EFFECT OF TRISODIUM CITRATE IN THE TREATMENT OF MASTITIS IN CATTLE

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Thesis submitted in partial fulfillment of the requirement for the degree of

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DECLARATION

I hereby declare that this thesis entitled **"EFFECT OF TRISODIUM CITRATE IN THE TREATMENT OF MASTITIS IN CATTLE"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that the thesis entitled **"EFFECT OF TRISODIUM CITRATE IN THE TREATMENT OF MASTITIS IN CATTLE"** is a record of research work done independently by **Smt. Sindhu.O.K.**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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Dedicated to My Family

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Introduction

1. INTRODUCTION

Mastitis remains as one of the most common and problematic disease affecting dairy animals. It causes huge economic losses to the farmers due to reduced milk production, cost of treatment and man power involved in the management of the disease. Often it results in loss of milk production and animal becomes unproductive even during the early lactation.

Mastitis is the inflammation of the parenchyma of the mammary gland. It is a disease complex having different causes, different degrees of intensity and variation in duration and residual effects (Schalm and Woods, 1953). It is characterized by pathological changes in the glandular tissue followed by physical and biochemical changes in milk

Bacteria are the etiological factor in the majority of cases and the use of antibiotic is the first line of therapy followed by anti-inflammatory agents. Antibiotics and anti-inflammatory agents have not provided panacea for the malady, rather their use has posed serious public health problems.

The pH of the normal milk is unsuitable for the optimum growth of the common pathogens. Maintenance of this pH prevents pathogenic bacteria to grow and establish in the udder. Generally mastitis milk has been found to be alkaline and hypocitreamic (Dhillon *et al.*, 1989).The alkaline pH is very much favorable for the multiplication of bacteria (Cruickshank *et al.*, 1969). Citrate a harbinger of lactogenesis is present in considerable amounts in the milk of cows and goats. Milk in mastitis is low in citric acid which is an important item in milk synthesis (Peaker and Linzell, 1975). According to Dhillon *et al.* (1995) restoration of pH and other constituents of milk is also important for the clinical management of mastitis and oral administration of tri-sodium citrate is recommended as a therapeutic measure.

With this background information, the study on "Effect of tri-sodium citrate in the treatment of mastitis in cattle" was under taken with the following objectives.

1. To study the pH changes of mastitis milk in cattle
2. To evaluate the level of citric acid, calcium, lactose and chloride in milk of cattle affected with mastitis.
3. To study the efficacy of oral administration of tri-sodium citrate as a therapeutic agent in subclinical mastitis of cattle.
4. To assess the efficacy of oral administration of tri-sodium citrate as a supportive treatment along with antibiotic in clinical mastitis of cattle.

Review of Literature

2. REVIEW OF LITERATURE

2.1 PATHOLOGY OF MILK IN MASTITIS

2.1.1 Physical Characters

2.1.1.1 Colour

Lohuis *et al.* (1990) reported a yellowish serous discharge from the mammary gland after experimental inoculation with *E. coli* organisms.

Morin *et al.* (1998) suggested that clear or white colour of milk, swelling of the udder and watery milk were the predictors of coliform mastitis.

Rai *et al.* (1998) studied 82 Bovine mastitis cases and opined that mastitis milk samples from early lactation were pale and samples from late lactation were creamy white. The isolation studies showed *Staphylococcus aureus* as the predominant organism followed by *Streptococcus uberis*.

A fourth lactation Friesian cow was presented with the history of hard and painful udder, scanty, watery and purplish red discharge from the udder. The organism isolated was *Bacillus cereus* (Parkinson *et al.*, 1999).

2.1.1.2 Consistency

In lactating cattle, 76 quarters affected with mastitis produced a watery secretion which later turned to a thick purulent material with a putrid smell. On culturing, majority of the organisms isolated were *Actinomyces pyogenes* (Saini *et al.*, 1992).

Orbitzhauser *et al.* (1995) reported that cows were more likely to have watery milk when clinical mastitis was caused by streptococci than it was caused by Enterobacteriaceae.

Factors identified as predictors of coliform mastitis were history of previous clinical mastitis in the affected quarter, clear or white colour of milk, swelling of the udder, watery milk, lack of previous mastitis in the other glands, lack of palpable udder abscesses and a high rectal temperature (Morin *et al.*, 1998).

