the middle phase of lactation (average of six cows).

Day	Potassium (ppm)	Sodium (ppm)	Potassium-sodium ratio
140	1358.33	520.00	2.61
150	1366.67	551.67	2.48
160	1341.67	583.33	2.43
170	1358.33	548.33	2.48
180	1383.33	565.00	2.45
190	1391.67	553.82	2.52
200	1375.00	546.67	2.52

Overall average ratio : 2.49.

Table 18. Content of potassium and sodium and potassium-sodium ratio in the late phase of lactation. (average of eight cows).

Day	Potassium (ppm)	Sodium (ppm)	Potassium-sodium ratio
250	1275.00	562.50	2.27
260	1206.25	606.25	1.99
270	1325.00	610.00	2.17
280	1306.25	598.75	2.18
290	1281.25	622.50	2.06
300	1321.88	611.25	2.16
310	1306.25	667.50	1.96
320	1314.29	688.57	1.91
330	1278.57	690.00	1.85

Overall average ratio : 2.06.

Table 19. Lactose content of mastitis milk (in percentage).

	Days	Crossbred cow No.				Average
		873	992	735	776	
Subclinical stage.	-5				2.57	2.57
	-4		4.00		3.36	3.68
	-3	3.75	3.87	3.64	2.13	3.35
	-2	2.50	1.55	2.44	3.00	2.37
	-1	2.10	1.50	3.21	2.14	2.44
Clinical stage.		2.10	0.97	1.80	1.71	1.65

Average:2.64

Table 20. Chloride content of mastitis milk (in percentage).

	Days	Crossbred cow No.				Average
		873	992	735	776	
Subclinical stage.	-5				0.239	0.239
	-4		0.211		0.224	0.218
	-3	0.182	0.223	0.221	0.223	0.212
	-2	0.190	0.243	0.211	0.231	0.219
	-1	0.210	0.242	0.226	0.231	0.227
Clinical stage.		0.200	0.243	0.223	0.239	0.226

Average : 0.223

Table 21. Contents of chloride and lactose and lactose-chloride ratio in the milk of infected udder.

	Days	Chloride %	Lactose %	Chloride-lactose ratio
Subclinical stage.	-5	0.239	2.57	9.31
	-4	0.217	3.68	5.90
	-3	0.212	3.35	6.33
	-2	0.210	2.37	8.86
	-1	0.227	2.24	10.13
Clinical stage.		0.226	1.65	13.70

Average: 9.04.

Table 22. Sodium content of mastitis milk (in parts per million).

	Days	Crossbred cow No.				Average
		873	992	735	776	
Subclinical stage.	-5				950	950
	-4		1050		900	975
	-3	850	1000	810	1100	940
	-2	800	1200	1050	1000	1050
	-1	950	1200	1200	1200	1137.5
Clinical stage.		900	1150	1350	1100	1125

Average : 1023.33

Table 23. Potassium content of mastitis milk (in parts per million).

	Days	Crossbred cow No. 873	992	735	776	Average
Subclinical stage.	-5				1200	1200
	-4		1200		1100	1150
	-3	1200	1000	1200	1100	1125
	-2	1200	1050	1100	1200	1137.5
	-1	1200	1200	1000	1200	1150
Clinical stage.		1150	1100	1000	1250	1125

Average : 1147.92.

Table 24. Contents of potassium and sodium and potassium-sodium ratio of mastitis milk.

	Days	Potassium (ppm)	Sodium (ppm)	Potassium-sodium ratio
Subclinical stage.	-5	1200	950	1.26
	-4	1150	975	1.10
	-3	1125	940	1.20
	-2	1137.5	1050	1.08
	-1	1150	1137.5	1.01
Clinical stage.		1112.5	1125	1.00

Average : 1.12.

Table 25. Lactose content of induced lactation milk (in percentage).

Day of Lactation	Cow Number 813	Cow Number 10286	Average
1	0.22	1.00	0.61
3	3.50	3.80	3.65
7	4.62	4.60	4.61
10	4.60	4.75	4.68
20	4.97	dried off.	
40	4.75		
60	4.60		
150	4.44		
152	4.71		
154	4.75		

Table 26. Chloride content of induced lactation milk (in percentage).

