SOMATIC CELL COUNT AND ITS INFLUENCE ON THE QUALITY OF MILK IN CROSS-BRED COWS

By
C. T. SATHIAN

THESIS

submitted in partial fulfilment of the requirement for the degree

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Faculty of Veterinary and Animal Sciences
Kerala Agricultural University

Department of Dairy Science
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DECLARATION

I hereby declare that the thesis entitled **"SOMATIC CELL COUNT AND ITS INFLUENCE ON QUALITY OF MILK IN CROSS-BRED COWS"** is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Mannuthy

C. T. SATHIAN

CERTIFICATE

Certified that this thesis entitled **"SOMATIC CELL COUNT AND ITS INFLUENCE ON QUALITY OF MILK IN CROSS-BRED COWS"** is a record of research work done independently by **Dr. C. T. Sathian** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship or fellowship to him.

27.3.2001

Mannuthy

Dr. M. Mukundan,
Associate Professor and Head i/c,
Department of Dairy Science,
College of Veterinary and Animal Sciences,
Mannuthy.

CERTIFICATE

We, the undersigned, members of the Advisory Committee of **Dr. C.T. Sathian** a candidate for the degree of **Doctor of Philosophy in Dairy Science** agree that the thesis entitled " **SOMATIC CELL COUNT AND ITS INFLUENCE ON QUALITY OF MILK IN CROSS-BRED COWS**" may be submitted by **Dr. C.T. Sathian** in partial fulfilment of the requirement for the degree.

27.3.2001

Dr. M. Mukundan,
Associate Professor and Head,
(Chairman, Advisory Committee)
Department of Dairy Science,
College of Veterinary and Animal Sciences,
Kerala Agricultural University,
Mannuthy.

Dr. Sosamma Iype,
Director (Retd.)
CAS in Animal Breeding
and Genetics,
(Member)

29/3/01

Dr. V. Prasad,
Special Officer,
College of Dairy Science-
and Technology,
Mannuthy
(Member)

Dr. M. R. Saseendranath,
Associate Professor and Head,
Dept.of Veterinary Epidemiology and
Preventive Medicine
(Member)

Dr. P. I. Geevarghese,
Associate Professor,
Department of Dairy Science,
(Member)

EXTERNAL EXAMINER

C. NARESH KUMAR
PROFESSOR
INSTITUTE OF FOOD AND DAIRY TECHNOLOGY
KODUVALLY. P.O ALANATHY
CHENNAI

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C.T. SATHIAN

DEDICATED

TO

MINI, APPU & RAJU

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LIST OF ABBREVIATIONS

ACTH	Adreno Cortico Tropic Hormone
ADV	Acid Degree Value
ANOVA	Analysis of Variance
AOAC	Association of Official and Analytical Chemists
AR	Analytical Reagent
BMTSCC	Bulk Milk Tank Somatic Cell Count
CBF	Cattle Breeding Farm
CMT	California Mastitis Test
DHAS	Quebec Dairy Herd Analysis Service (France)
DMSCC	Direct Microscopic Somatic Cell Count
DNA	Deoxy Ribo Nucleic Acid
EEC	European Economic Community
EU	European Union
FDA	Food and Drug Administration
FFA	Free Fatty Acid
GOT	Glutamate Oxaloacetate Transaminase
IS	Indian Standard
LRS	Livestock Research Station
MF-DNA	Membrane Filter- DNA Method
NA GASE	N-acetyl-β-D-glucosaminidase
PA	Plasminogen Activator
PAI	Plasminogen Inhibitor
PFA	Prevention of Food Adulteration
SCC	Somatic Cell Count
SNF	Solids-not-fat
SRL	Sisco Research Laboratories
TCA	Trichloro Acetic Acid
UHT	Ultra High Temperature
ULF	University Livestock Farm
WBC	White Blood Corpuscles
WMT	Wisconsin Mastitis Test

INTRODUCTION

1. INTRODUCTION

Quality of raw milk is the most important criteria which decides on the final quality of market milk and dairy products. In recent years, the presence of bovine body cells excreted through milk is a matter of interest to Dairy Scientists. Legislation was enforced in many developed countries stipulating maximum permissible number of somatic cells in milk (Early, 1998). Further these standards are modified periodically lowering the limits along with the adoption of better practices of hygienic milk production.

In India, much debate is being held on quality of raw milk, but role of somatic cell count is rarely discussed. Moreover little research work has been done in this respect .Bulk milk having a high somatic cell count does not necessarily have a high bacterial count. The dreadful fact is that bulk milk with a high content of mastitic milk may be graded as of high quality when assessed on bacterial count alone (Schalm *et al.*, 1971). India being the world leader in milk production, has necessarily to enter the export of milk and milk products. Many of the importers like European Union has formulated stringent standards on somatic cell count in raw milk. Hence it is mandatory for our dairy industry also to maintain and monitor this aspect of milk quality before entering the global market.

The somatic cell count has got an important bearing on udder health and it is well known that mastitis is prevalent in our herds. The disease has resulted in huge loss of milk production and quality. According to an estimate in 1956 the total loss to Dairy industry due to mastitis in United states was in excess of $ 245 million

(Van Houweling, 1957) while the estimate in 1996 was $ 1.7-2 billion. The latter figure corresponds to 11 per cent of total cost for milk production in U.S. (Jones, 1998). In India according to an estimate in 1994, mastitis of cows lead to a financial loss of 889.51 crores (Singh *et al.*, 1994).

Detection of clinical mastitis is possible at farmer's level and can be successfully treated once the causative agent is identified by the veterinarian. But prevalence of subclinical mastitis in a herd often goes undetected, but is capable of affecting milk yield and composition in a drastic way. According to Robinson (1993) the loss of milk yield and milk solids begin when somatic cell count exceeds 1 x 10^5 cells / ml and counts less than 5 x 10^5 cells / ml are termed as subclinical mastitis. The intensity of this problem is aggravated by the fact that the farmers may be convicted for adulteration of milk (low milk solids on testing milk) under Prevention of Food Adulteration (PFA) act while actual reason for low milk solids is subclinical mastitis. Radhika and Iype (1999) conducted a survey in Thrissur District of Kerala and observed that 47 per cent of the cross-bred cows milk do not meet the prescribed minimum 8.5 per cent Solids-not-fat (SNF) in their milk . The prevalence of subclinical mastitis in the herd which is dominated by cross-breeds may be one of the causes for this phenomenon. Singh *et al.* (1994) reported a prevalence of 43.9 per cent for subclinical mastitis in a study conducted in eight dairy farms in Punjab. Survey carried out in the state of Madhya Pradesh indicated that 49.75 per cent of the cows were infected with subclinical mastitis (Tiwari *et al.*, 2000). In Kerala prevalence of subclinical mastitis was 22.3 per cent as reported by Biju (1996).

Apart from the negative effect on yield and composition of milk number of somatic cells is critical in processing of milk. There is concrete evidence in literature indicating deterioration in quality of dairy products if high somatic cell count milk was used for their preparation. In case of products like cheese most of the processing parameters like rennet clotting time, strength of coagulum, whey expulsion and yield are affected and Grandison and Ford (1986) concluded that even a small increase in somatic cell count is detrimental to cheese composition and quality.

The study was undertaken with the following objectives.

1. To find out correlation if any, between somatic cell count and chemical composition of milk in terms of percentages of total solids, solids-not-fat, lactose, total protein and chloride.

2. To evaluate the effectiveness of California mastitis test (CMT) in detecting subclinical mastitis with regard to somatic cell count

3. To find out whether milk sample preservatives like formalin and potassium dichromate will be effective in preserving cells in milk.

4. To study the influence of age of the cow, parity, stage of lactation and season on somatic cell count of milk.

5. To assess the difference in concentration of somatic cells between fractions of milk (foremilk, mid stream milk and strippings) collected during milking.

6. To compare the beneficial effects of mopping of udder and teat dipping in controlling high somatic cell count of milk.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1. General/ History

The cells in milk was first described by Savage (1907). An approved method for counting somatic cells was devised by Prescott and Breed (1910) which is a reference method even today under the name "Breed method". Blackburn and Macadum (1954) Classified the cells in milk into epithelial cells, granulocytes and agranulocytes. The extensive review on cells in milk (Cullen, 1966) deals with type of cells, their functions, method of counting and related subjects. The terms somatic cell count and leucocyte count were distinguished by Schalm and Lasmanis (1968). Apart from somatic cells, cell fragments are also present in milk. The shape, size and internal structure of these fragments in bovine milk vary from fragments in goat's milk (Brooker, 1978). In an extensive review, Munro *et al.* (1984) highlighted the effects of mastitis on processing properties of milk and on the yield and quality of milk products. The importance of somatic cells as indicators of mastitis was pointed out by Rossi (1993).

2.2. Somatic cell count and mastitis

Out of different forms of mastitis, existence of inflammation in the absence of gross signs of the inflammation is referred as subclinical form. This form can be detected only by tests for demonstration of products of inflammation (WBC, fibrin clots etc.) or by changes in physical and chemical nature of secretion (Schalm *et al.*, 1971).

Mastitis control programme involving teat dipping, correction of faulty milking machines and infusion of intramammary antibiotics at drying off resulted in increased milk yield, improvement in total solids content and reduction in incidence of clinical mastitis in cows(Brander, 1975). Paape and Corlett (1984) recommended use of an intramammary device containing copper to increase somatic cell count and to prevent infection of quarter. Such devices will not lead to hydrolytic rancidity of milk. Schukken *et al.* (1990) noticed a higher incidence of disease associated with poor cubicle cleanliness, leaky teat etc. Robinson (1993) published the classification of mastitis by International Dairy Federation based on somatic cell count. The protective role of somatic cells against udder infection was explained by Kehrli and Shuster (1994).Harmon (1994) described the pathogenesis of mastitis and how the somatic cell count increased during an infection. He concluded that reduced synthetic activity, compositional changes and elevated somatic cell count are the series of events in subclinical mastitis. Infected cow's mammary secretion before calving also showed higher somatic cell count (Hallberg *et al.*, 1995). Subclinical mastitis was defined as a minimal period of one week in which the somatic cell count was less than 5 lakh cells/ml (Nielen *et al.*, 1995). Biju (1996) detected subclinical cases of mastitis and cultured positive milk samples. He found that most predominant causative organism was staphylococci comprising of coagulase positive and coagulase negative groups. The avearge somatic cell count of infected milk samples in his research work was 0.9×10^5 cells/ml. Kirk *et al.* (1996) have expressed a controversial opinion that somatic cell count is not a sensitive or specific measure of infection status in subclinical mastitis. In the dairy industry, bovine mastitis is the most economically important disease and estimates indicate

economic losses exceed 2 billion US $/ year in the USA and 200 million NZ $/year in New Zealand (Buddle, 1997). A study conducted in the state of Madhya Pradesh by screening 400 lactating cows revealed that 49.75 per cent of the animals were subclinically infected and out of total quarters examined 23.1 per cent were affected with subclinical mastitis (Tiwari, *et al.*, 2000). Ibraheem Kutty *et al.* (2000) conducted a cross sectional survey among farmers in Kerala and found that complete cure of mastitis was obtained only with 51 per cent of clinical cases due to development of microbial resistance. Vijayan and Umashankar (2000) investigated incidence and aetiology of clinical mastitis in cows and concluded that mastitis had a significant association with season.

2.3. Somatic cell count and tests for mastitis

There are some cow side tests for detecting subclinical mastitis based on high somatic cell count in mastitic milk. Most widely used tests are California Mastitis Test (CMT), Wisconsin Mastitis Test (WMT) and Whiteside test. The CMT is dependant upon a reactivity of reagent with deoxyribonucleic acid (DNA) and a CMT reaction 3 indicated more than 5 X 10^6 cells/ ml of milk while a negative reaction indicated less than 0.15 X 10^6 cells/ ml of milk (Schneider and Jasper, 1964). According to Thompson and Postle (1964) Wisconsin mastitis test (WMT) is a better indirect indication of somatic cell count than CMT because in latter only nucleated cells will contribute to gel formation. Schalm *et al.* (1971) described various modifications of California Mastitis Test (CMT). Kitchen *et al.* (1980) recommended N-acetyl-β-D-glucosaminidase test as most suitable for measuring mammary gland epithelial damage. Biju (1996) detected subclinical mastitis using

CMT and found that 22.23 per cent of the milk samples were infected. He reported a direct correlation between somatic cell count and CMT scores. Electrical conductivity/ resistance of milk can also be tested for the presence of infection, but Seguya and Mansell (2000) opined that this is of limited use because of overlapping of readings between infected and non-infected quarters.

2.4.Somatic cells and herd improvement

There are contrary reports indicating ineffectiveness of selection of sires based on somatic cell count because the trait showed low heritability (Kennedy *et al.*, 1982b).The possibility of creating a selection index incorporating udder health as a criterion was pointed out by Jahnke *et al.* (1990). Sire evaluation based on daughter performance (progeny records) with respect to somatic cell count was suggested by Banos and Shook (1990).The genetic evaluation of bulls based on somatic cell scores of their progenies for reducing mastitis incidence was discussed by Cassell (1994). Selection based on somatic cell count was more efficient than selection based directly on clinical mastitis (Philipsson *et al.*, 1995). Bahr *et al.* (1995) found antagonistic genetic correlation between somatic cell count and milk yield while phenotypic correlation between milk ability and somatic cell count was low.

2.5.Legislation on somatic cell count

Jones (1986) expressed a hope that lowering of acceptable limit of somatic cell count from 1.5 million/ml to 1 million/ml by FDA will reduce chances of mixing of mastitic milk with bulk milk. The Quebec federation of milk producers

(France) has implemented a programme for bringing down somatic cell count in which farmers who supply milk which has a somatic cell count > 75,000 cells/ml will face a penalty (Lacroix, 1993) . As per European Union (EU) regulation Bulk milk tank somatic cell count (BMTSCC) of a farmer should not exceed 4lakh cells/ml for receiving full payment(Nielen *et al.*, 1995).

The United Kingdom Dairy products (Hygiene) regulations (1995) require that whole milk intended for processing into milk based products shall have a plate count at 30°C of ≤ 100,000 CFU/ ml and somatic cell count of ≤ 400 000 cells/ml. (Early, 1998). Kroll *et al.* (2000) noted the microbiological classification of raw milk and cytological classification followed by dairy co-operatives in Poland as per the directive of European Economic Community (EEC).

2.6.Estimation of somatic cell count

An approved method for counting somatic cells by staining milk smear was devised by Prescott and Breed (1910) which is known as Direct microscopic somatic cell count (DMSCC). It is only a reference method known as "Breed method". Paape *et al.* (1963) were of the opinion that DMSCC is not a practical method because it is necessary to make approximately 200 smears from a milk sample to obtain reasonable accuracy. Paape *et al.* (1965) estimated the coefficient of variation for DMSCC as 19 per cent which was higher than other methods of somatic cell count .Standard procedure for DMSCC approved by National Mastitis Council was described by Brazis (1968). Later International Dairy Federation published a similar standard procedure (Anon, 1984).Possible errors inherent in

Breed method was explained by Smith (1969). Pettipher and Rodrigues (1981) improved DMSCC by filtering milk and staining cells concentrated on membrane using fluorescent acridine orange dye. Vines *et al.* (1986) noted variability among laboratories in estimation of somatic cell count, fat per cent and protein per cent.

Introduction of electronic cell counters lead to rapid and accurate counting of somatic cells. Different models like Coulter counter, Fossomatic counter are available. Pearson *et al.* (1974) and Greer and Pearson (1976) described factors affecting somatic cell count by Coulter counter. Schmidt (1975) obtained a correlation coefficient of 0.922 between electronic cell counter and DMSCC. Chances for counting cytoplasmic particles as somatic cells are high in electronic counters (Vassiliadou *et al.*, 1992).Electronic counters show good repeatability of counts but reproducibility of counts are low (Faust and Timms, 1995). Cost of the instrument is very high and often not affordable by the industry.

Paape *et al.*(1965) compared different methods for somatic cell count and they recommended Feulgen-DNA-reflectance spectrophotometry as a reliable method. Membrane Filter-DNA (MF-DNA) method is a simple, precise and less expensive method for estimation of somatic cells. Many scientists have suggested procedures based on this principle. Hutjens *et al.* (1970) standardised a method using diphenylamine as colour developing reagent. Ward and Schultz (1973) replaced diphenylamine with indole reagent for better results. Bremel *et al.* (1977) suggested use of tetrasodium salt of Ethylene Diamine Tetra Acetic acid (EDTA) along with detergent in the above procedure. Sathian and Mukundan (2000) modified MF-DNA method by including

trichloroacetic acid for solubilising DNA and incorporating a standard curve using pure DNA. This procedure gave a correlation coefficient of 0.95 between optical density and DMSCC against 0.94 reported by Hutjens *et al.* (1970).

2.7.Preservation of milk sample and somatic cell count

Schmidt (1975) reported potassium dichromate (0.05 per cent) as a good preservative for milk samples for counting with electronic counters while freezing of samples resulted in large variation in somatic cell count by this method.Clarke *et al.* (1996) showed that samples for somatic cell count can be preserved with bronopol for 10 days under refrigeration even though IDF has stipulated a maximum storage period of 3 days. Sathian and Mukundan (2000) did not find any significant variation when cells were estimated by MF-DNA method in frozen samples of milk.