Rai *et al.* (1998) reported that mastitis milk samples studied in cows in the early stage of lactation had curd like flakes and in the late stage of lactation had fewer flakes. *Staphylococcus aureus* was the predominant isolate obtained from those cases.

Occasional appearance of the clots and wateriness in the first streams were the characteristic signs of staphylococcus mastitis and presence of clots in watery foremilk may be the only apparent abnormality in streptococcal mastitis (Radostits *et al.*, 2000).

2.1.1.3 Abnormal Constituents

Wani *et al.* (1988) reported a case of mastitis in a Jersey cow discharging milk mixed with blood tinged pus. The organism isolated from this case was *Corynebacteruim bovis.*

After the inoculation of *E. coli* cultures intramammarily, appearance of the secretions from the mammary gland varied widely from normal with some fine clots in cows with moderate clinical signs, to yellowish serous secretions containing large clots in cows with severe clinical signs (Lohuis *et al.*, 1990).

Pyorala *et al.* (1994) reported serous or clotty milk in 17 cows in experimentally induced *E. coli* mastitis.

Rai *et al.* (1998) showed that some of the mastitic samples from the cows in early lactation was blood tinged and no blood was there from the cows in late lactation.

2.1.2 Chemical Characters

2.1.2.1 pH

Schalm *et al.* (1971) reported that normal cow milk had a range of pH from 6.4 to 6.8 with an average of about 6.6. The acidity of the milk was due to free acidic groups of casein, citrate and phosphate as well as to the presence of dissolved CO_2. In mastitis due to increased permeability, there was dilution of milk with more alkaline blood components principally bicarbonate, making milk alkaline.

Dhillon *et al.* (1989) reported that the normal milk pH was 6.5 which might be prohibitive for bacterial growth.

In most of the crossbred cows the pH of the normal milk was 6.62 (Yadav *et al.*, 1991).

A mean pH of 6.61 ± 0.02 were obtained for the CMT positive milk samples in cows (Kalorey *et al.*, 1993).

Dhillon *et al.* (1995) studied six cases of mastitis and found out that when the pH was dropped to 6.5, a reduction in bacterial count was observed due to creation of unfavourable conditions for their growth.

Singh *et al.* (1997) cited that pH of the mastitis milk ranged between 7.5 and 8.5 before treatment and dropped to 6.5 after recovery.

The overall pH of buffalo milk averaged 6.64 ± 0.02 and stage of lactation, lactation number and season of calving did not influence it significantly during the lactation (Dubey *et al.*, 1998).

Mulkalwar *et al.* (1999) cited that normal ewe's milk had a pH of 6.42 – 6.58 and got increased in subclinical (+6.49 – 6.62, ++6.5 – 6.73 and +++6.72 – 6.95) and clinical mastitis (6.8 – 7.23).

Reddy *et al.* (1999) reported that in mastitis, after treatment with trisodium citrate, pH value (7.4) of mastitis milk decreased to 6.5.

Dhillon *et al.* (2000) studied ten high yielding buffaloes suffering from mastitis and stated that milk pH ranged between 7.5 to 8.5 before treatment and a value of 6.5 was obtained in all the quarters after successful treatment with tri-sodium citrate.

2.1.2.2 Chloride

Chloride levels in milk showed significant increase along with the pH of the milk as the CMT reaction became more intense (Ashworth *et al.*, 1967).

Fernando *et al.* (1985) cited that chloride level of subclinical mastitis milk in a cow was 0.105 g/dl in fore milk and 0.13 g/dl in stripping milk. It was also suggested that the electrical conductivity, chloride and sodium content of milk were more accurate for predicting infection status of quarters.

Yadav *et al.* (1991) reported that in most of the crossbred cows the chloride concentration in normal milk was 0.093 g/dl.

The milk chloride in Murrah buffaloes were 0.063 ± 0.033 g/dl during first month, decreased to lowest of 0.050 ± 0.033 g/dl during fifth month and increased thereafter during the subsequent months of lactation to 0.059 ± 0.005 g/dl during 10^{th} month (Dubey *et al.*, 1998).

Singh *et al.* (1998) recorded significant increase in sodium and decrease in potassium content of milk from infected quarters. They also reported an increase of chloride content to 0.179 ± 0.007 g/dl and 0.231 ± 0.02 g/dl in subclinically and clinically affected quarters respectively.