Day of lactation	Cow Number 813	Cow Number 10286	Average
1	0.265	0.280	0.273
3	0.239	0.235	0.237
7	0.180	0.175	0.178
10	0.158	0.150	0.154
20	0.132	dried off.	
40	0.135		
60	0.165		
150	0.149		
152	0.140		
154	0.136		

Table 27. Sodium content of induced lactation milk (in parts per million).

Day of lactation	Cow Number 813	Cow Number 10286	Average
1	1500	1800	1650
3	1050	1240	1145
7	720	600	660
10	720	600	600
20	630	dried off.	
40	620		
60	560		
150	600		
152	640		
154	620		

Table 28. Potassium content of induced lactation milk (in parts per million).

Day of lactation	Cow Number 813	Cow Number 10286	Average
1	800	400	600
3	950	1000	975
7	1150	1300	1225
10	1200	1400	1300
20	1150	dried off.	
40	1400		
60	1400		
150	1400		
152	1400		
154	1400		

Table 29. Correlations between the estimated components of milk.

Phase of lactation	Lactose-chloride	Lactose-sodium	Lactose-potassium	Sodium-chloride	Sodium-potassium
Early	-0.76	-0.83	0.86	0.77	-0.83
Middle	-0.34	-0.30	0.16	0.46	-0.44
Late	-0.43	-0.27	0.26	0.03	-0.14

ILLUSTRATIONS

FIGURE 1. LACTOSE AND CHLORIDE CONTENT OF MILK DURING THE THREE PHASES OF LACTATION.