2.8.Management practices and somatic cell count

Implementation of mastitis control measures will reduce somatic cell count. Mc Donald (1970) recommended washing the udder with running tap water, drying with paper towels and teat dipping with chlorhexidine as the best management practice for preventing infection. Moxley *et al.*(1978) concluded that two important practices reducing somatic cell count are teat dipping and mopping the udder to dryness before milking. They arrived on this conclusion from results of a survey by Quebec Dairy Herd Analysis Service (DHAS, France). This survey indicated that only 50 per cent farmers used a teat cup, 55 per cent used a separate towel for drying , 18 per cent dried udder after washing, 35 per cent rinsed teat cups in a disinfectant and 50 per cent used a teat dip . Hoare *et al.* (1979) also made similar

observations. McKinnon *et al.* (1983) compared the effect of different methods of teat washing on bacterial count of milk. They found that washing the teat with sodium hypochlorite and drying with paper towels gave best results. In Northern Ireland the Milk Marketing Board could reduce the somatic cell count in the farmer's milk by comprehensive mastitis advisory programme (Taylor, 1991).

The influence of three milking machine factors namely vacuum fluctuations, vacuum level and pulsator rate on somatic cell count were studied by Olney *et al.* (1983) and they concluded that these factors did not influence somatic cell count in case of uninfected cows. Dry cow therapy is considered to be an important measure to reduce somatic cell count in milk (Macmillan *et al.*, 1983). Management factors like milking practice, housing, bedding and manure handling are also influencing somatic cell count (Reneau, 1986). Wichtel *et al.* (1994) considered administration of intraruminal selenium pellets helpful in reducing somatic cell count and improving milk yield and composition. Maintenance of milking machine was considered to be important in controlling BMTSCC by Fenlon *et al.* (1995). Burmeister *et al.* (1995) noticed higher incidence of teat chapping when no conditioner was used in iodophore teat dip. Reneau (1997) pointed out that attachment of milking machine only after proper milk let down as well as removal of teat cups soon after milking will reduce somatic cell count and chances for new infection.. Segregation or separate milking of cows that were positive for *Staphylococcus aureus* mastitis by microbiological tests reduced prevalence of infection from 29.5 per cent to 16.3 per cent . This practice reduced BMTSCC from 600,000 to 345,00 cells/ml (Wilson *et al.*, 1995).

2.9.Other factors affecting somatic cell count

Stage of lactation, stress conditions, age, season and diurnal variation are reported to have influence on somatic cell count . The loss of mammary cells during advancing lactation from normal udders was reported by Tucker (1969) and he also opined that trophic hormones released from pituitary during milking are beneficial in mammary cell maintenance. But Duitschaever and Ashton (1972) could not find any relationship between age of the cow and somatic cell count . Bodoh *et al.* (1976) prepared statistical models involving factors affecting somatic cell count in milk. They found that somatic cell count is influenced by season, age of the cow and stage of lactation. Lin and Chang (1994) fully agreed with the above finding. Emmanuelson (1988) reported that somatic cell count changed with lactation period and lactation number. Within a lactation somatic cell count was highest shortly after calving which rapidly declined within 25 to 45d and then showed an inclining trend throughout remainder of lactation (Kennedy *et al.*, 1982a). Schultz *et al.* (1990) prepared lactation curves for somatic cell count , protein per cent and fat per cent . They found that somatic cell count increased with parity while fat per cent and protein per cent decreased with parity. It is noteworthy that the influence of stage of lactation on somatic cell count was not at all proved in the experiments done by Paape *et al.* (1979); Duitschaever and Ashton (1972) and Natzke *et al.* (1972). But in an uninfected udder these factors have limited influence and fail to produce a significant impact on somatic cell count (Harmon, 1994). Gonzalo *et al.*(1994) included parity and type of birth in addition to abovesaid factors as affecting somatic cell count .

Paape *et al.* (1973) could not find any relation between heat stress and somatic cell count of uninfected animals. According to (Kennedy *et al.*, 1982a) and Nelson *et al.* (1967) there was a profound influence for season on somatic cell count and they observed increased frequency of high somatic cell count samples in summer. Cullen (1968) noticed a periodic rise and fall in somatic cell count in weekly milk samples from infection free udders of cows and recorded a fall in cell counts during first two weeks of lactation and count reached maximum towards end of lactation. It is suspected that there is a physiological mechanism involving regular desquamation of epithelial cells. Environmental heat stress caused a modest increase in somatic cell count and such cows showed a correlation between concentration of neutrophils in blood and somatic cell count of milk. According to Arave *et al.*(1974) crowding had no effect on somatic cell count and the count was higher for cows having more lot size. Moore *et al.* (1980) compared somatic cell count of foremilk, midstream milk and strippings and found that strippings contained more cells compared to foremilk. Other scientists like Schalm and Lasmanis (1968); Smith and Schultz (1967) and Nader *et al.* (1995) also agreed with above finding.Wegner *et al.* (1976) also have reported similar findings. In contrary to above findings Harmon (1994) could not find any influence for age, stage of lactation, season and various stresses on somatic cell count, but he reported some diurnal variation on somatic cell count . Hamann (1993) also could not find any effect on somatic cell count by stress. Guidry *et al.* (1975) reported that oestrus did not affect somatic cell count .

Stresses like isolation or chasing by a dog increased somatic cell count of

milk from individual quarters with previous history of infection (Whittlestone *et al.*, 1970). Teat end shape and teat morphology have got influence on somatic cell count of milksample according to Slettbakk *et al.* (1990); Seykora and McDaniel(1985). Administration of agents like ACTH in cows increased number of circulating leucocytes but it did not influence their number in milk (Paape *et al.*, 1974). But according to Wegner *et al.*(1976) corticotropin injection brought about a modest increase in somatic cell count. Variation in somatic cell count is possible with time of sampling. Strippings contained more cells compared to foremilk. (Smith and Schultz, 1967) and the cell count showed a decreasing trend with ageing of sample (Kennedy *et al.*, 1982a).

2.10.Effect of somatic cell count on milk yield

Philpot (1967) found out a correlation between CMT results and losses in milkyield. He noticed a reduction of percentages of 2.8, 11.4 , 25.6 and 45.5 in milkyield when CMT scores were T, 1, 2 and 3 respectively.Duitschaever and Ashton (1972) were unable to find any relationship between milk yield and somatic cell count. Raubertas and Shook (1982) estimated loss in milk production associated with subclinical mastitis and the average values were 135 kg in first lactation and 270 kg in all other lactation for unit increase in log somatic cell count . Anderson (1982) estimated that the average loss of 259 litres per cow per lactation when cell count was over 1000 X 10^3. Similar findings were reported by Kennedy et al(1982b), Jones (1986) and Robinson (1993). Kirk (1984) developed a programmable calculator programme for estimating loss of milk yield attributable to mastitis from somatic cell count. Jones *et al.* (1984) discovered that decrease in

milk yield was linear with increase in somatic cell count for herds averaging below 7700 kg milk. Daily reduction in milk yield was 0.5kg and 0.7kg for first and later lactations respectively when somatic cell count increased from 2X 10^5 to 4 X 10^5 cells per ml (Batra, 1986). Jones (1986) postulated that on an average 15 per cent reduction in milk yield is possible when somatic cell count was above 1 million/ml. According to Robinson (1993) reduction in milk yield was 10 per cent and 30 per cent when somatic cell count were 50,00,00 cells/ml (subclinical mastitis) and 10,00,000 cell/ml (clinical mastitis) respectively.

2.11.Effect of somatic cell count on milk composition

Asby *et al.* (1977) noticed a variation in milk solids content contributed by variation in somatic cell count and they opined that maintenance of low cell count will be beneficial in increasing milk solids in herd milk. Jones (1986) reported following changes in milk composition when cell count was higher in milk. He could notice a decrease in percentages of fat, SNF, casein, potassium and lactose while a corresponding increase was noticed in contents of total protein, whey protein, sodium and chloride in the above said milk samples.

2.11.1.Total solids:- Ashworth *et al.* (1967) reported a significant decrease of 1.07 per cent in total solids in association with subclinical mastitis. Schultz (1977)quantified the total solids per cent of high somatic cell count milk samples as 92 per cent of the normal. Asby *et al.* (1977) estimated a reduction of 0.0266 units in total solids for each lakh rise in somatic cell count.

2.11.2. Milk fat:- Ashworth *et al.* (1967) reported a decrease of 0.45 per cent in fat

content in association with subclinical mastitis. The effect of mastitis on milk fat per cent is not clear in that researchers vary in their finding from negative correlation between somatic cell count and fat per cent (King, 1978) to no correlation (Ingr, 1973); Kennedy *et al.*(1982b) and to low positive correlation (Ng-Kwai-Hang *et al.*, 1982). Schultz (1977)quantified the fat content of high somatic cell count milk samples as 88 per cent of the normal. Asby *et al.* (1977) noticed possibility of 4 per cent variation in fat contributed by variation in somatic cell count. Robinson (1993) noticed a 7 per cent reduction in fat yield during subclinical mastitis. But a recent report showed an increase in fat content for increase in somatic cell count of milk (Cooney *et al.* 2000).

2.11.3. Fat composition:- There are reports indicating following changes with mastitis a) Reduction in number of fat globules. b) changes in composition of fat globule membrane c) fatty acid composition . Bachman *et al.* (1988) reported that milk with high somatic cell count showed higher acid degree value indicating increased fat hydrolysis.

2.11.4. Solids-not-fat(SNF):-The reduction in SNF in association with mastitis is well known. McKenzie *et al.* (1958) found that most of the cows showing SNF <8.5 per cent had cell counts > 2.5 lakhs/ml. Ashworth *et al.* (1967) reported a decrease of 0.57 per cent in SNF in association with subclinical mastitis. Asby *et al.* (1977) noticed a 11-15 per cent variation in SNF contributed by variation in somatic cell count. Slater (1991) noticed a reduction of SNF by 0.2 per cent during subclinical mastitis, mainly due to drop in lactose content.

2.11.5. Milk protein:- Total protein content was not affected in studies of Schultz (1977) while some workers reported a significant positive correlation between cell count and total protein content (Weaver and Kroger, 1977; Kennedy *et al.*, 1982b). Ng-Kwai-Hang *et al.* (1980) considered somatic cell count as an important factor influencing protein content and composition of milk. Ng-Kwai-Hang *et al.* (1982) noticed a slight increase of 0.099 per cent in total protein content with unit increase in log somatic cell count.

2.11.6. Casein:- Weaver and Kroger(1977) could not find any correlation between somatic cell count and casein content of milk. Schultz (1977) quantified the casein content of high somatic cell count milk samples as 82 per cent of the normal.The total casein content generally declines with increasing cell count. Ng-Kwai-Hang *et al.* (1982) noticed a slight decrease of 2.79 per cent in casein content with unit increase in log somatic cell count . The loss of casein was upto 9 per cent during subclinical mastitis as reported by Robinson (1993). Among fractions of casein α-casein and β-casein showed a decreased concentration while content of γ-casein and κ-casein increased with mastitic infection.

2.11.7. Whey proteins:- Whey protein concentration increases during mastitis because the permeability barriers between blood and milk breaks down leading to entry of greater amounts of blood proteins into milk. Singh and Ganguli (1975b) studied changes in the whey protein fractions and concluded that their content is lower in mastitic milk. Schultz (1977) quantified the whey protein concentration of high somatic cell count milk samples as 162 per cent of the normal. Serum albumin level increases with mastitic infection and a positive correlation of 0.80 is reported

between cell count and concentration of this protein fraction in milk (Kitchen *et al.*, 1980). Ishikawa *et al.* (1982) noticed a 56 per cent increase in total whey protein per cent as CMT score increased from 0 to 3. Ng-Kwai-Hang *et al.* (1982) and Weaver and Kroger (1977) found a positive correlation between cell count and serum protein per cent and they estimated a 0.16 per cent increase in serum protein concentration for each lakh increase in cell count. Concentration of α-lactalbumin and β-lactoglobulin decreased while that of immunoglobulins increased with increasing cell count (Ishikawa *et al.*, 1982). Increased proteolytic activity was observed in milk with high cell count (Derham and Andrews, 1982).

2.11.8. Lactose:- The concentration of lactose decreases with mastitis. Ashworth *et al.* (1967) reported a decrease of 0.77 per cent in lactose in association with subclinical mastitis. Janzen (1970) estimated a decrease of 0.1 per cent lactose for each unit increase in CMT. Renner(1975) suggested that decreased concentration of lactose is an indicator for mastitis with a threshold level of 4.6 per cent . Walstra and Jenness (1984) noticed that reduction in lactose content is the best indicator of mastitis than somatic cell count.A massive loss of 10 per cent in lactose content was observed by Robinson (1993) during subclinical mastitis.

2.11.9. Minerals in milk:- Calcium which is significant in rennin coagulation decline in concentration with mastitis. Singh and Ganguli(1975a) reported a lesser level of calcium and phosphorus in micellar casein during infection. Higher concentration of chloride ions in milk is regarded as an indicator for mastitic infection and many researchers have confirmed this fact. Sodium ion level also change in line with chloride ions and are a function of the need to maintain osmotic balance. Schultz

(1977)quantified the chloride , sodium and potassium content of high somatic cell count milk samples as 161 per cent , 136 per cent and 91 per cent respectively of the normal.

2.11.10. Milk enzymes:- Schultz (1977) quantified the lipase content of high somatic cell count milk samples as 116 per cent of the normal. The levels of many enzymes in milk increase as a result of mastitis and these changes have found application in the design of routine diagnostic tests for mastitis (Kitchen, 1981). The important enzymes which show a significant positive correlation with CMT or somatic cell count are N-Acetyl-β-D-glucosominidase(NAGase), catalase, xanthine oxidase and lipase. Berning *et al.* (1987) assayed different milk fractions for NAGase and they found that there was no significant difference in somatic cell count or NAGase activity between foremilk and bucketmilk in uninfected cows.

Fitz-Gerald *et al.* (1981) postulated that higher free fatty acid (FFA) content of mastitic milk was not due to increased lipase activity but due to higher FFA levels on secretion of that milk. Literature shows evidence for increased activity of alkaline and acid phosphatase, aldolase, GOT, lactate dehydrogenase and different esterases in mastitic milk. Somatic cells are having higher plasminogen activator(PA) activity during mastitis. This will be activating inactive plasminogen in milk to plasmin (Gilmore *et al.*, 1995; Politis and Ng-kwai-hang, 1989). When somatic cell count increased from 50,000/ml to 1000000/ml, PA activity increased eight fold (Zachos *et al.*, 1992). There is a PA inhibitor (PAI-1) in the mammary epithelial cell lines as reported by Zavizion *et al.* (1996). During mastitis, epithelial cell damage may lead to decrease in inhibitor activity.

2.11.11. Other compositional changes:- Glycogen, glucocorticoids and lactoferrin showed an increasing trend with increasing cell count while riboflavin and ascorbic acid show a decline in concentration.

2.12.Properties of milk:- Electrical conductivity was greatest in fore milk, less in composite milk and least in strippings which can have a correlation with somatic cell count (Jackman *et al.* ,1980). An important physical change in mastitic milk is elevated pH and it is the basis for many cowside tests for mastitis like CMT. It is a well known fact that mastitic milk is alkaline in reaction (Barry and Donnelly,1981). Schultz (1977)observed the pH of high somatic cell count milk samples as 105 per cent of the normal. He also noticed an increase in acid degree value (fat hydrolysis) of milk by 183 per cent . Similar reports were published by Bachman *et al.* (1988). Marschke and Kitchen (1982) found an increase of pH from 6.75 to 7.51 when cell count increased from 843 X 10^3 to 3788 X 10^3.

2.13.Effect of somatic cell count on fluid milk quality

Nielen *et al.* (1995) noted that a single cow with a period of elevated somatic cell count may cause a rise of bulk milk tank somatic cell count (BMTSCC) level. The control of somatic cell count in bulk milk is possible only by stringent legislation at production and processing of milk. Lowering of acceptable limit of somatic cell count from 1.5 million/ml to 1 million/ml by FDA will reduce chances of mixing of mastitic milk with bulk milk (Jones, 1986). The proportion of free fatty acids in milk, measured by acid degree value (ADV) increases with mastitis contributing to rancidity in milk and its products (Agarwal and Narayanan, 1976).

Lipolysis in mastitic milk on storage is increasing upto a cell count of 1000 X 10^3 and above that threshold lipolysis was decreasing probably due to breakdown of lipoprotein lipase at high cell counts (Jurczak and Sciubisz, 1981). According to Csapo *et al.* (1995) subclinical mastitic milk contained more D-amino acid than normal raw milk thereby reducing digestibility, bioavailability and form some toxic products.

2.14.Influence of somatic cell count on processing of milk and Dairy products

2.14.1.Pasteurised milk:- Janzen (1972) noticed a highly significant correlation between somatic cell count and flavour scores of pasteurised milk. The milk from cows infected with streptococcal mastitis (somatic cell count ≥ 750,000) lacked freshness, was unclean, was less sweet and smelled differently from normal milk (Jones, 2000).

2.14.2.Homogenised milk:-The clarification of milk is an important step before homogenisation to avoid sedimentation (brown sediment at the bottom of package). This sediment primarily contains somatic cells (Harding, 1994).

2.14.3.UHT milk :- Age gelation in UHT milk started earlier with high BMTSCC milks due to elevated proteolytic activity (Auldist *et al.*, 1996).

2.14.4.Evaporated milk:- Altered heat stability of subclinical mastitic milk lead to problems in manufacture of evaporated milk (Feagan *et al.*, 1966).

2.14.5.Cream:- Needs *et al.* (1988) prepared cream testing 38 per cent fat using

milks containing different levels of inclusion of high somatic cell count milk. It was noticed that whipping time and stiffness increased and overrun decreased as proportion of high somatic cell count milk increased.

2.14.6.Butter:- Jones (1986) reported lesser content of milk fat globule membrane in butter made from high somatic cell count milk which is a protective agent against lipolysis. Lipolysis of milk fat produces free fatty acids which act as foam depressants which depress whipping properties of cream (Harding, 1994).The literature cites instances of slower acid development, flavour defects and longer churning time when butter was made from mastitic cream. Such product showed rapid deterioration on storage.

2.14.7. Cultured dairy products:- In milk there are some natural inhibitors (antibodies) which are active against certain lactic acid bacteria. It was postulated that these factors are transferred to milk from blood (Randolph and Gould, 1968). This may be one important reason for slower acid production and flavour production when mastitic milk was used for propogation of lactic acid bacteria. It was observed that diacetyl production is inhibited thereby impairing flavour in yogurt when milk with somatic cell count above 5×10^5 cells/ml was used. (Harding, 1994).

2.14.8. Cheese :- There are so many reports on influence of mastitic milk on cheese because mastitis affects many properties of milk which are important in cheese manufacture including rennet clotting time, curd firmness, whey expulsion and rate of acid development. The mastitic milk being alkaline will lead to increased clotting time and Ali *et al.* (1980) noticed a positive correlation betwee

clotting time and somatic cell count . These workers also noticed a reduction in yield of cheese when milk with subclinical mastitis was used for cheese manufacture possibly due to lower solids-not-fat. Rate of curd firming was slow with mastitic milk. Grandison and Ford(1986) prepared cheddar cheese from milks containing different levels of cells. They noticed a reduction in coagulum strength and an increase in moisture content with increase in somatic cell count. Cooney *et al.* (2000) noticed low solids content in Swiss cheese prepared from mastitic milk and the protein losses in whey was higher in this case.

Organoleptic quality of cheese is also affected by mastitic milk . Increased proteolytic activity was observed in milk with high cell count and this led to problems in cheese making (Derham and Andrews, 1982). Politis *et al.* (1989) noticed the aberrations in coagulation for cheese due to increased plasmin activity in high cell count milk. This is reflected in the loss of protein in whey and lower solids in cheese made from such milk as reported by Politis and Ng-kwai-hang(1988a). Same authors have published another report indicating increased rennet clotting time and slower rate of curd forming with higher somatic cell count (Politis and Ng-kwai-hang, 1988b). Similar observations were also made by Grandison and Ford (1986) and they concluded that even a small increase in somatic cell count is detrimental to cheese composition and quality. Camembert and Tilsit cheeses made from milk with high somatic cell count had a bitter flavour which becomes more pronounced during ripening (Harding, 1994).

2.14.9.Dried milk :- Feagan *et al.* (1966) reported problems in manufacture of skim milk powder while milk contained varying proportions of subclinical mastitis milk. Abbot *et al.* (1974) found out that high somatic cell count milk will lead to lower keeping quality of spray-dried whole milk.

2.15.Somatic cell count in milk from other species .

Conner (1979) observed a striking difference in composition of breast milk when women were affected with mastitis and he pointed out that salty milk from breast with chronic mastitis may be a cause for poor nursing. Singh and Singh (1981) examined the relationship between somatic cell count and composition of buffalo milk and noted that fat, SNF and lactose was low and chloride was more when somatic cell count was high. Dhakal and Kapur(1993) found that lactose content is low in foremilk and strippings from buffaloes infected with subclinical mastitis. They derived an inverse relationship between lactose content and somatic cell count while a positive correlation was noticed between lactose and epithelial cell count.

Peris *et al.* (1991) found that strippings contained more cells compared to foremilk in case of ewe's milk. Charon(1993) investigated the relationship between teat measurements and somatic cell count in sheep. Wu *et al.* (1985) recognised somatic cell count and CMT as the most efficient diagnostic test for mastitis in goats. Similar opinion was expressed by Lin and Chang (1994) as well as by Vassiliadou *et al.* (1992). Lee *et al.* (1994) derived a positive correlation between somatic cell count of goat milk and its chloride content. Lin and Chang (1994)

reported a positive correlation between pH, specific gravity and electrical conductivity of goat milk and its somatic cell count while a negative correlation existed with fat lactose and milk yield. Lee *et al.* (1994) noted a positive correlation between somatic cell count and NAG-ase activity of goat milk.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. Standardisation of Membrane-Filter-DNA method of somatic cell count

Twenty morning milk samples were collected from individual cows of University livestock farm (ULF), Mannuthy. The samples were analysed for somatic cells both by Direct Microscopic Somatic Cell Count (DMSCC) and Membrane Filter-DNA (MF-DNA)method of somatic cell count. DMSCC was carried out as per the procedure recommended by (Schalm *et al.*, 1971). Estimation of somatic cells was done by MF-DNA method described by Sathian and Mukundan (2000). This procedure is a modification of indole method suggested by Ward and Schultz (1973). In this modified procedure, the colour development with the cells in milk sample was correlated with colour development by pure DNA (Ex calf thymus). Using DMSCC as reference MF-DNA method was standardised. The detailed procedure is given below.

Reagents used

1. Tritron-X-100 (E.Merck, Germany) 0.1 per cent solution prepared in normal saline. (0.9 per cent sodium chloride solution in distilled water)

2. Trichloro acetic acid (E.Merck, India) 5 per cent solution in distilled water.

3. Hydrochloric acid 5N solution

4. Indole A.R.(SRL) 0.06 per cent solution in distilled water . Water is to be heated to 80°c for dissolving indole. Indole reagent is prepared by mixing together one part of indole, one part of Hydrochloric acid and two parts of distilled water.

Plate 1. MF-DNA method - membrane filtration apparatus with vacuum pump

Plate 2. MF-DNA method- modified arrangement by Sathian and Mukundan (2000).

The arrangement for filtration of milk using vacuum pump and water vacuum is shown in plates 1 and 2 respectively. The membrane filter is arranged in the syringe filter holder and a barrel of 20ml glass syringe is attached. For applying vacuum, filter holder is mounted on a suction flask with a 14 gauge needle inserted in the stopper . Side arm of the suction flask may be connected to water vacuum or to a vacuum pump. To prepare blank 15 ml of hot Tritron solution is added to syringe barrel. Vacuum is applied until the liquid is completely filtered. This membrane will serve as blank. Procedure is same for filtration of milk samples. In this case 2 ml well mixed milk sample is pippetted into syringe barrel containing hot Tritron solution and mixed with another syringe before filtration.

After filtration the membrane filters carrying cells as well as blank filter are transferred to glass tubes which can be stoppered. To each tube 5 ml of indole reagent and 3 ml of Trichloro acetic acid (TCA) was added and mixed. All the tubes were stoppered and immersed in a vigorously boiling water bath exactly for 10 minutes. They were transferred to a chilled water bath for 5 minutes. The optical density was measured at 490 nm in a Spectrophotometer. DNA content of the sample was calculated from standard curve shown in Fig 2 . From the micrograms of DNA cell numbers can be estimated on the assumption that nine microgram of DNA correponds to one million cells (Hutjens *et al.*, 1970).

Note:-

a) all reagents should be filtered through What man No.1 filter paper before use.

b) The membrane filter should be absolutely dry before transferring to test tube.

c) Filter holder and syringe barrel should be thoroughly washed between milk samples.

3.2. Somatic cell count and California Mastitis Test (CMT)

A total of 200 cows maintained at ULF, Mannuthy and Cattle Breeding Farm (CBF), Thumburmuzhy and Livestock Research Station (LRS), Thiruvazhamkunnu were screened for CMT as per the procedure suggested by Schalm *et al.*, (1971). Composition of CMT reagent was as follows.

Sodium lauryl sulphate - 4g

Teepol^{R} - 15 ml

Bromcresol purple - 0.01g

Distilled water - 100 ml

Simultaneously milk samples were collected from individual quarters of these cows and somatic cell count was estimated by MF-DNA method.

3.3. Somatic cell count and milk composition

Pooled Milk samples were collected from cows belonging to ULF, Mannuthy, ICAR Progeny testing Scheme field units at Parappur and Ramavarmapuram. The data on milk yield, age, parity and stage of lactation of these cows were also recorded. The fresh samples were subjected to analysis of somatic cell count as well as percentages of fat, TS, SNF, Protein, lactose and chloride by procedures described below.

Fat per cent:- Estimation of fat content in milk was done by Gerber method as per the procedure described in IS:1224 - Part I (1977).

Total solids per cent:- Total solidsper cent was estimated by gravimetric method described in IS: 1479-Part II, (1961).

Solids-not-fat(SNF) per cent:- Solids-not-fat (SNF) content of milk was determined by finding the difference between total solids content and fat content of milk.

Protein per cent:- Total protein content in milk was estimated by Dye-binding method described by Dolby (1961). Standardisation of method was done by the following procedure using condensed and diluted milks according to Sathian *et al.* (2000).

Ten litre of pooled milk was divided into two equal fractions, half the quantity of milk was condensed at 55-60°C, 64 cm of Hg using vacuum condenser (Anhydro,UK).

Samples were drawn at 5, 10, 15 and 20 minutes intervals. The other half was diluted in the following proportions. 10 ml milk + 0 ml water; 10 ml milk + 5 ml water; 10 ml milk + 10 ml water; 10 ml milk +15 ml water and 10 ml milk + 20 ml water. All the samples were subjected to dye binding as well as Kjeldahl estimation of total nitrogen (AOAC, 1980). Do-Dx values were calculated according to Dolby (1961) where Do= optical density of the blank and Dx= optical density of sample. A standard curve was plott ed between total proteinper cent (Kjeldahl) on Y axis and Do-Dx on X axis. Correlation coefficient 'r' was calculated as 0.951 by fitting a regression equation.

Lactose per cent :- Lactose concentration in milk was estimated by colorimetric method described by Feitosa *et al.* (1978).

Chloride per cent:- The chloride content of milk was estimated by titration method

according to Jenness(1988) by titrating milk with 0.1 N Silver nitrate solution with potassium chromate as indicator. Titre value was multiplied by 0.0355 to obtain chlorideper cent of the sample.

Koestler number:- This factor was calculated by the following formula suggested by Fox and McSweeney(1998)

$$\text{Koestler Number} .= \frac{\text{chloride per cent}}{\text{lactose per cent}} \text{ X } 100$$

Somatic cell count :- The somatic cell count was estimated by MF-DNA method according to Sathian and Mukundan (2000) as per the procedure described elsewhere.

The correlation between somatic cell count and individual chemical components of milk was analysed statistically.

3.4. Somatic cell count and milk yield

The milk yield of above animals were noticed and it was tested whether somatic cell count has any influence on milk yield by suitable statistical methods.

3.5. Influence of age, parity and stage of lactation on somatic cell count

The data on age, parity and stage of lactation of the cows from which samples were collected was recorded . The animals were divided into groups on the basis of age, parity and stage of lactation. The influence of these parameters on somatic cell count was analysed statistically by finding out correlation coefficients between milk constituents within each group. Comparative study between groups were done by Analysis of variance (Snedecor and Cochran (1967).

3.6. Influence of season on somatic cell count

The influence of seasons in Kerala as per the classification of Somanathan (1980) on somatic cell count were studied by collecting morning pooled/ bulk milk samples from milk co-operatives at Ayyapankavu, Poochatty and Ramavarmapuram at fortnightly intervals throughout the year. The samples were distributed among different seasons as follows.

Cold and wet - June, July and August.

Warm and Wet - May , September , October and November.

Warm and Dry - December and January.

Hot and dry - February, March and April.

The samples were subjected to somatic cell count by MF-DNA method. The influence of season on somatic cell count and other milk constituents was analysed statistically by finding out correlation coefficients between milk constituents within each season. Comparative study between seasons were done by Analysis of variance (Snedecor and Cochran (1967).

3.7. Relation between Somatic cell count and stage of milking.

Samples of milk were collected at three different stages of milking namely foremilk, midstream milk and strippings from 30 cows at ULF Mannuthy which are hand milked. Number of somatic cells in these samples were estimated by MF - DNA method. The cell counts in each fraction were compared using T-test to assess the relationship between them.

3.8. Impact of milk sample preservatives on somatic cell count

Six pooled milk samples of one litre were collected from Kerala Agricultural University Dairy plant. Each sample was divided into two equal fractions. Preservatives were added to each fraction at the following dose rate.

Formalin - 0.4 ml/ 100 ml of milk

Potassium dichromate -0.2 g/ 100 ml of milk.

Somatic cell count of fresh milk sample as well as samples to which preservatives were added were estimated. During the course of study it was found that preserved samples cannot be subjected to MF-DNA method of somatic cell count because the preservatives interfered with colour development and the results were erroneous. Hence somatic cell count of fresh as well as preserved samples were done by Direct microscopic somatic cell count (DMSCC). The count was repeated on samples at 3 days intervals upto 1 month. The sample preserved with potassium dichromate showed signs of spoilage from 10 days onwards because of change in colour, viscosity and cells were absent on microscopic examination. Hence somatic cell count could be taken only upto 10 days for milk samples preserved with potassium dichromate. With formalin treated samples somatic cell count was estimated upto one month at 3 day intervals. The results were statistically analysed using T-test to find out the suitability of these preservatives for milk samples meant for somatic cell count.

3.9. Influence of milking management practices on somatic cell count

Eighteen cows with high somatic cell count ($\geq 0.5 \times 10^6$ cells/ml) were

selected from the field for this study after primary screening. They were divided into three groups of six cows each. One group was subjected to mopping of the udder after washing with mild antiseptic lotion before milking while another group was subjected to post-milking teat dipping with Povidone Iodine (WOKADINE^R) at a final concentration of 0.05per cent. Third group served as control. The practices were continued for one month. Milk samples were collected before treatment on three occasions and at three day intervals during treatment period from all the cows. Somatic cell count of the samples was estimated by MF-DNA method.

3.10. Statistical analysis of data

Statistical analysis of data was done using following procedures as described by Snedecor and Cochran (1967).

3.10.1 Standardisation of MF-DNA method:- The correlation between optical density of MF- DNA method and DMSCC was tested. Correlation was also tested between optical density and μg of pure DNA for second standardisation.

3.10.2 Somatic cell count and California Mastitis Test (CMT):- The results were analysed by finding out correlation between CMT scores and somatic cell count.

3.10.3 Somatic cell count and milk composition:- Data from all the samples collected were subjected to tests for correlation between them and results are expressed as that of pooled data. Means of observations were compared by Analysis of variance. These data were classified into groups as shown below.

Fat per cent :- <3 per cent, 3 to 3.49 per cent, 3.5 to 4.5 per cent and >4.5 per cent

SNF per cent :- <8 per cent, 8 to 8.49 per cent, 8.5 to 8.99 per cent and ≥9 per cent

Somatic cell count :- < 1 X 10^5, 1 X 10^5-2.9 X 10^5, 3 X 10^5-4.9 X 10^5, 5 X 10^5-9.9 X 10^5, ≥ 10 X 10^5 cells/ml of milk.

Correlations between parameters were tested within these groups and mean values of groups were compared by Analysis of variance.

3.10.4 Somatic cell count and milk yield:- The entire data were classified into following groups depending on production potential.

Milk yield / day :- <5kg, 5-8.9kg and ≥9kg

Correlations were tested between milk constituents within these groups. Mean values of milk constituents within groups were compared by Analysis of variance.

3.10.5 Influence of age, parity and stage of lactation on somatic cell count :-

Entire data were grouped based on following criteria.

Parity of cow :-1,2,3 and 4

Age of cow:- ≤5yr, 5-8 yr and >8 yr

Stage of lactation:- Early lactation, Mid lactation and Late lactation.

Correlations were tested between milk constituents within these groups. Mean values of milk constituents within groups were compared by Analysis of variance.

3.10.6 Influence of season on somatic cell count :- Data on somatic cell count of pooled (bulk) milk samples were classified into those belonging to four seasons referred under 3.6. Correlation's were tested between milk constituents within each season. Mean values of milk constituents within seasons were compared by Analysis of variance.

3.10.7 Relation between Somatic cell count and stage of milking:- The somatic cell count of three fractions of milk collected during milking was compared by Paired 't'-test.

3.10.8 Impact of milk sample preservatives on somatic cell count :- The somatic cell count of fresh and preserved samples were compared for significant difference, if any by Student's 't'-test.

3.10.9 Influence of milking management practices on somatic cell count :- The Changes in somatic cell count brought about by management practices were compared within three groups of cows by Analysis of co-variance.

RESULTS

The results are presented under various headings. The data on all the milk samples collected were statistically analysed and observations are recorded as belonging to pooled data. These data were grouped and analysed as shown under 2.10 and results are given.

Under each heading (From section **4.3.** to **4.6.**) correlation between milk constituents and somatic cell count in pooled data is given first. Then significant relationships between different parameters within different groups of data are presented. Later results on comparison between mean values of groups by ANOVA are given.

4.1 Standardisation of MF-DNA method for estimation of somatic cell count

The MF-DNA method was modified as described in section 2.1. Modified MF-DNA method was standardised with Direct Microscopic Somatic Cell Count (DMSCC). The results of standardisation is represented in Fig. 1. as a standard curve between optical density of MF-DNA method and DMSCC for a number of milk samples. Standardisation of the modified procedure was also done between Pure DNA and optical density at 490 nm as shown in Fig 2. It was also possible to carry out standardisation in a photocolorimeter at 470 nm. The regression equations fitting to these curves and corresponding 'r' values are noted in Table 1.