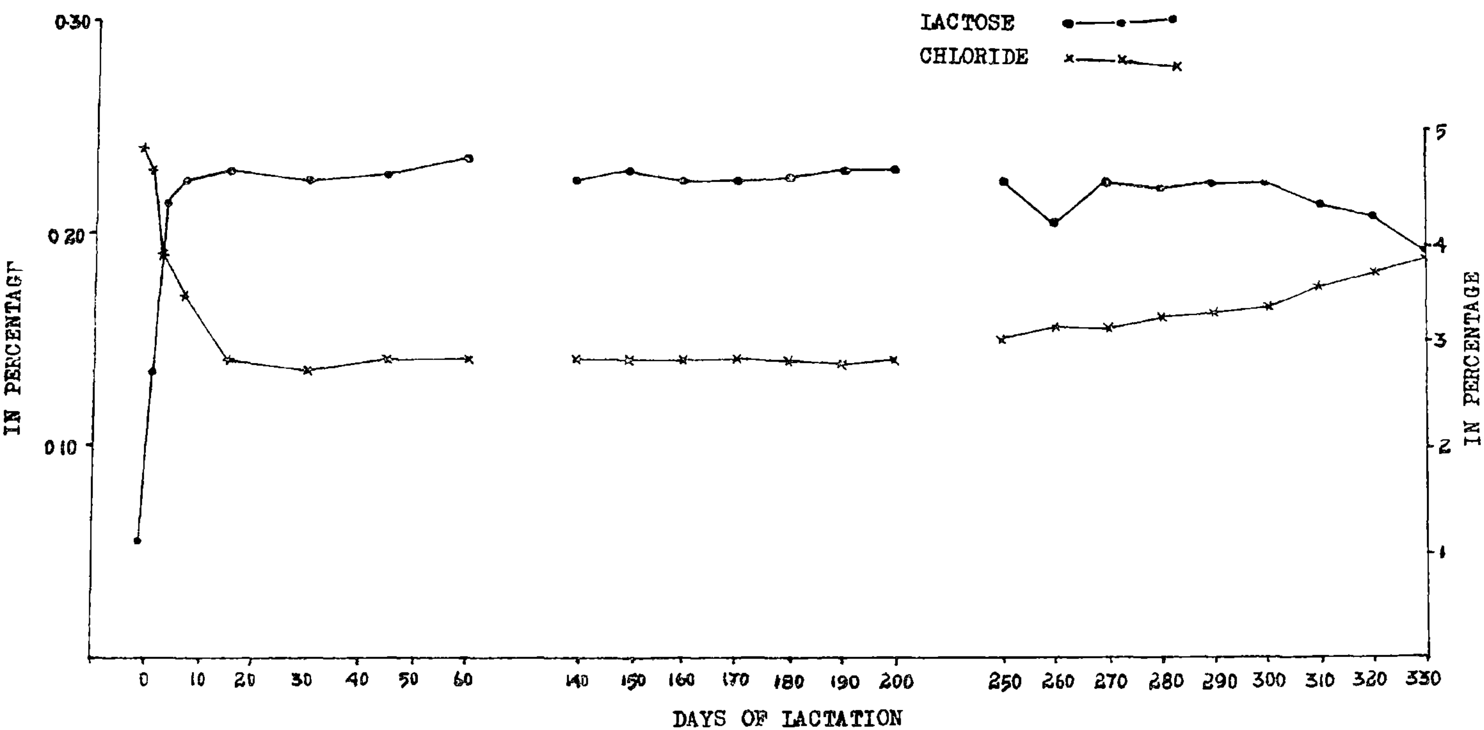
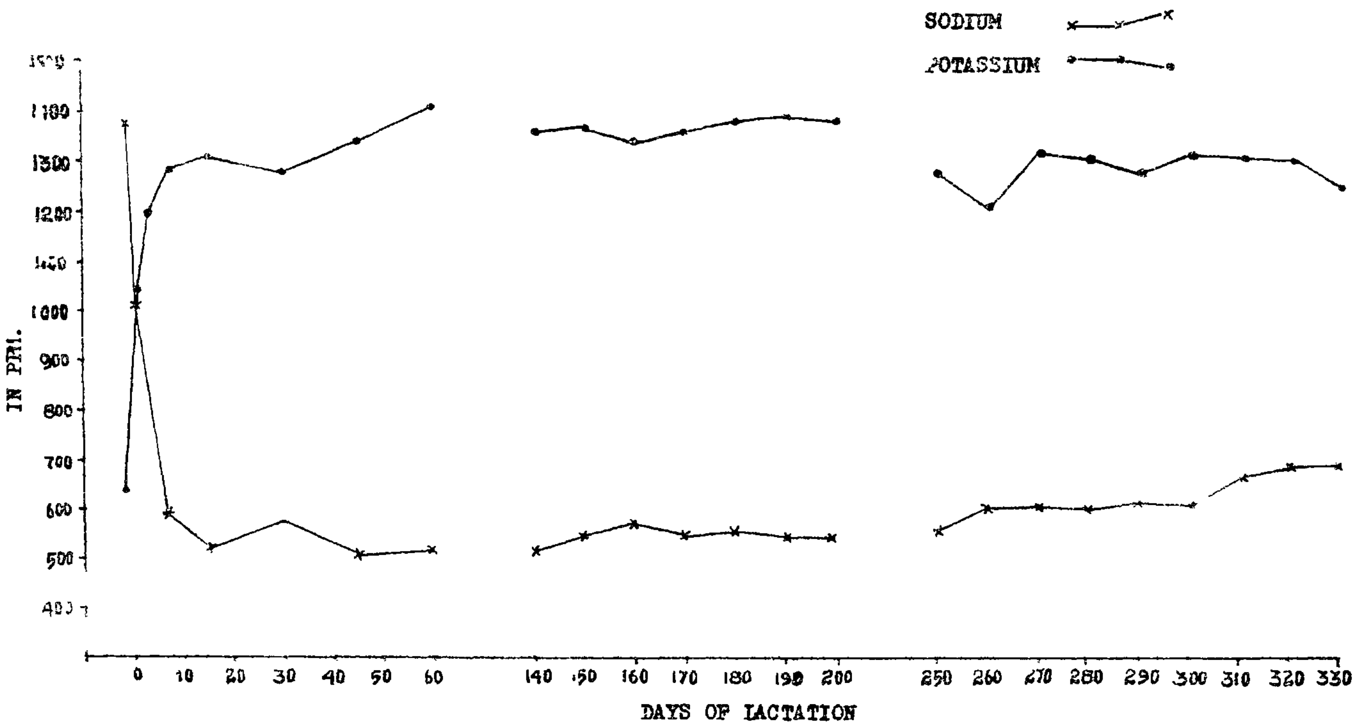


FIGURE 2. SODIUM AND POTASSIUM CONTENT OF MILK DURING THE THREE PHASES OF LACTATION.