4.2 Somatic cell count and California mastitis test (CMT) scores

The results of screening cows for CMT at ULF, Mannuthy; CBF,

Fig. 1. Standard curve between DMSCC and Optical density(MF-DNA)

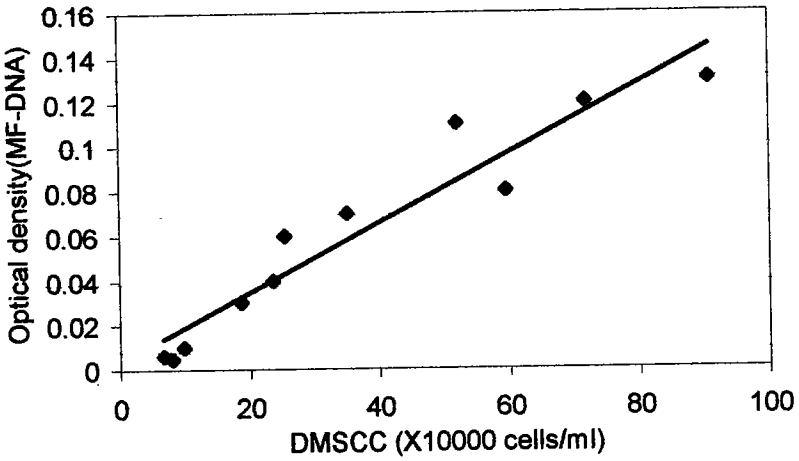


Fig. 2. Standard curve for estimation of DNA in Somatic cells (Spectrophotometer)

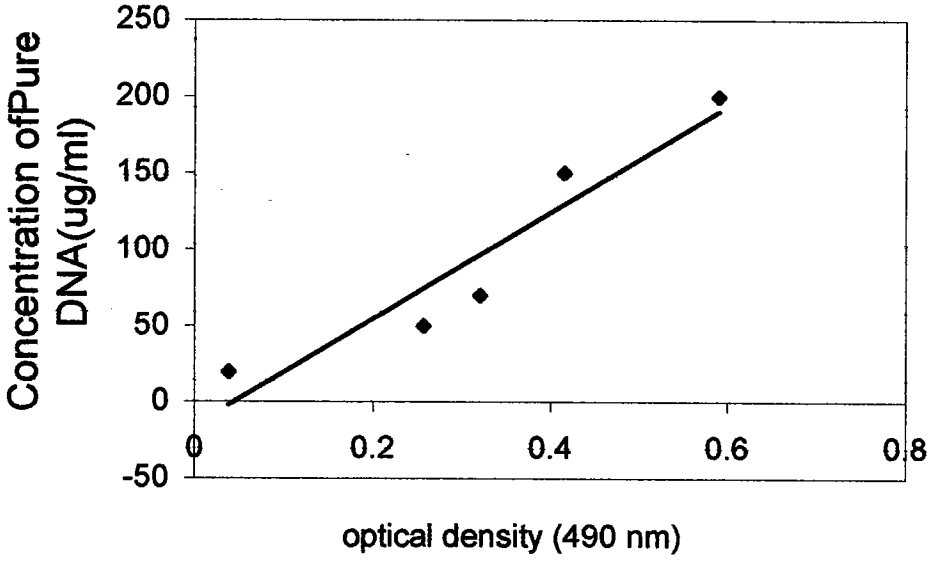


Table 1. Regression equations and 'r' values of standardization of MF-DNA method

Parameter	Regression equation	'r' value
MF-DNA method v/s DMSCC method	Y = 1.2595 + 586.7251X	0.955
DNA (μg) v/s Optical Density (490 nm)	Y = -14.8305 + 347.3848X	0.975
DNA (μg) v/s Optical Density (470 nm)	Y = -8.6593 + 1572.5521X	0.965

Table 2. Results of screening milk samples for CMT

Source	No. of cows	No. of quarters	California Mastitis Test Scores			
			0	1	2	3
ULF, Mannuthy	75	287	142	60	70	15
CBF, Thumburmuzhy	54	203	104	25	60	14
LRS, Thiruvazhamkunnu	71	279	175	40	50	14
Total	200	769	421	125	180	43

Table 2A. Average somatic cell count for California Mastitis Test (CMT) scores

CMT score	SCC# Present study	SCC# Report 1	SCC# Report 2
Zero	0.83	0.21	0.9
1	3.88	5.41	6.9
2	4.19	9.91	16.0
3	5.52	24.1	50.0

#- x 10^5 cells / ml
Report 1: Biju (1996)
Report 2: Schneider and Jasper (1964)

Thumburmuzhy and LRS, Thiruvazhamkunnu are represented in Table 2. Two hundred cows were screened and 760 quarters were tested. The correlation coefficient between CMT scores and somatic cell count was +0.626 which was significant ($P<0.01$). The average somatic cell count corresponding to each CMT score were '0' for 0.83 $X10^5$; '1' for 3.88 $X10^5$; '2' for 4.19 $X10^5$; '3' for 5.52 $X10^5$ and '4' for 8.01 $X10^5$.

4.3. Milk composition

A total of 600 milk samples were collected from ULF, Mannuthy and from field units of ICAR Progeny testing scheme . Results of analysis of these samples are given below.

4.3.1. Somatic cell count and fat percentage

Averages of milk fat per cent are represented in Table 3. and depicted in Fig. 3. A non-significant positive correlation of +0.013 was noticed between fat per cent and somatic cell count when pooled data were analysed as shown in Table 3A.

4.3.2. Somatic cell count and protein percentage

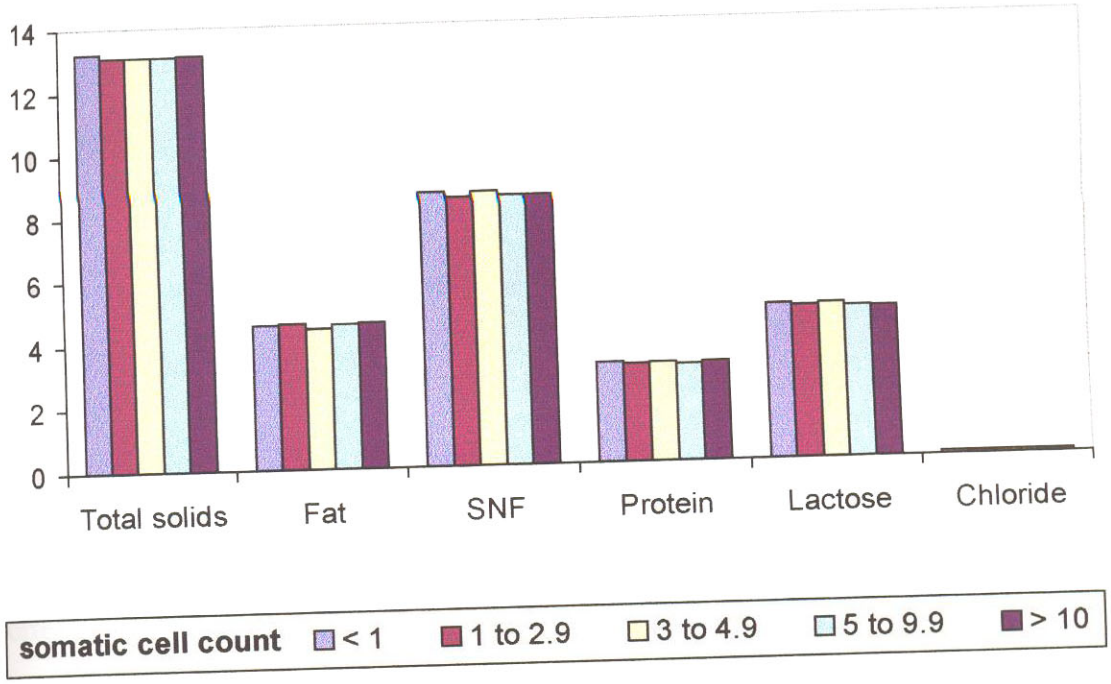
Averages of milk protein per cent are represented in Table.3. and depicted in Fig. 3. It is clear from Table 3A that a non-significant positive correlation of +0.001 was recorded between protein per cent and somatic cell count on analysis of pooled data.

Table 3. Average values of somatic cell count, milk yield and percentage of milk constituents

Source	No. of samples	SCC*	Yield (kg)	TS	SNF	Fat	protein	Lactose	Chloride
ULF, Mann uthy	433	9.18	7.29	13.23	8.59	4.64^{a}	3.13^{a}	4.81	0.106
Field units	167	11.38	4.3	12.95	8.34	4.60^{b}	3.00^{b}	4.77	0.105

* - x 10^5 cells / ml
Means bearing different superscripts are significantly different ($P<0.05$)

Fig. 3. Influence of somatic cell count# on milk constituents.



- x 10^5 cells / ml

When data were classified under different levels of milk yield as shown in Table 6A there was significant positive correlation ($P<0.05$) between milk protein per cent and somatic cell count in milk for cows yielding 5 - 8.9 kg of milk/day when data were grouped under different levels of milk fat per cent as shown in Table 4. When data were grouped under different age groups of cows there was significant positive correlation ($P<0.05$) between milk protein per cent and age for cows above 8 years of age as shown in Table 7.

4.3.3. Somatic cell count and total solids percentage

Averages of milk total solids per cent are represented in Table.3. and depicted in Fig 3. A non-significant negative correlation of -0.025 was noticed between total solids per cent and somatic cell count when pooled data were analysed as shown in Table 3A.

Total solids were negatively correlated with somatic cell count for milk samples having fat per cent between 3.5 and 4.5 as shown in Table 4 where correlation coefficient is -0.192 which is significant ($P<0.05$).

4.3.4. Somatic cell count and solids-not-fat (SNF)

Percentage

Averages of SNF per cent are represented in Table.3. and depicted in Fig. 3. A non-significant negative correlation of -0.059 was present between SNF per cent and somatic cell count on analysis of pooled data as shown in Table 3A.

Table 3A. Correlation coefficients between milk constituents in the pooled data

TS x Fat	**0.791****
„ x SNF	**0.553****
„ x Protein	**0.442****
„ x Lactose	**0.398****
„ x Chloride	0.041
„ x SCC	-0.025
Fat x SNF	-0.073
„ x Protein	-0.024
„ x Lactose	-0.072
„ x Chloride	-0.004
„ x SCC	0.013
SNF x Protein	**0.753****
„ x Lactose	**0.550****
„ x Chloride	0.072
„ x SCC	-0.059
Protein x Lactose	**0.246****
„ x Chloride	0.048
„ x SCC	0.001
Lactose x Chloride	0.021
„ x SCC	**-0.172****
Chloride x SCC	-0.002
SCC x Milk yield	-0.131*

* - $P < 0.05$
** - $P < 0.01$

Table 4. Correlation between milk constituents for milk samples under different ranges of fat per cent.

Parameter	< 3	3 - 3.5	3.5 - 4.5	> 4.5
TS x Fat	NS	NS	**0.373****	**0.553****
TS x SNF	**0.859****	**0.979****	**0.950****	**0.707****
TS x Protein	**0.809****	**0.780****	**0.738****	**0.502****
TS x Lactose	**0.859****	NS	**0.516****	**0.397****
TS x Chloride	NS	NS	NS	NS
TS x SCC	NS	NS	**-0.192***	NS
Fat x SNF	NS	NS	NS	**-0.199****
Fat x Protein	NS	NS	NS	**-0.153***
Fat x Lactose	NS	NS	NS	NS
Fat x Chloride	**-0.596***	**-0.689****	NS	NS
Fat x SCC	NS	NS	NS	NS
SNF x Protein	**0.927****	**0.789****	**0.738****	**0.721****
SNF x Lactose	**0.929****	**0.631****	**0.517****	**0.479****
SNF x Chloride	**0.559***	NS	NS	NS
SNF x SCC	NS	NS	**-0.201***	NS
Protein x Lactose	**0.873****	NS	NS	**0.156***
Protein x Chloride	NS	NS	NS	NS
Protein x SCC	NS	NS	NS	NS
Lactose x Chloride	NS	NS	NS	NS
Lactose x SCC	NS	**0.582***	**-0.294***	**-0.150***
Chloride x SCC	NS	NS	NS	NS

* - $P<0.05$
** - $P<0.01$

Table 4A. Correlation between milk constituents for milk samples under different ranges of solids-not-fat (SNF) per cent

Parameter	< 8	8 - 8.49	8.5 - 8.99	≥ 9
TS x Fat	**0.838****	**0.991****	**0.990****	**0.958****
TS x SNF	**0.419****	NS	NS	**0.321****
TS x Protein	**0.308***	NS	NS	NS
TS x Lactose	**0.603****	NS	NS	NS
TS x Chloride	NS	NS	NS	NS
TS x SCC	NS	NS	NS	NS
Fat x SNF	NS	NS	NS	NS
Fat x Protein	NS	NS	NS	NS
Fat x Lactose	NS	NS	NS	NS
Fat x Chloride	NS	NS	NS	NS
Fat x SCC	NS	NS	NS	NS
SNF x Protein	**0.491****	**0.428****	NS	**0.393****
SNF x Lactose	**0.669****	NS	**0.251****	NS
SNF x Chloride	NS	NS	NS	**0.250***
SNF x SCC	NS	NS	**-0.208***	NS
Protein x Lactose	**0.474****	**-0.573****	**-0.458****	**-0.394****
Protein x Chloride	NS	NS	NS	NS
Protein x SCC	NS	NS	NS	NS
Lactose x Chloride	NS	NS	NS	NS
Lactose x SCC	NS	NS	NS	**-0.273***
Chloride x SCC	NS	NS	NS	NS

* - $P<0.05$
** - $P< 0.01$

Table 5. Mean and SE of milk constituents under different levels of somatic cell count

Milk constituent		SCC# <1	SCC# 1 to 2.9	SCC# 3 to 4.9	SCC# 5 to 9.9	SCC# ≥ 10	Overall
Total solids	Mean	13.23	13.11	13.11	13.08	13.13	13.13
	± SE	0.171	0.157	0.171	0.174	0.120	0.071
Fat	Mean	4.56	4.61	4.44	4.56	4.61	4.57
	± SE	0.171	0.111	0.166	0.107	0.100	0.059
SNF	Mean	8.66	8.49	8.66	8.52	8.52	8.56
	± SE	0.111	0.123	0.103	0.102	0.068	0.043
Protein	Mean	3.15	3.08	3.11	3.06	3.13	3.11
	± SE	0.031	0.058	0.044	0.053	0.035	0.020
Lactose	Mean	4.87^{abc}	4.80^{abcde}	4.88^{abcd}	4.78^{bcde}	4.75^{bde}	4.81
	± SE	0.027	0.034	0.039	0.045	0.025	0.015
Chloride	Mean	0.108	0.104	0.108	0.103	0.105	0.106
	± SE	0.002	0.002	0.003	0.002	0.002	0.001

* - x 10^5 cells / ml

Means bearing different superscripts are significantly different ($P<0.05$)

Table 5A.Correlation between different parameters under different levels of somatic cell count#

Parameter	< 1 lakh	1-2.9 lakh	3-4.9 lakh	5-9.9lakh	≥10 lakh
TS x Fat	**0.796****	**0.623****	**0.814****	**0.840****	**0.821****
TS x SNF	**0.374****	**0.718****	**0.349***	**0.824****	**0.548****
TS x Protein	NS	**0.634****	**NS**	**0.700****	**0.396****
TS x Lactose	**0.273***	**0.382****	**0.345***	**0.600****	**0.376****
TS x Chloride	NS	NS	NS	**0.273***	NS
TS x SCC	NS	NS	NS	NS	NS
Fat x SNF	**-0.265***	NS	NS	NS	NS
Fat x Protein	NS	NS	NS	**0.340****	NS
Fat x Lactose	NS	NS	NS	**0.305***	NS
Fat x Chloride	NS	NS	NS	NS	NS
Fat x SCC	NS	NS	NS	NS	NS
SNF x Protein	**0.719****	**0.722****	**0.733****	**0.837***	**0.767****
SNF x Lactose	**0.474****	**0.445****	**0.587****	**0.703***	**0.541****
SNF x Chloride	**NS**	NS	NS	NS	NS
SNF x SCC	NS	NS	NS	NS	NS
Protein x Lactose	**0.286***	NS	NS	**0.421****	NS
Protein x Chloride	NS	NS	NS	NS	NS
Protein x SCC	NS	NS	NS	NS	NS
Lactose x Chloride	NS	NS	NS	NS	NS
Lactose x SCC	NS	NS	NS	NS	NS
Chloride x SCC	NS	NS	NS	NS	NS
SNF x Koestler No.	NS	NS	NS	NS	**-0.206***
SCC x Koestler No.	NS	NS	NS	NS	**0.178***

* - P<0.05
** - P < 0.01

Table 6. Mean and SE of milk constituents and milk yield for cows varying in production potential.

Milk constituent		Milk yield < 5 kg	Milk yield 5 to 8.9 kg	Milk yield ≥ 9 kg
Total solids	Mean	13.24	13.21	13.06
	± SE	0.33	0.09	0.13
Fat	Mean	4.71	4.57	4.49
	± SE	0.25	0.08	0.11
SNF	Mean	8.53	8.63	8.56
	± SE	0.24	0.05	0.07
Protein	Mean	3.08	3.16	3.08
	± SE	0.09	0.02	0.04
Lactose	Mean	4.76	4.80	4.88
	± SE	0.06	0.02	0.03
Chloride	Mean	0.116^{a}	0.104^{b}	0.105^{c}
	± SE	0.005	0.001	0.002
SCC*	Mean	11.78	9.060	7.120
	± SE	0.315	0.073	0.120
Milk yield	Mean	2.15	4.13	5.69
	± SE	0.10	0.05	0.10

*- x 10^5 cells / ml

Means bearing different superscripts are significantly different ($P<0.05$)

Table 6A.Correlation between milk constituents at different levels of milk yield

Parameter	< 5 kg	5 - 8.9 kg	≥ 9 kg
TS x Fat	**0.699****	**0.803****	**0.852****
TS x SNF	**0.658****	**0.498****	**0.503****
TS x Protein	**0.650****	**0.331****	**0.359****
TS x Lactose	**0.693****	**0.290****	**0.434****
TS x Chloride	NS	NS	NS
TS x SCC	NS	NS	NS
Fat x SNF	NS	NS	NS
Fat x Protein	NS	NS	NS
Fat x Lactose	NS	NS	NS
Fat x Chloride	NS	NS	NS
Fat x SCC	NS	NS	NS
SNF x Protein	**0.817****	**0.634****	**0.75****
SNF x Lactose	**0.770****	**0.378****	**0.509****
SNF x Chloride	**0.398***	NS	**-0.328****
SNF x SCC	NS	NS	NS
Protein x Lactose	**0.741****	NS	NS
Protein x Chloride	NS	NS	**-0.380****
Protein x SCC	NS	**0.180***	NS
Lactose x Chloride	NS	NS	NS
Lactose x SCC	NS	**-0.166***	NS
Chloride x SCC	NS	NS	NS

* - P< 0.05
** - P< 0.01

Fig. 4. Influence of milk production potential on milk composition and somatic cell count

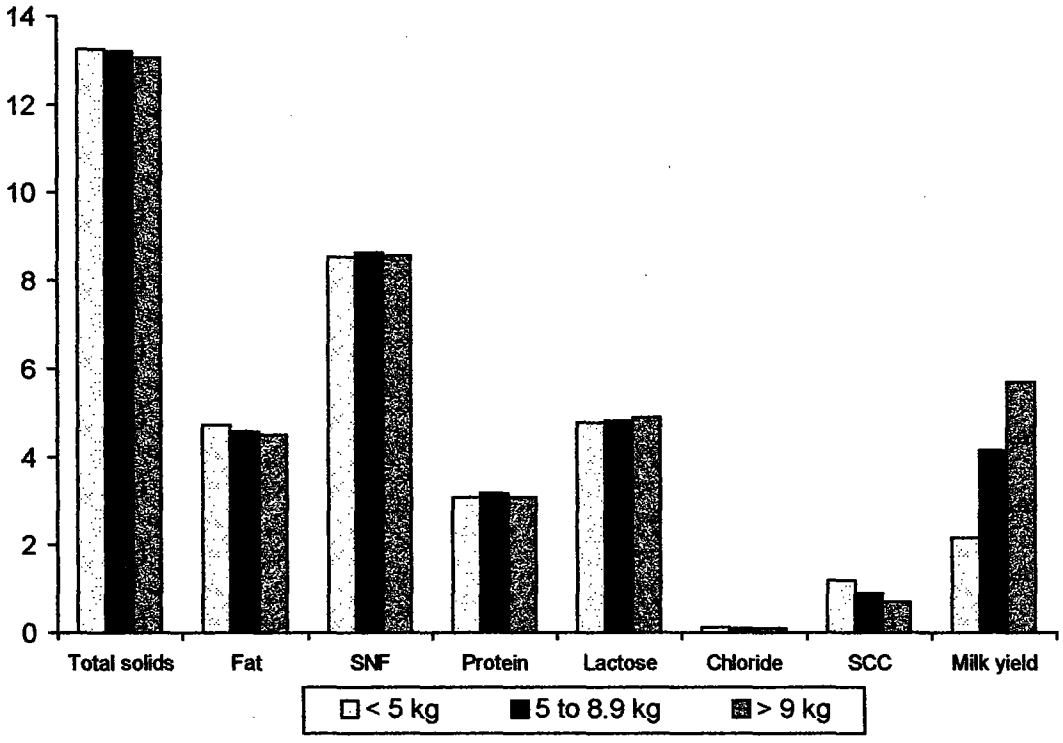


Table 7. Correlation between milk constituents under different age groups

Parameter	≤ 5	6 - 8	> 8
TS x Fat	NS	**0.785****	**0.860****
TS x SNF	NS	**0.642****	NS
TS x Protein	NS	**0.496****	NS
TS x Lactose	NS	**0.518****	NS
TS x Chloride	NS	NS	NS
TS x SCC	NS	NS	NS
Fat x SNF	NS	NS	**-0.610***
Fat x Protein	NS	NS	NS
Fat x Lactose	NS	**0.205***	NS
Fat x Chloride	NS	NS	NS
Fat x SCC	NS	NS	NS
SNF x Protein	NS	**0.767****	NS
SNF x Lactose	NS	**0.581****	**0.536***
SNF x Chloride	NS	NS	NS
SNF x SCC	NS	NS	**-0.697****
Protein x Lactose	NS	**0.267****	NS
Protein x Chloride	NS	NS	NS
Protein x SCC	NS	NS	**+0.516***
Lactose x Chloride	NS	NS	NS
Lactose x SCC	NS	NS	NS
Chloride x SCC	NS	NS	NS

* - P<0.05
**- P<0.01

Table 8. Mean and SE of milk constituents and milk yield for cows under different parity.

Milk constituent		Parity I	Parity II	Parity III	Parity IV
Total solids	Mean	13.23	13.24	12.89	13.44
	± SE	0.15	0.10	0.19	0.28
Fat	Mean	4.63	4.63	4.29	4.67
	± SE	0.14	0.07	0.16	0.24
SNF	Mean	8.60	8.60	8.60	8.77
	± SE	0.09	0.06	0.11	0.23
Protein	Mean	3.13	3.12	3.13	3.31
	± SE	0.03	0.03	0.05	0.03
Lactose	Mean	4.86	4.83	4.75	4.69
	± SE	0.02	0.02	0.03	0.06
Chloride	Mean	0.102	0.105	0.108	0.107
	± SE	0.002	0.002	0.003	0.002
SCC*	Mean	7.780	9.690	8.790	10.110
	± SE	0.114	0.108	0.132	0.253
Milk yield	Mean	4.56	4.16	4.00	4.52
	± SE	0.13	0.12	0.14	0.30

* - x 10^5 cells / ml

Table 8A. Correlation between milk constituents under different parities of cows

Parameter	I	II	III	IV
TS x Fat	**0.797****	**0.771****	**0.822****	**0.614***
TS x SNF	**0.408****	**0.646****	**0.576****	**0.575***
TS x Protein	**0.352****	**0.515****	**0.380****	NS
TS x Lactose	**0.411****	**0.371****	**0.541****	NS
TS x Chloride	NS	**0.233****	NS	NS
TS x SCC	NS	NS	NS	NS
Fat x SNF	NS	NS	NS	NS
Fat x Protein	NS	NS	NS	NS
Fat x Lactose	NS	NS	**0.318***	NS
Fat x Chloride	NS	NS	NS	NS
Fat x SCC	NS	NS	NS	NS
SNF x Protein	**0.676****	**0.742****	**0.776****	NS
SNF x Lactose	**0.365****	**0.581****	**0.494****	**0.635***
SNF x Chloride	NS	**0.250****	NS	NS
SNF x SCC	NS	NS	NS	NS
Protein x Lactose	NS	**0.213***	NS	NS
Protein x Chloride	NS	**0.181***	NS	NS
Protein x SCC	NS	NS	NS	NS
Lactose x Chloride	NS	**0.198***	NS	NS
Lactose x SCC	NS	NS	NS	NS
Chloride x SCC	**-0.238***	NS	NS	NS

* - $P<0.05$
** - $P<0.01$

Fig. 5. Influence of parity on milk composition, milk yield and somatic cell count

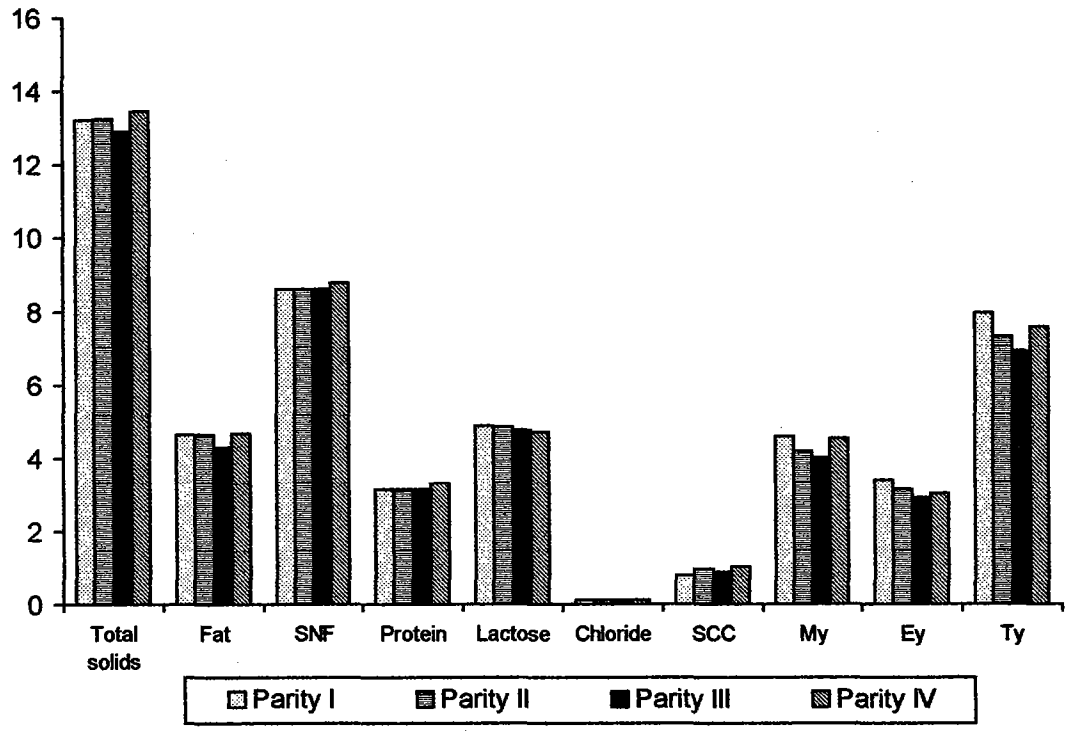


Table 9. Mean and SE of milk constituents and milk yield during different stages of lactation.

Milk constituent		Early lactation	Mid lactation	Late lactation
Total solids	Mean	12.94[a]	12.92[ab]	13.52[c]
	± SE	0.20	0.10	0.10
Fat	Mean	4.32[a]	4.39[ab]	4.86[c]
	± SE	0.18	0.08	0.08
SNF	Mean	8.61	8.53	8.66
	± SE	0.94	0.06	0.08
Protein	Mean	3.15[abc]	3.04[ab]	3.20[c]
	± SE	0.04	0.03	0.03
Lactose	Mean	4.80	4.82	4.81
	± SE	0.03	0.02	0.02
Chloride	Mean	0.108	0.104	0.105
	± SE	0.003	0.002	0.002
SCC*	Mean	9. 100	9.980	9.340
	± SE	0.126	0.133	0.090
Milk yield	Mean	4.96[a]	4.49[b]	3.69[c]
	± SE	0.15	0.11	0.11

*- x 10^5 cells / ml

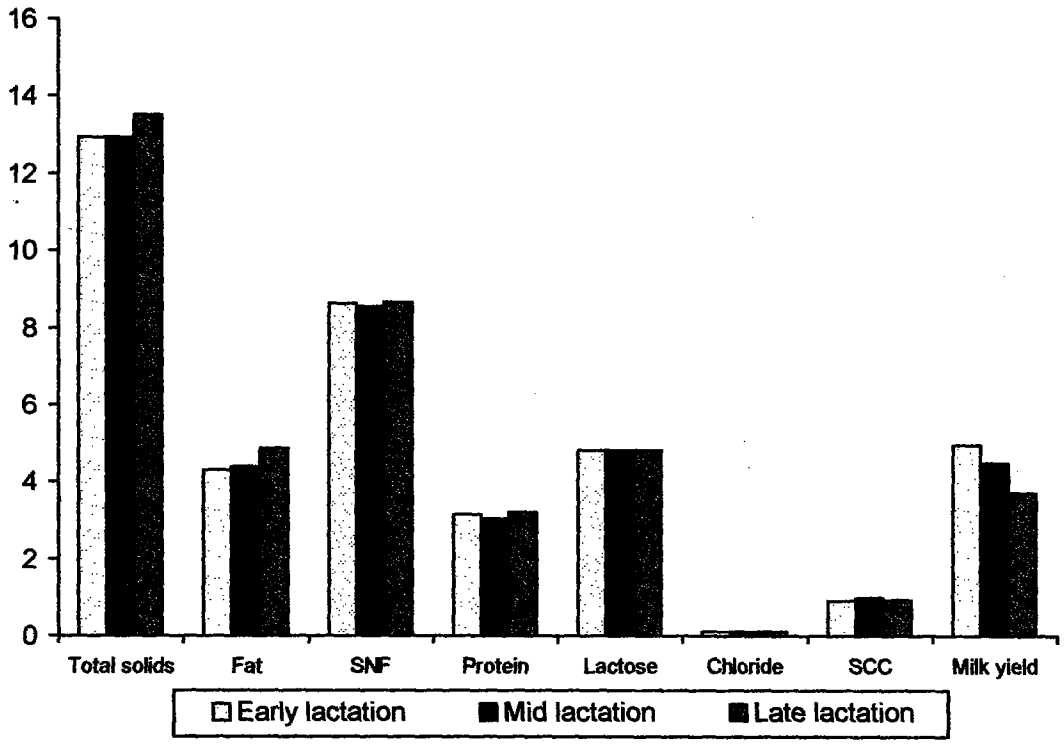
Means bearing different superscripts are significantly different ($P<0.05$)

Table 9A. Correlation between milk constituents during different stages of lactation

Parameter	Early lactation	Mid lactation	Late lactation
TS x Fat	**0.885****	**0.796****	**0.653**
TS x SNF	**0.388****	**0.598****	**0.657**
TS x Protein	NS	**0.391****	**0.584**
TS x Lactose	**0.482****	**0.402****	**0.422**
TS x Chloride	NS	NS	**0.308**
TS x SCC	NS	NS	NS
Fat x SNF	NS	NS	NS
Fat x Protein	NS	NS	NS
Fat x Lactose	NS	NS	NS
Fat x Chloride	**-0.340***	NS	**0.237***
Fat x SCC	NS	NS	NS
SNF x Protein	**0.692****	**0.719****	**0.741****
SNF x Lactose	**0.572****	**0.445****	**0.541****
SNF x Chloride	NS	NS	NS
SNF x SCC	NS	NS	NS
Protein x Lactose	NS	NS	**0.348****
Protein x Chloride	NS	NS	NS
Protein x SCC	NS	NS	NS
Lactose x Chloride	NS	NS	NS
Lactose x SCC	**-0.311***	NS	NS
Chloride x SCC	NS	NS	NS

* - $P<0.05$
**- $P<0.01$

Fig. 6. Influence of stage of Lactation on milk composition, milk yield and somatic cell count.



A significant negative correlation (P<0.05) existed between SNF per cent and somatic cell count for milk samples with a fat content between 3.5 and 4.5 as shown in Table 4. where correlation coefficient was -0.201. There was a significant negative correlation (P<0.05) between SNF per cent and somatic cell count for milk samples having a SNF content between 8.5 and 8.99 where correlation coefficient was -0.208 as shown in Table 4A.

From Table 7 it is clear that a highly significant negative correlation (P<0.01) existed between SNF per cent and somatic cell count for milk samples collected from cows above 8 years of age.

4.3.5. Somatic cell count and lactose percentage

Averages of lactose per cent are represented in Table.3. and is depicted in Fig.3. Lactose was negatively correlated with somatic cell count when pooled data were analysed with a correlation coefficient of -0.172 which was highly significant (P<0.01) as shown in Table 3A.

A significant negative correlation (P<0.05) was revealed between lactose content in milk and somatic cell count for samples from cows in their early lactation(Table 9A), cows having daily milk yield between 5 and 8.9 kg(Table 6A), cows having milk fat per cent 3 and above(Table 4) and cows having milk SNF per cent 9 and above(Table 4A). Significant negative correlation was also noticed between lactose and somatic cell count during warm and wet season (P<0.05) and during hot and dry season (P<0.01) as shown in Table 10A.

4.3.6. Somatic cell count and chloride content of milk

Averages of chloride in milk are represented in Table 3. and is depicted in Fig.3. The negative correlation of -0.002 between chloride content and somatic cell count was non-significant when pooled data were analysed.

There existed a significant negative correlation ($P<0.05$) between chloride per cent of milk and its somatic cell count in the milk from primiparous cows as shown in Table 8A.

4.3.7. Somatic cell count and Koestler number

A non-significant positive correlation of +0.07 was noticed between Koestler number and somatic cell count when pooled data were analysed.

Results of statistical analysis showed a significant positive correlation ($P<0.05$) of +0.178 between somatic cell count and Koestler number in samples having somatic cell count 10 X 10^5 cells/ml and above as shown in Table 5A. From same Table it can be understood that Solids-not -fat per cent of milk showed a negative correlation ($P<0.05$) with Koestler number in this group of samples.