DISCUSSION

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DISCUSSION

The major minerals like sodium, potassium and chloride and the milk sugar lactose are involved in an important function of maintaining the osmotic pressure of milk in equilibrium with blood, so as to perform the synthesis and secretion of milk in the normal manner. These osmotically active substances are interrelated and vary in an integrated manner during different phases of lactation and in conditions like mastitis.

In the present study, the milk collected at different phases of lactation, from mastitis effected udder and from induced lactation were analysed to observe the changes occuring to the above mentioned osmotically active constituents of milk.

Lactose content of milk.

The lactose content was 1.14 ± 0.31% in the colostrum drawn one day prior to parturition. This value was very low when compared to the mid-lactation values. Colostrum samples had a mean lactose content of 3.53 ± 0.30%, which was lower than the mean lactose content obtained in the early phase of lactation (4.56 ± 0.02%). The lactose contents obtained in the early phase of lactation and middle phase were slightly less than the values reported by

Comberg *et al.* (1967) and Barakat *et al.* (1969) probably because of breed differences.

The lactose content decreased from the normal to 4.33 ± 0.04% in the late lactation and it reached 3.86 ± 0.17% on the 330th day. This variation in content of lactose in the milk in late lactation may be associated with physiological factors and related to the process of synthesis of milk. It may be possible that at the tail end of lactation along with the decrease in the amount of secretory tissue there may be a depression in the ability of the existing tissues to synthesise lactose. As the lactose content of the milk was decreasing the total quantity of milk produced also decreased.

The milk from lactation induced animals also showed similar trends in lactose content as that of post-partum milk. This finding is in agreement with the reports of Joseph and Pavithran (1979 a & b) that the major constituent in milk are similar in induced milk and post-partum milk.

Lactose content of mastitis milk was 2.64%. This value is in agreement with the findings of Walsh and Neave (1968). This decrease in the level of lactose is expected, as the disease condition damages the integrity of the cellular structure, more of plasma exudate may enter the alveolar lumen causing an increase in osmotic pressure. To compensate this increase, synthesis of lactose is reduced (Schmidt, 1971).

Chloride content of milk.

Mammary secretion drawn one day prior to parturition had higher chloride content (0.234 ± 0.003%). The colostrum contained 0.227 ± 0.008% of chloride on the first day of parturition. This value is slightly less than the reported value of 0.257% (Sebela et al. 1976). The high chloride content of colostrum may be due to the greater rate of passage of chloride into the milk from blood in the late pregnancy (Peaker, 1974).

The chloride content remained steady in the middle phase of lactation and amounted to 0.141 ± 0.002%. This value is slightly higher than the reported normal values but almost in agreement with the estimated value of Tapernoux and Maget (1967).

Chloride content was increased in the late phase of lactation to 0.165 ± 0.002% and reached upto 0.191 in the 330th day. This increase combined with the decrease of lactose indicated a compensatory mechanism in maintaining the osmotic pressure of milk in equilibrium. Mastitis also increased the chloride content of milk (0.223%). Ingr et al. (1973) suggested a critical limit of 0.15% for the diagnosis of mastitis. Jenness and Patton (1959) and Mielke (1975) also have reported an increase in chloride content of milk in mastitis condition. This must be due to increased vascularity in the affected tissues and increased effussion of plasma exudate from the damaged tissues to the alveolar lumen which get mixed up with the secreted milk.

The milk from induced lactation had a chloride content of 0.271% on the first day and was higher than the normal post-partum colostral values. This increased level of chloride in the induced lactation might be a consequence of the hormone treatment at high doses which might have altered the cellular permeability of the secretory cells.

Sodium content of milk.

Sodium content of mammary secretion collected one day prior to parturition was 1380 ± 197.59 ppm and colostrum contained only 857.5 ± 87.75 ppm. Both the figures were higher than the normal values for milk. Vinovrski et al. (1976) also obtained high contents of sodium in the first three days of calving. This may be due to the greater permeability of mammary ducts to sodium ions in late pregnancy (Linzell and Peaker, 1974).

The mean sodium content of milk in the early phase except that of colostrum period remained almost similar to that of the middle phase of lactation and was 547.86 ± 10.12 ppm. This value is almost similar to the reported sodium content of 50 mg/100 ml (Ganguli, 1979). But Pinto et al. (1978) reported a low sodium content of 35 mg/100 ml in milk.

The content of sodium was higher in the late phase (626.86 ± 7.6 ppm) than the middle phase of lactation. The content of sodium showed the same trend as that of chloride in different phases of lactation.