4.3.8. Comparison between samples belonging to different levels of somatic cell count

Mean and standard error for milk constituents of samples with different levels of somatic cell count are presented in Table.5. Means which are significantly different on comparison by analysis of variance are denoted by

different superscripts. Results showed that lactose content in milk with cell count less than 1 x 10^5 cells/ml significantly differed from lactose content in samples with cell count 5 x 10^5 cells/ml and above. There was no significant difference between percentages of total solids, SNF, fat, protein, and chloride content between milk samples belonging to different levels of somatic cell count.

4.4. Milk yield

4.4.1. Somatic cell count and Milk yield

Average values of milk yield are represented in Table.3. A significant negative correlation of -0.131 ($P<0.05$) existed between morning milk yield and somatic cell count as shown in Table 3A .

4.4.2. Comparison between milk samples from cows differing in production potential

Mean and standard error for milk constituents of samples collected from cows with different production potential are presented in Table.6. Means which are significantly different on comparison by analysis of variance are denoted by different superscripts. Influence of milk production potential on milk composition and somatic cell count is depicted in Fig. 4. Results showed that there was significant difference ($P<0.01$) between samples belonging to above three groups of samples in their chloride content. The differences between three groups of samples in percentages of total solids, fat, SNF, protein, lactose and somatic cell count were non-significant.

4.5. Age, parity and stage of lactation

4.5.1. Somatic cell count and age of the cows.

Age had only an insignificant positive correlation with somatic cell count (+0.008) when pooled data were analysed.

The correlation between milk constituents in samples from cows having age 5 years and below, 6-8 years and above 8 years are given in Table 7. Mean and standard error of milk constituents for samples collected from cows belonging to different age groups are presented in Table 7. Means which are significantly different on comparison by analysis of variance are denoted by different superscripts.

When data were grouped under different parities of cows percentages of total solids, fat and lactose were positively correlated ($P<0.01$) with age of the cows for the primiparous cows. Total solids in milk and fat per cent had a significant positive correlation with age for cows ($P<0.05$) yielding milk between 5 kg and 8.9 kg.

4.5.2. Somatic cell count and parity of the cows

The average values for somatic cell count and other milk constituents for cows different in parity are given in Table .8. The influence on parity of the cows and different milk constituents are depicted in Fig.5. Parity had only an insignificant positive correlation with somatic cell count (+0.050) when pooled data were analysed .

4.5.2.1. Parity of the cow and milk constituents

The correlation between milk constituents in samples from cows belonging to first, second, third and fourth parities are presented in Table.8A.

Total solids was significantly correlated with fat and SNF ($P<0.01$) in all the parities. Significant correlation ($P<0.01$) was present between total solids and protein as well as lactose in parities other than fourth parity. In primiparous cows there was a significant negative correlation of -0.238($P<0.05$) between somatic cell count and chloride content of milk. From Table 8 it is clear that no significant difference existed between parities with respect to levels of remaining milk constituents.

4.5.2.2.Comparison between different parities of the cows

Mean and standard error of milk constituents for samples collected from cows belonging to different parities are presented in Table.8. Means which are significantly different on comparison by analysis of variance are denoted by different superscripts. Analysis of variance between first, second, third and fourth parities on percentages of total solids, fat, SNF, protein, lactose , chloride, somatic cell count and milk yield showed that none of these variables differed significantly between parities of the cow.

4.5.3. Somatic cell count and stage of lactation of the cows

The influence of stage of lactation on milk constituents are depicted in Fig.6. Stage of lactation had only an insignificant positive correlation with somatic cell count (+0.032) when pooled data were analysed.

4.5.3.1. Stage of lactation and milk constituents

The correlation between milk constituents in samples from cows belonging to early, mid and late lactations are presented in Table.9A. Total solids had a significant correlation with milk solids other than protein ($P<0.01$) in all the three stages of lactation. Milk protein had no significant correlation with total solids during early lactation. SNF had significant correlation ($P<0.01$) with milk protein and lactose in all the three stages of lactation. The somatic cell count had a negative correlation of -0.311 with lactose content of milk during early lactation($P<0.05$). Protein and lactose contents were positively correlated ($P<0.01$) only during late lactation. The correlation coefficient between stage of lactation and milk fat per cent was +0.263 which was significant ($P<0.01$)

4.5.3.2. Comparison between different stages of lactation

Mean and standard error of milk constituents for samples collected from cows belonging to early, mid and late lactations are presented in Table.9. Means which are significantly different on comparison by analysis of variance are denoted by different superscripts. Fat content of milk during late lactation was significantly different ($P<0.05$) from that of early and mid lactation. Milk yield during late lactation was significantly different ($P<0.05$) from that of early and mid lactation. Analysis of variance indicated that total solids content of milk during late lactation was significantly different ($P<0.05$) from that of early and mid lactation. This analysis also showed that there is significant difference ($P<0.05$) in

fat per cent between cows belonging to late lactation and cows belonging to other stages of lactation. Protein content of milk differed significantly ($P<0.05$) between cows belonging to mid lactation and late lactation.

4.6. Season

4.6.1. Somatic cell count and season

The influence of season of the year on different milk constituents are depicted in Fig.7. The correlation between milk constituents in milk samples collected during cold and wet, warm and wet, warm and dry and hot and dry seasons from Milk co-operatives at Parappur, Ramavarmapuram and Poochatty are presented in Table.10A. Season had a significant negative correlation ($P<0.05$) of -0.126 with somatic cell count in pooled data.

4.6.2. Season and milk constituents

Total solids had a significant correlation with other milk solids ($P<0.01$) in all the four seasons as shown in Table 10A.. SNF had significant correlation ($P<0.01$) with milk protein and lactose in all the four seasons. The somatic cell count had a negative correlation of -0.250 with lactose content of milk during warm and wet season($P<0.05$) and -0.355 ($P<0.01$) during hot and dry season. Protein and lactose contents were positively correlated ($P<0.01$) during cold and wet season as well as warm and wet season. Fat content of milk showed a positive correlation with lactose content during cold and wet season ($P<0.05$).

4.6.3. Comparison between different seasons

Mean and standard error of milk constituents for samples collected during

Table 10. Mean and SE of milk constituents during different seasons

Milk constituent		Cold and wet	Warm and wet	Warm and dry	Hot and dry
Total solids	Mean	12.90^{ad}	13.49^{bd}	13.07^{acd}	13.10^{abcd}
	± SE	0.19	0.156	0.10	0.13
Fat	Mean	4.36^{ad}	4.89^{bd}	4.48^{acd}	4.60^{abcd}
	± SE	0.15	0.13	0.08	0.11
SNF	Mean	8.53	8.60	8.58	8.50
	± SE	0.11	0.10	0.06	0.08
Protein	Mean	3.04	3.16	3.13	3.10
	± SE	0.05	0.03	0.03	0.04
Lactose	Mean	4.80	4.83	4.80	4.79
	± SE	0.03	0.02	0.02	0.02
Chloride	Mean	0.110^{ab}	0.107^{abc}	0.104^{bcd}	0.101^{cd}
	± SE	0.003	0.002	0.001	0.002
SCC*	Mean	10.800	12.110	9.670	7.290
	± SE	0.184	0.147	0.092	0.076

* - x 10^5 cells / ml

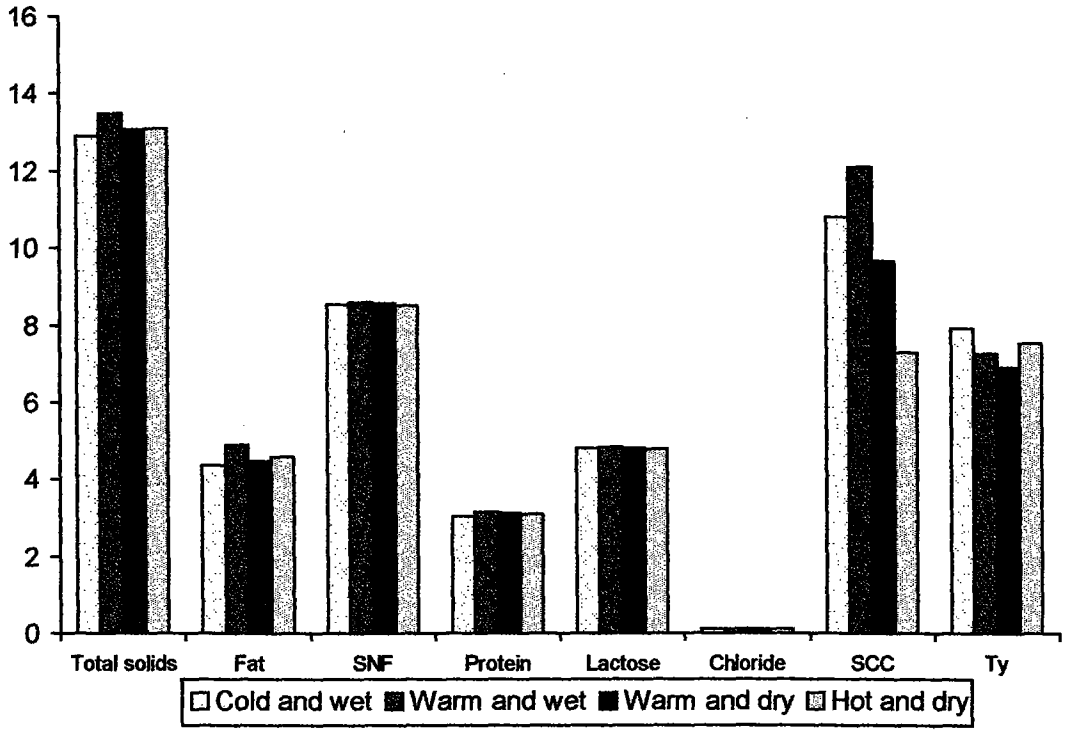
Means bearing different superscripts are significantly different ($P<0.05$)

Table 10A. Correlation between milk constituents during different seasons

Parameter	Cold and wet	Warm and wet	Warm and dry	Hot and dry
TS x Fat	**0.817****	**0.746****	**0.778****	**0.800****
TS x SNF	**0.610****	**0.550****	**0.555****	**0.503****
TS x Protein	**0.518****	**0.418****	**0.427****	**0.348****
TS x Lactose	**0.647****	**0.357****	**0.258****	**0.341****
TS x Chloride	NS	NS	NS	NS
TS x SCC	NS	NS	NS	NS
Fat x SNF	NS	NS	NS	NS
Fat x Protein	NS	NS	NS	NS
Fat x Lactose	**0.276***	NS	NS	NS
Fat x Chloride	NS	NS	NS	NS
Fat x SCC	NS	NS	NS	NS
SNF x Protein	**0.868****	**0.748****	**0.644****	**0.770****
SNF x Lactose	**0.742****	**0.543****	**0.445****	**0.491****
SNF x Chloride	NS	NS	NS	NS
SNF x SCC	NS	NS	NS	NS
Protein x Lactose	**0.564****	**0.317****	NS	NS
Protein x Chloride	NS	NS	NS	NS
Protein x SCC	NS	NS	NS	NS
Lactose x Chloride	NS	NS	NS	NS
Lactose x SCC	NS	**-0.250***	NS	**-0.355****
Chloride x SCC	NS	NS	**NS**	NS
SNF x Koestler No.	NS	NS	**-0.213***	NS

* - $P<0.05$
** - $P< 0.01$

Fig. 7. Influence of season on milk composition and somatic cell count



different seasons are presented in Table.10. Means which are significantly different on comparison by analysis of variance are denoted by different superscripts. Results of Analysis of variance indicated that total solids content of milk during warm and wet season differed significantly ($P<0.05$) from total solids content noticed in cold and wet season as well as warm and dry season. There was no significant difference between total solids content of milk during warm and wet season and that of hot and dry season.

Results of ANOVA revealed that fat per cent during warm and wet season differed significantly ($P<0.05$) from fat per cent noticed in cold and wet season as well as warm and dry season. There was no significant difference between fat per cent of milk during warm and wet season and that of hot and dry season. Chloride content in milk showed a significant difference ($P<0.05$) between cold and wet season and warm and dry season as well as hot and dry season. It was also observed that chloride content in milk showed a significant difference ($P<0.05$) between warm and dry season and hot and dry season.

4.7. Somatic cell count at different stages of milking

Results of statistical analysis by paired T-test showed that the fractions fore milk, mid stram milk and strippings did not differ significantly between them in the somatic cell count.

4.8. Influence of milk sample preservatives on somatic cell count

Formalin and potassium dichromate were the two preservatives used in this study. Addition of these preservatives made the sample unfit for estimation of

Table 11. Effect of formalin on somatic cell count by DMSCC

Batch	Without preservative	Immediately on addition	Week 1	Week 2	Week 3	Week 4
I	1.536	1.516	1.061	0.468	0.53	0.323
II	0.606	0.753	0.682	0.454	0.227	0.303
III	0.985	1.061	0.606	0.379	0.379	0.227
IV	0.227	0.151	0.758	0.498	0.447	0.214
V	0.578	0.553	0.531	0.533	0.542	0.502
VI	1.031	0.991	0.956	0.972	0.934	0.911

Table 11A. Effect of potassium dichromate on somatic cell count by DMSCC

Batch	Without preservative	Immediately on addition	Week 1	Week 2	Week 3	Week 4
I	1.516	0.987	0.909	0.758	0.606	0.379
II	0.606	0.464	0.227	0.758	0	0
III	0.454	0.227	0.075	0	0	0
IV	0.224	0.015	0.075	0	0	0
V	0.725	0.510	0.312	0.156	0	0
VI	0.510	0.472	0.259	0.201	0	0

somatic cell count by MF-DNA method because erroneous values of optical density were noticed in both cases. Hence counting was done by DMSCC . The observations on somatic cell count on addition of formalin and potassium dichromate and 't' values obtained are presented in Table.11 and 11A respectively. Addition of potassium dichromate lead to drastic reduction of somatic cell count immediately. Further, this sample was fit for examination only upto 10 days and during this period cell count was further reduced. potassium dichromate could not preserve cells in milk samples. Samples containing formalin showed variations in somatic cell count during observations upto one month but these counts were not significantly different from each other on analysis.

4.9. Influence of milking management on somatic cell count

Mean values of somatic cell count for the control group of cows, group of animals subjected to mopping of udder prior to milking and the group subjected to post milking teat dipping are presented in Table.12. Analysis of the data by analysis of co-variance showed that the cows whose udders were dipped in povidone iodine compounds after milking showed a significantly ($P<0.05$) lower cell count than control group. There was no significant difference between control group and group subjected to mopping of the udder in somatic cell count.

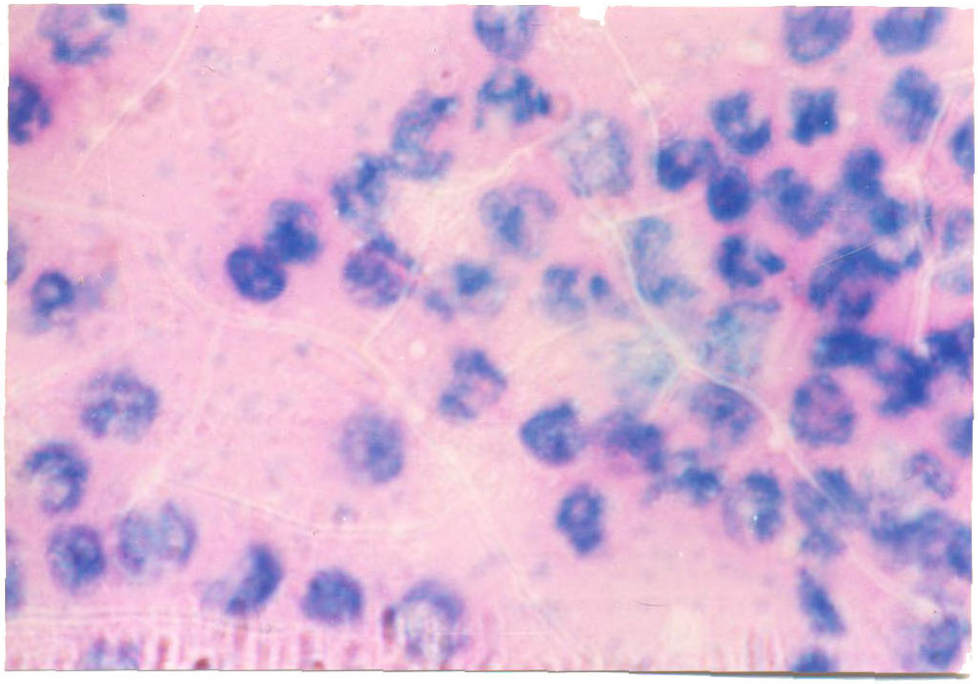
Table 12. Influence of milking management practices on somatic cell count of milk

	Control group		Mopping udder		Teat dipping	
	Initial Mean of SCC[#]	Final mean of SCC#	Initial Mean of SCC#	Final mean of SCC#	Initial Mean of SCC#	Final mean of SCC#
	5.12	4.56	6.21	5.92	5.82	3.11
	7.21	8.12	8.19	8.05	8.93	6.51
	5.32	5.51	11.21	10.51	10.21	9.18
	6.13	6.11	9.35	8.52	5.12	4.9
	8.99	8.85	5.32	5.01	6.97	6.89
	10.23	11.03	6.89	5.91	8.21	8.2
Adjusted means[$]		7.724[a]		6.978[ab]		6.445[b]

- x 10^5 cells / ml
$ - by analysis of co-variance

Plate 3. Milk smear (DMSCC) - Clinical mastitis (Stain Broadhurst-Paley., x 1000)

Plate 4. Milk smear (DMSCC)- Subclinical mastitis. (Stain Broadhurst-Paley., x 1000)



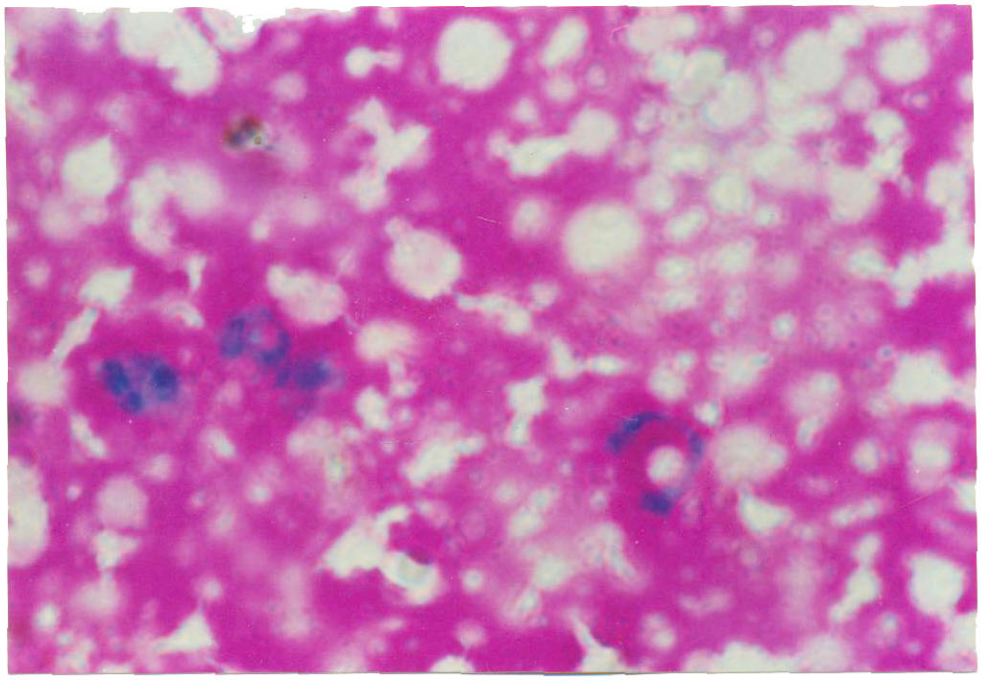
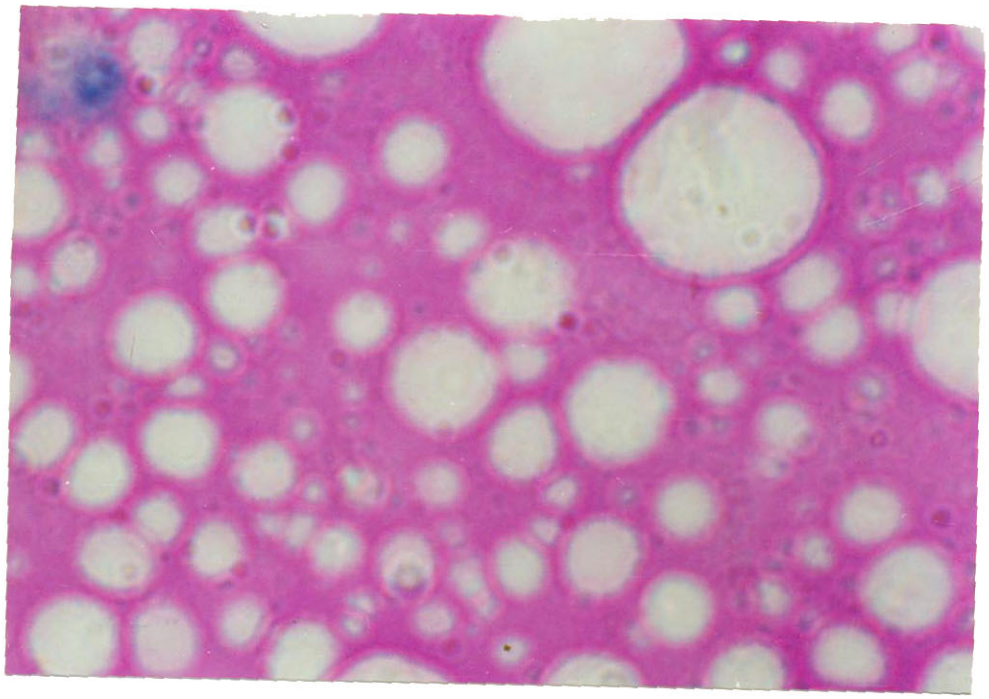


Plate 5. Milk smear (DMSCC)- Normal milk (Stain Broadhurst-Paley., x 1000)

Plate 6. Milk smear (DMSCC)- Milk preserved with formalin (after one week) (Stain Broadhurst-Paley., x 1000)



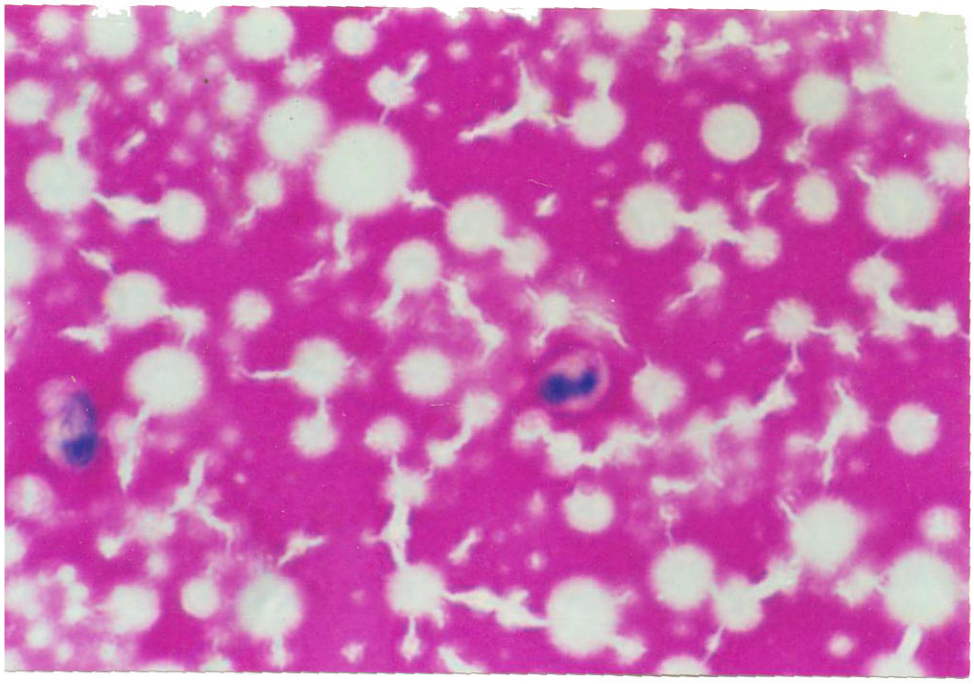
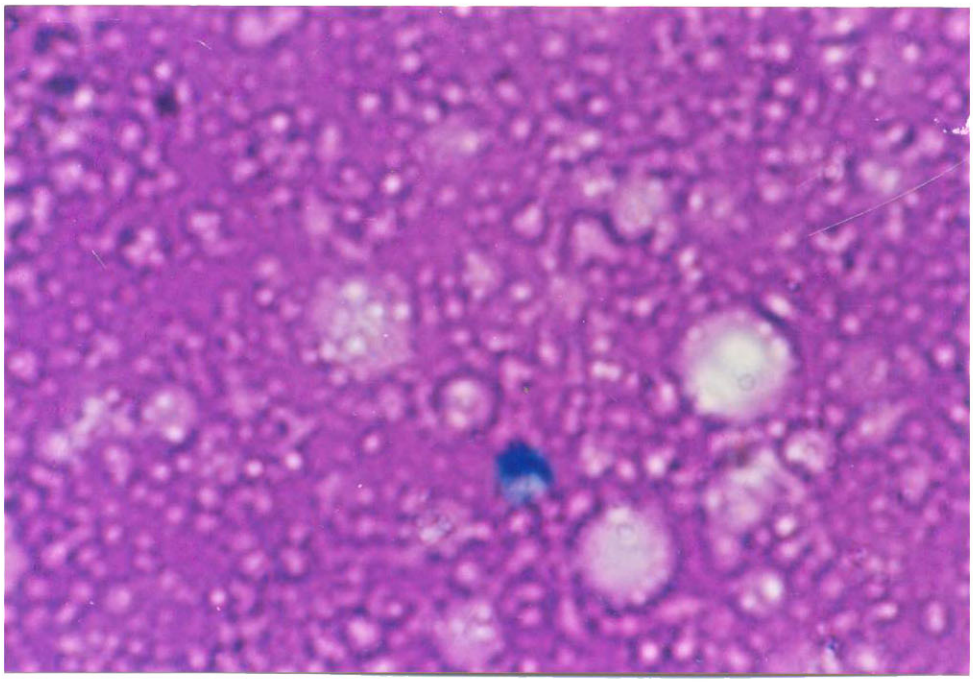
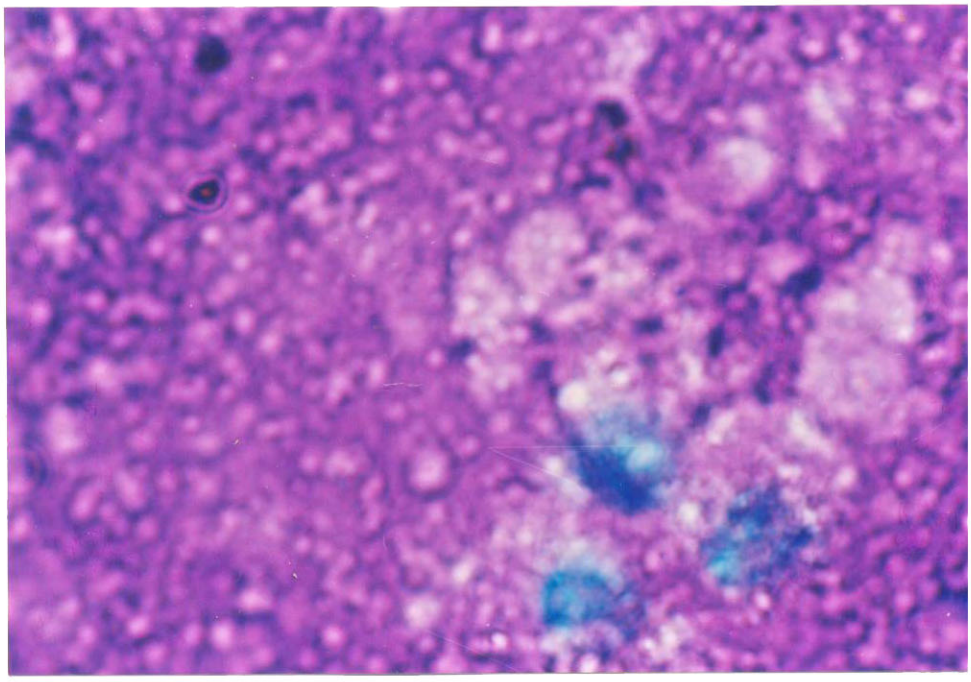


Plate 7. Milk smear (DMSCC)- Milk preserved with potassium dichromate. (Stain Broadhurst-Paley., x 1000)

Plate 8. Milk smear (DMSCC)- Milk preserved with formalin (after one month) (Stain Broadhurst-Paley., x 1000)





DISCUSSION

5. DISCUSSION

5.1 Standardisation of MF-DNA method for estimation of somatic cell count

The MF-DNA described by Ward and Schultz (1973) was modified because preliminary trials with MF-DNA method indicated that colour development with indole reagent was less intense and sensitivity of method was lower. Hence MF-DNA method was modified as described by Sathian and Mukundan(2000). Addition of trichloroacetic acid to the reagent resulted in solubilisation of DNA and better colour development. Modified MF-DNA method was standardised with two reference procedures.

A) Standardisation with Direct Microscopic Somatic Cell Count (DMSCC) :- Standard curve depicted between optical density of MF-DNA method and DMSCC for a number of milk samples is shown in Fig.1. had a correlation coefficient 0.955 between optical density of MF-DNA method and somatic cell count by DMSCC.

B) Standardisation with pure DNA:- This procedure is helpful to reduce errors arising from intra laboratory variation in DMSCC as reported by Smith (1969). In this standardisation procedure the correlation coefficient between optical density of the milk sample and that of pure DNA was 0.975. This proved that modified method was sensitive enough to detect small changes in somatic cell count . The regression equations fitting to these curves and corresponding 'r' values are noted in Table. 1.

5.2. Somatic cell count and California Mastitis Test (CMT) scores

The correlation coefficient between CMT scores and somatic cell count was +0.626 which was significant. In this study the procedure adopted for somatic cell count was based on Deoxyribonucleic acid (DNA) content of somatic cells. This confirms the reliability of this correlation because CMT is dependant upon a reactivity of reagent with deoxyribonucleic acid (DNA) as reported by Schneider and Jasper (1964) and Schalm *et al.*(1971). The average somatic cell count corresponding to each CMT score were given in section 4.2. These thresholds are lower than those reported by Schneider and Jasper, (1964) and Biju (1996)as revealed in Table .2. Schneider and Jasper (1964) have reported extremely high values for somatic cell count but the composition of the CMT reagent is unknown. Most probably they might have used anionic detergent of different type which was the practice in those years as pointed out by Schalm *et al.* (1971). Biju (1996) used sodium hydroxide instead of sodium lauryl sulphate in preparation of CMT reagent which may be the reason for variation in reaction. The thresholds for somatic cell count observed in this study are well suited for selection of milk for processing into various products.

5.3. Milk composition

5.3.1. Somatic cell count and fat percentage

The correlation between fat per cent and somatic cell count in pooled data was negative and non-significant. Fat content in milk is the parameter showing maximum variation (Walstra and Jenness, 1984). Hence the contribution of somatic

cell count in the concentration of fat is difficult to assess as opined by Kennedy *et al.* (1982b) who could not find any change in fat content of milk corresponding to changes in somatic cell count . Results in this study do not agree with reports of Ng-Kwai-Hang *et al.* (1982) who reported a low positive correlation between fat content and somatic cell count. A negative correlation was observed between somatic cell count and milk yield in this study. The reduction in milk yield will definitely enhance fat content of milk and this will mask the actual impact of somatic cell count on fat per cent (Schultz,1977).

5.3.2. Somatic cell count and protein percentage

Results indicate a non-significant negative correlation between protein per cent and somatic cell count in pooled data. This result fully agrees with the findings of Ng-Kwai-Hang *et al.* (1982) who noticed a slight increase in protein per cent with increase in somatic cell count. Many workers have noticed that subclinical mastitis reduced casein content of milk while it increased concentration of whey proteins . According to Schultz (1977) these changes may lead to a slight increase in total protein content in milk. Impact of these changes on processing of milk, especially cheese making has been the topic for research for Ali *et al.* (1980); Derham and Andrews (1982) and Cooney *et al.* (2000).

A significant positive correlation ($P<0.05$) was noticed between milk protein per cent and somatic cell count for cows yielding 5 - 8.9 kg of milk/day and for cows above 8 years of age. This is an important finding because a large share of cattle population in this state falls under these groups.

5.3.3. Somatic cell count and total solids percentage

The correlation between total solids per cent and somatic cell count when pooled data was negative and non-significant. The negative correlation between total solids per cent and somatic cell count in milk samples having fat per cent between 3.5 and 4.5 was significant . Results showed that total solids are lower in samples with high cell count in a large share of samples. This finding is in full agreement with reports of Ashworth *et al.* (1967) and Schultz (1977).

5.3.4. Somatic cell count and solids-not-fat (SNF) percentage

Somatic cell count was negatively correlated with SNF per cent in pooled data but, correlation was non-significant.

A significant negative correlation existed between SNF per cent and somatic cell count for milk samples with a fat content between 3.5 and 4.5 A significant negative correlation between SNF per cent and somatic cell count was also found in milk samples having a SNF content between 8.5 and 8.99. A highly significant negative correlation existed between SNF per cent and somatic cell count for milk samples collected from cows above 8 years of age. These three groups of cows represent a large share of cattle population in our area and results demand an investigation into probable reasons for high cell count in cows. The survey conducted by Radhika and Iype(1999) in Kerala revealed that 47 per cent of the cross-bred cows milk do not meet the prescribed minimum 8.5 per cent SNF in their milk. It can be concluded that high somatic cell count arising from

subclinical infection of udder is one of the reasons for low SNF in milk from crossbred cows.

5.3.5. Somatic cell count and lactose percentage

Results indicated that lactose is the only milk solid which exhibits a significant negative correlation with somatic cell count in pooled data as well as in most of the groups of data. These results are in full agreement with findings of Ashworth *et al.* (1967) and Janzen (1970). But the massive reduction in lactose content upto 10 per cent as reported by Robinson (1993) is not found in this study.