Induced lactation showed higher sodium content (1650 ppm) in the colostrum period than normal post-partum colostrum. For the rest of the period of the early phase also this trend prevailed. The mid-lactation values of sodium were also higher than the normal and amounted to 620 ppm. The relationship with sodium and chloride in milk of induced lactation was same as that of post-partum lactation. The higher level of these ions in the milk of induced lactation might have been due to increased cellular permeability. It is curious to note that the level of lactose had not decreased and the corresponding affect on the osmotic pressure of milk need further study.

Mastitis increased the sodium content. Average sodium content was 1023.33 ppm in mastitis milk samples. This may be due to the derangement in the process of synthesis in the infected udder. The sodium and chloride contents were simultaneously increased in mastitis and there was a consequent depression in the lactose content. As generally understood, this phenomenon is compensatory mechanism in the maintenance of osmotic pressure of milk.

Potassium content of milk.

The potassium content was low in the mammary secretion collected one day prior to lactation (640 ± 69.64 ppm). Steady increase of potassium was noticed from the colostrum period in relation with decreasing sodium to reach a normal after seventh day of calving.

The colostral values of potassium were lower than the values reported by Sebela *et al.* (1976) and Vinovrski *et al.* (1976).

The mid-lactation value (1367.86 ± 6.10 ppm) obtained is slightly lower than the reported values of Kani *et al.* (1971), Comberg *et al.* (1967) and Ganguli (1979).

The value obtained for potassium content in the late phase of lactation was lower than the mid-lactation values

(1290.36 ± 8.86 ppm). It was observed that the level of potassium started at a lower level at the beginning of lactation slowly increased and then slightly reduced towards the end of lactation. The trend of lactose was also similar but that of chloride was on the reverse.

The potassium content of milk of lactation induced animals showed similar trends as that of normal post-partum milk. It may be assumed that the treatment meted out in the induction procedure has no effect on the potassium concentration.

Infection of the udder decreased the potassium content of milk which amounted to 1147.92 ppm. This is in agreement with the reports of Wheelock *et al.* (1966). This reduction in potassium concentration may be for maintaining the osmotic pressure of milk due to the other changes in concentrations of osmotically active constituents of milk.

<u>Interrelationships between lactose, chloride, sodium and potassium.</u>

The chloride-lactose ratio was very high in the colostrum (6.36). The Koestler number decreased steadily to arrive at 3.06 on the 15th day of lactation. This value is within the range of ratio reported by Sonkar *et al.* (1976). The ratio is higher than the range (1.5 to 3.0) reported by Jenness and

Patton (1959). The average ratio was 3.09 in mid-lactation and 3.87 in the late lactation.

Correlations between lactose and chloride were negative (r = -0.76 for early, r = -0.36 for middle and r = -0.43 for late phases of lactation).

Mastitis increased the Koestler number to 9.04 due to the higher chloride and lower lactose contents of mastitis milk.

Potassium-sodium ratio of milk was calculated for early, middle and late phases of lactation as 2.44, 2.49 and 2.06, respectively. The ratio was 1.36 in the colostrum. Mastitis decreased the ratio to 1.12. This change in the ratio is due to the slight reduction in potassium and the sizeable increase in the sodium during mastitis. The present observations are in agreement with the findings of Ingr <u>et al.</u> (1973) that a ratio of 2.0 can be considered as a critical value for potassium-sodium ratio, below which it may be considered that the udder is affected with mastitis. But care should be taken to avoid mixing of colostrum in the milk as the ratio in colostrum is also low.

Sodium and potassium in the milk were correlated negatively in all the three phases of lactation (r = -0.83, r = -0.44 and r = -0.14 for early, middle and late phases of lactation respectively).

Correlations between lactose and sodium contents of milk were negative throughout the lactation (r = -0.83 for early, r = -0.30 for middle and r = -0.27 for late phases, respectively). This was expected as lactose and sodium were negatively correlated and sodium and chloride were positively correlated. Naturally this is the mechanism in maintaining the osmotic pressure.

Positive correlations were observed between lactose and potassium in the three phases of lactation namely early (r = 0.86), middle (r = 0.16) and late (r = 0.26). As lactose and sodium were negatively correlated it could be deduced that sodium and potassium might be having a negativ correlation. This inference was found to be true as the correlation between sodium and potassium was -0.83, -0.44 and -0.14 respectively, in the three phases of lactation studied.