There was a significant negative correlation between lactose per cent and somatic cell count for samples from cows in their early lactation, cows having daily milk yield between 5 and 8.9 kg, cows having milk fat per cent 3 and above, cows having milk SNF per cent 9 and above. Significant negative correlation was also noticed between lactose and somatic cell count during warm and wet season and during hot and dry season. The use of lactose as an indicator in mastitis as suggested by Renner (1975) is confirmed by the findings of this study. Similar observations were also reported by Dhakal and Kapur (1993).

5.3.6. Somatic cell count and chloride content of milk

The results showed that negative correlation between chloride content and somatic cell count was non-significant when pooled data were analysed. This result is contrary to findings of many researchers like Renner (1975); Kitchen (1981) and Munro *et al.* (1984). But Oshima *et al.* (1974b) opined that chloride level alone is

not an effective indicator of infection or high somatic cell count but a combined change in lactose; sodium and chloride ions is more reliable. Another suggestion is estimation of ratio of potassium ions to sodium ions instead of chloride ions (Kitchen, 1981). Further, the sensitivity of titration method of estimation of chloride is less compared to potentiometric method. The physiological mechanism involved in the increase in chloride content of milk is to maintain a constant osmotic pressure for milk. Constraints on the osmotic pressure of milk cause a strong negative correlation between concentration of lactose and dissolved salts. Walstra and Jenness (1984) recorded that osmotic pressure is not exactly constant as revealed by the fact that coefficient of variation of freezing point depression in genuine cow milk samples throughout the year was 1 per cent . So this relation may not hold true in all cases. The measurement of electrical conductivity which is an indirect representation of chloride ions in milk was not effective in detecting subclinical mastitis (Seguya and Mansell, 2000).

There existed a significant negative correlation between chloride per cent of milk and its somatic cell count in the milk from primiparous cows.

5.3.7. Somatic cell count and Koestler number

Results showed that the positive correlation of +0.07 between Koestler number and somatic cell count in pooled data was non-significant. According to Fox and McSweeney (1998) a Koestler number greater than 3 indicates abnormal milk. Mean value for Koestler number for pooled data was 2.13 and was 0.178 for samples having somatic cell count 10×10^5 cells/ml and above in this study. Hence Koestler number is not much useful as an indirect indication of high cell count.

A significant positive correlation existed between somatic cell count and Koestler number in samples having somatic cell count 10 X 10^5 cells/ml and above. In this group of samples Solids-not -fat per cent of milk showed a significant negative correlation with Koestler number.

5.3.8. Comparison between samples belonging to different levels of somatic cell count

Results showed that lactose content in milk with cell count less than 1 X 10^5 cells/ml significantly differed from lactose content in samples with cell count 5 X 10^5 cells/ml and above. This result confirm the finding that lactose is the most affected constituent when somatic cell count is high thereby influencing SNF of high cell count milk. This finding is fully supported by Schultz(1977) who opined that lactose accounted for essentially all the drop in SNF. Further, there was no significant difference between chloride content of samples different in cell count pointing out the inefficacy of chloride as an indicator of high somatic cell count. The threshold level of somatic cell count above which lactose concentration of milk will be lowered is 5 X 10^5 cells/ml as per results of this study. This level of cell count is the limit for classifying udder infection as subclinical mastitis (Nielen, *et al.* , 1995). Hence it is an important finding in this study supporting the inference that lactose is most important indicator of high somatic cell count or indirectly udder infection. From Table 5, it is clear that level of none of the remaining milk solids including chloride did not differ significantly between different levels of somatic cell count. This finding does not support the use of chloride level as an indicator for abnormal milk.

Results revealed a significant negative correlation between milk yield and somatic cell count. This finding is in full agreement with the earlier research reports by Raubertas and Shook(1982); Kennedy *et al.* (1982b) and Robinson (1993).

Table 9 shows that somatic cell count did not differ between lactations while milk yield showed a significant difference between them. Table 6A revealed that correlation between somatic cell count and milk yield was non significant within each stage of lactation. Hence it is proved that the negative influence of somatic cell count is not limited to any particular lactation and it contributes to loss in milk yield throughout lactation period.

5.4.1. Comparison between milk samples from cows differing in production potential

Results in Table 6 showed that there was significant difference between samples belonging to above three groups of samples with reference to their chloride content. There is no evidence in literature supporting this finding. Chloride being a fully ionised radical will be diluted well in the total milk. So it is rational to assume that concentration of chloride is much dependant on dilution or total milk available in normal udder. Further research is required to study this relationship utilising more precise methods for estimation of chloride in milk. If there is a good correlation between chloride content and milk yield that can be used as an index for assessing milk production potential of cows.

5.5. Impact of age, parity and stage of lactation on somatic cell count

5.5.1. Somatic cell count and age of the cows.

Results showed that age had only an insignificant positive correlation with somatic cell count when pooled data were analysed. The correlation between somatic cell count and age of cows belonging to different age groups were also insignificant. This finding is in agreement with the report of Duitschaever and Ashton (1972). In their study the average somatic cell count was $2X10^5$ cells/ml while it was 9.8 $X10^5$ in this study. Hence it is concluded that influence of age on somatic cell count is negligible at low as well as high levels of somatic cell count. This result does not agree with the findings of Bodoh *et al.* (1976); Lin and Chang (1994) and Gonzalo *et al.* (1994) who reported significant correlation between age of the cows and somatic cell count in milk.

5.5.1.1. Age of the cow and milk constituents

Results showed that age of the cow is positively correlated with milk fat per cent. This confirms the established finding reported by Walstra and Jenness (1984). Positive correlation was noticed between percentages of total solids, fat and lactose and age in primiparous cows. Percentages of total solids and fat had a significant positive correlation with age in cows yielding milk between 5 kg and 8.9 kg. For explaining these findings detailed studies are required.

5.5.2. Somatic cell count and parity of the cows

Results in Table 8A showed that parity had only an insignificant positive correlation with somatic cell count when pooled data were analysed. This finding

was not in agreement with the report by Gonzalo *et al.* (1994). Factors like age at first calving, Intercalving period are different for cattle population in which these authors carried out their research and may influence the results.

5.5.2.1. Parity of the cow and milk constituents

Results indicate that there was no significant relationship between total solids and protein or lactose in milk collected from cows in their fourth parity. From Table 8. it is clear that there is a definite increase in protein per cent as well as definite reduction in lactose content in fourth parity. In primiparous cows there was a significant negative correlation between somatic cell count and chloride content of milk. This finding is well explained under section 5.3.6. There was a significant negative correlation between lactose content and parity in cows yielding 5-8.9 kg. Fat per cent had a significant negative correlation with parity for cows yielding less than 5 kg milk/day. Explanation of these results demand further study.

5.5.2.2.Comparison between different parities of the cows

Comparison between first, second, third and fourth parities on percentages of total solids, fat, SNF, protein, lactose , chloride, somatic cell count and milk yield showed that none of these variables differed significantly between parities of the cow. This indicates that parity had little influence on milk composition even though some workers like Schultz (1990) reported a marked change in milk composition with advancing parity.

5.5.3. Somatic cell count and stage of lactation of the cows

Stage of lactation had only an insignificant positive correlation with somatic cell count in the pooled data. This finding is in full agreement with the reports of Paape *et al.* (1979); Duitschaever and Ashton (1972) and Natzke *et al.* (1972) who could not find any relationship between somatic cell count and stage of lactation. But, results of this study is contrary to the findings of Bodoh *et al.* (1976) who described stage of lactation as an important factor affecting somatic cell count.

5.5.3.1. Stage of lactation and milk constituents

One outstanding observation is lack of correlation between total solids per cent and protein per cent during early lactation which requires detailed study about fractions of milk protein involved. The somatic cell count had a significant negative correlation with lactose content of milk during early lactation. This observation is in line with the increased incidence of mastitic infection during early lactation. The significant positive correlation between stage of lactation and milk fat per cent is an established fact in most of the reports (Walstra and Jenness, 1984).

5.5.3.2. Comparison between different stages of lactation

Results showed that milk yield as well as fat per cent were significantly different between early lactation and mid lactation. Results showed that there is significant difference in total solids per cent between cows belonging to late

lactation and cows belonging to other stages of lactation. Protein content of milk differed significantly between cows belonging to mid lactation and late lactation.

According to Walstra and Jenness (1984) The precise influence of stage of lactation on milk constituents cannot be determined because of concurrent changes in season and feeding regime.

5.6. Influence of season on somatic cell count

5.6.1. Season and milk constituents.

Results revealed a significant negative correlation between season and somatic cell count. This result is in full agreement with the findings of Bodoh *et al.* (1976) and Gonzalo *et al.* (1994). Highest counts in temperate region is noticed during summer as reported by Nelson *et al.* (1967) and Wegner *et al.* (1976). But distribution of season in Kerala is quite different and from Table 10. it is clear that maximum cell count was recorded during cold and wet season (rainy season) which gradually decline towards summer months in this study. This finding may be correlated with finding of Vijayan and Umashankar (2000) that in Kerala mastitis is most prevalent during rainy season.

Results showed a negative correlation between somatic cell count and lactose content of milk during warm and wet season during hot and dry season. But from Table 10. it can be understood that maximum variation in lactose per cent is only 0.04 which cannot be considered as reasonable.

5.6.2. Comparison between different seasons

Results indicated that total solids content of milk during warm and wet season differed significantly from total solids content noticed in cold and wet season as well as warm and dry season. There was no significant difference between total solids content of milk during warm and wet season and that of hot and dry season. Results also revealed that fat per cent during warm and wet season differed significantly from fat per cent noticed in cold and wet season as well as warm and dry season. From Table 10. it is clear that both total solids and fat per cent show an increasing trend towards hotter months. Under prevailing condition the milk yield will be lower during hot season thereby improving concentration of milk solids.

It was also observed that chloride content in milk showed a significant difference between warm and dry season and hot and dry season. The level of chloride was higher during rainy season than in hot season.

5.7. Somatic cell count at different stages of milking

Results showed that the fore milk, mid stream milk and strippings did not differ among them in the somatic cell count. This result is not in agreement with the findings of Moore *et al.* (1980) and Nader *et al.* (1995). Probable reasons may be

1) the average somatic cell count of samples with which these scientists worked was below 2 X 10^5 cells/ml while average of samples in this study was 9.18 X 10^5 cells/ml.

2) They were collecting fractions from milking machine while in this study hand milking was done.

One important inference that can be made from this result that CMT can be done on any fraction of milk against the time old concept that CMT is to be done on either fore milk or strippings (Gray and Schalm, 1960).

5.8. Influence of milk sample preservatives on somatic cell count

From results it can be seen that potassium dichromate could not preserve cells in milk samples. Samples containing formalin showed gradual reduction in somatic cell count during observations up to one month but these counts were not significantly different from each other on analysis by T-test. From a practical standpoint the samples preserved with formalin can be used for DMSCC for one week.

5.9. Influence of milking management on somatic cell count

Results clearly indicated that the cows whose udders were dipped in povidone iodine compounds after milking showed a significantly lower cell count than control group. There was no significant difference between control group and group subjected to mopping of the udder in somatic cell count . This finding is supported by large number of publications. Baby *et al.* (1982) from a study at University Livestock Farm found that there was significant reduction in fresh infection with mastitis in cows undergoing post-milking teat dipping. Similar findings were reported by Moxley *et al.* (1978) and Hoare *et al.* (1979). It is

important to note that Iodine compound in teat dip is effective in preventing fresh colonization of bacteria on udder surface there by reducing chances of microbial entry into teat canal. This is important because each mammary gland of dairy cow undergo a subclinical response many times after each milking when small numbers of microbes may gain access to mammary gland before teat sphincter tightly seals the teat canal (Kehrli and Shuster, 1994).

SUMMARY

6. SUMMARY

A research project was undertaken to study correlations if any, between somatic cell count and chemical composition of cross-bred cow milk; the influence of age of the cow, parity, stage of lactation and season on somatic cell count of milk; evaluate the effectiveness of California Mastitis Test (CMT) in detecting subclinical mastitis; find out whether chemical preservatives will be effective in preserving cells in milk; assess the difference in concentration of somatic cells between fractions of milk collected during milking and to evaluate the effects of mopping of udder and teat dipping in controlling high somatic cell count in milk.

For determining somatic cell count, MF-DNA method was modified and standardised both by DMSCC and by pure DNA. Six hundred individual morning milk samples were collected and chemically anlysed for somatic cell count , percentages of TS, SNF, fat, protein, lactose and chloride. Age, parity, milk yield and stage of lactation of these cows were also recorded. Entire data were analysed as pooled data. Data were also classified based on somatic cell count of the samples,fat and SNF content, milk yield, age, parity and stage of lactation. Correlations between milk constituents were tested in each group.

Results showed that lactose per cent is negatively correlated with somatic cell count in pooled data as well as in many groups of data. TS and SNF per cent of milk had a negative correlation with somatic cell count for samples with fat per cent between 3.5 and 4.5 and for samples with SNF per cent between 8.5 and 8.99.

Chloride content of milk had no significant correlation with somatic cell count. Koestler number (percentage ratio between lactose and chloride) was correlated with cell count only in samples with somatic cell count more than 10 X 10^5 cells/ml. Somatic cell count had a negative correlation with milk yield of cows.

Pooled milk samples were collected from three milk co-operatives at fortnightly intervals for one year and analysed. When data were grouped under four seasons of Kerala TS per cent was significantly lower during cold and wet season (June, July and August) than warm and dry season (December and January).

Foremilk, mid-stream milk and strippings were separately collected during milking of thirty cows and were analysed for somatic cell count. It was observed that there was no significant difference among these fractions in somatic cell count.

Effectiveness of formalin (0.4 per cent) and potassium dichromate (0.2 per cent) in preserving somatic cells in milk was tested.

Six batches of pooled milk samples were used for each preservative. Somatic cell count was determined by DMSCC because preserved milk samples gave erratic values with MF-DNA method. Counts were taken at three day intervals for a period of one month. In case of formalin there was a gradual decline in somatic cell count but these decreases in counts were not significantly different from each other. Immediately on addition of potassium dichromate there was drastic reduction in somatic cell count and this trend continued leading to absence of cells by tenth day of experiment.

Three groups of cows with somatic cell count higher than 5 X 10^5 were selected for studying the influence of two milking management practices. In one group udder was mopped to dryness prior to milking while in other group post-milking teat dipping with Povidone Iodine (0.5 per cent) was practiced. Third group served as control. These practices were continued for one month. On analysis of results it was found that teat dipping lead to significant reduction in somatic cell count while mopping had no significant effect.

Results of this study revealed that somatic cell count in milk influenced milk composition and higher counts will lead to significant drop in lactose per cent of milk. This effect was most marked among cows with fat per cent 3.5 to 4.5 as well as SNF per cent 8.5 to 8.99 in their milk. Above findings may be regarded as one of the reasons for widespread reduction in SNF per cent of milk in cross-bred cows. Low lactose content may be used as an indicator for high somatic cell count in milk while chloride per cent cannot be regarded as a reliable index. Total solids content showed a reduction during cold and wet season.

This research work confirmed that California Mastitis Test (CMT) has a positive correlation with somatic cell count and is useful in selecting low count milk for processing. There was no significant difference in somatic cell count among fractions of milk collected during milking. Formalin may be used as a preservative for milk samples meant for somatic cell count by DMSCC. Teat dipping was an effective practice in controlling high somatic cell count in milk.

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SOMATIC CELL COUNT AND ITS INFLUENCE ON THE QUALITY OF MILK IN CROSS-BRED COWS

By
C. T. SATHIAN

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Kerala Agricultural University

Department of Dairy Science
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
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ABSTRACT

A study was undertaken to find out correlation's if any, between somatic cell count and chemical composition of cross-bred cow milk; the influence of age of the cow, parity, stage of lactation and season on somatic cell count of milk; evaluate the effectiveness of California Mastitis Test (CMT) in detecting subclinical mastitis; find out whether chemical preservatives, formalin and potassium dichromate will be effective in preserving cells in milk; assess the difference in concentration of somatic cells between fractions of milk collected during milking and to evaluate the effects of mopping of udder and teat dipping in controlling high somatic cell count in milk.

A modified procedure of Membrane Filter- Deoxyribo Nucleic Acid (MF-DNA) method was developed and standardised with Direct Microscopic Somatic Cell Count as well as using pure DNA for estimation of somatic cell count. This method was precise and suitable for field use. California Mastitis Test (CMT) scores has good correlation with somatic cell count and is useful for selecting milk for processing into various products based on somatic cell count. On analysis of six hundred individual morning milk samples it was found that lactose per cent is negatively correlated with somatic cell count. Total Solids (TS) and Solids-Not-Fat (SNF) per cent of milk had a negative correlation with somatic cell count in samples with fat per cent between 3.5 and 4.5 or SNF per cent between 8.5 and 8.99. Above groups of samples constituted a major share of total samples collected.

Chloride content of milk had no significant correlation with somatic cell count. Use of low lactose level in milk as an indicator for high somatic cell count is recommended. Somatic cell count had a negative correlation with milk yield of cows. A study on pooled milk samples from milk co-operatives revealed that TS per cent was significantly lower during cold and wet season (June, July and August) than warm and dry season (December and January).

There was no significant difference among foremilk, mid-stream milk and strippings in somatic cell count and CMT may be done on any fraction of milk. Study revealed that formalin (0.4 per cent) may be used as a preservative for milk samples meant for Direct Microscopic Somatic Cell Count (DMSCC) while potassium dichromate is not fit for this purpose. None of these preservatives were not suitable for milk analysis by MF-DNA method of somatic cell count. The experiment proved that teat dipping was an effective practice in comparison with mopping of udder prior to milking in controlling high somatic cell count in milk.