The correlation between sodium and chloride in the mil were positively correlated throughout the lactation. The correlations worked out as described above are in close agreement with the findings of Praphulla and Anantha-krishnan (1970).

SUMMARY

A study was undertaken to ascertain the pattern of secretion of lactose, sodium, potassium and chloride in the milk at different phases of lactation, in mastitis condition and in induced lactation.

The lactose content was very low in colostrum drawn one day prior to lactation and increased in colostrum and attained the normal level by seventh day. The lactose contents of milk in early phase and middle phase were almost remaining constant but lowered in late phase of lactation. Lactose of induced lactation milk also showed similar trends. Lowering of lactose was noted in the mastitis samples. The potassium in milk also behaved like that of lactose. The sodium and chloride had an opposite trend in milk than that of lactose and potassium.

In the induced lactation milk the sodium and chloride contents were higher and might be due to the alteration in the permeability of the mammary alveolar epithelium.

The chloride-lactose ratio was higher in colostrum and in mastitis milk, while it was around 3.00 in the normal milk.

Potassium-sodium ratio was around 2.5 in the milk. The ratio was lowered below 2.00 in mastitis condition of the udder.

The correlation between lactose and chloride, lactose and sodium and sodium and potassium were negative. Positive correlation was obtained between sodium and chloride. Lactose and potassium also showed positive correlation in all the three phases of lactation.

From the study conducted, it was understood that the osmotically active constituents of milk were interrelated in their behaviour to attain osmotic balance in the milk. These substances showed variation in different phases of lactation and in infection of the udder in a special manner to restore equilibrium in osmotic pressure. This was also true in the cases of induced lactation milk.

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CHEMICAL CONSTITUENTS AFFECTING OSMOLALITY OF MILK IN CROSSBRED CATTLE

BY

SIMON DANIEL

ABSTRACT OF A THESIS

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Faculty of Veterinary and Animal Sciences
Kerala Agricultural University

Department of Dairy Science
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
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ABSTRACT

A study was planned to ascertain the behaviour of osmotically active components in normal milk, milk collected from animals induced to lactate and milk collected from infected udder. An exhaustive review on the literature available on the topic was made.

The samples of milk were collected from three groups of crossbred cows which were in three phases of lactation namely, early, middle and late. Milk samples were also collected from two infertile animals which were induced to lactate with the administration of steroid hormones and from four cows affected with mastitis. All the samples were analysed for lactose, chloride, sodium and potassium by standard methods described.

The lactose andppotassium contents of the milk were low in the colostrum. The lactose attained the normal leve by seventh day of calving and potassium by 15th day. The mean lactose content was 4.54% in mid-lactation and 4.33% in the late phase. Potassium showed the similar trend and decreased to 1290.36 ppm from a mid-lactation value of 1367.86 ppm. The lactose and potassium were positively correlated in all the three phases of lactation.

The sodium and chloride content varied in the milk in an opposite manner than that of lactose and potassium. The

sodium contents were 546 ppm in early, 547.86 ppm in middle and 626.86 ppm in late phases of lactation. Colostrum contained 857.5 ppm of sodium. Chloride was also higher in colostrum and decreased to an average early phase chloride of 0.147% from 0.209%. Middle phase had a chloride content of 0.141% and in late phase it was 0.165%.

All the components behaved in the similar manner to that of milk in the milk from induced lactation. But the chloride and sodium were high. Mastitis lowered the lactose and potassium to 2.14% and 1147.92 ppm respectively while sodium and chloride increased to 1023.33 ppm and 0.223%.

The chloride-lactose ratio was 6.36 in colostrum, 3.21 in early phase, 3.09 in middle phase and 3.87 in late phase of lactation. Potassium-sodium ratio were amounted to 2.44, 2.49 and 2.06 respectively for early, middle and late phases of lactation. The ratio was 1.36 in colostrum. Mastitis lowered the potassium-sodium ratio while increased the chloride-lactose ratio.

The correlation between lactose and chloride, lactose and sodium and sodium and potassium were negative while positive correlations were observed between sodium and chloride and lactose and potassium.

These observations were discussed with reference to other reported values.