Investigations on 200 milk samples revealed that estimation of sodium ions and chloride ions has got greater reliability to diagnose infected quarters compared to estimation of potassium ions (Singh *et al.*, 2000).

Chloride content increased significantly from the normal value of 83.05 ± 8.73 mg per cent to 277.10 ± 1.67 mg per cent in clinical mastitis milk samples of cows (Charjan *et al.*, 2001).

2.1.2.3 Lactose

Average decrease of 0.77 per cent of Lactose was noted between one milk sample ranked negative in CMT and one ranked three in CMT (Ashworth *et al.*, 1967).

Schalm *et al.* (1971) opined that impaired lactose production during mastitis was probably due to altered osmotic equilibrium induced due to more concentration of sodium chloride in the milk. Osmotic equilibrium was maintained with that of blood by a reduction in the secretion of lactose.

Fernando *et al.* (1985) cited that lactose percentage of subclinical mastitis milk samples were low (foremilk 4.8 g per cent and strippings – 4.4 g per cent).

A study involving 50 Sahiwal cows and 26 Murrah buffaloes revealed that cow milk samples with CMT negative scores had average lactose of 4.99 g per cent and CMT positive had lactose of 3.7 g per cent (Hirpurkar *et al.*, 1987).

Yadav *et al.* (1991) reported that in most of the crossbred cows the lactose concentration in milk was 4.53 per cent. Within the crossbreds, lactose content in milk of three bred crosses was lesser than that of their respective interse's and half breds.

Normal level of lactose obtained in cow's milk was 4.13 g per cent and it got reduced to 3.07 g per cent during subclinical mastitis (Mert *et al.*, 1992).

On the basis of both specificity and sensitivity, lactose (71.43 per cent and 82.01 per cent), lactose + AST (86.96 per cent and 71.64 per cent) and lactose + SCC (79.66 per cent and 71.64 per cent) were more effective indirect tests to monitor subclinical udder infection (Pednekar *et al.*, 1992).

Dhakkal and Kapur (1993) found that lactose content of mastitis milk in buffaloes (foremilk was 3.62 ± 0.13 g per cent and stripping milk was 3.31 ± 0.13 g per cent) was significantly low when compared to normal milk (foremilk 4.25 ± 0.03 g per cent and stripping milk 4.18 ± 0.03 g per cent). Lower concentration of lactose was also noted in CMT positive samples. An inverse relationship was recorded between lactose content and total cell, neutrophil, viable cell and bacterial counts in milk.

Mean value of lactose in milk samples taken from cows affected with acute or subacute mastitis was found to be 4.09 ± 0.23 g per cent (Singh *et al.*, 1997).

Singh *et al.* (1998) opined that a significant reduction in lactose content was observed in subclinically (4.6 ± 0.07 g per cent) and clinically (3.12 ± 0.02 g per cent) infected quarters in cross bred dairy cows.

Mulkalwar *et al.* (1999) suggested that pH and lactose in ewe's milk could serve as the best indicators to assess the condition of udder health, if their threshold levels were established.

Dhillon *et al.* (2000) reported a low level of lactose (3.74 ± 0.12) in mastitis milk of buffaloes. After treatment the level reached to a normal level of 5.62 ± 0.0179 g per cent.

Charjan *et al.* (2001) stated that the average lactose concentration in normal milk of cow was 3.6 ± 0.04.In subclinical mastitis (1+) it was 3.1 ± 0.07, subclinical mastitis (2+) it was 2.91 ± 0.04, subclinical mastitis (3+) it was 2.64 ± 0.24 and clinical mastitis it was 1.55 ± 0.13 g/100 ml.

Sathian (2001) recommended that low level of lactose in milk could be used as an indicator of high somatic cell count. Normal milk contained a lactose per cent of 4.8 ± 0.3 g per cent.

Coulon *et al.* (2002) opined that clinical signs of mastitis were accompanied by higher SCC, lower lactose concentration and higher protein concentration.

2.1.2.4 Citric Acid

Schalm *et al.* (1971) observed that citrate was present in levels of about 150 mg/100 g milk. It functions as a chelating agent in milk, sequestering some 15 to 20 per cent of the calcium. It also impart acidity to normal milk.

The final stage of lactogenesis was preceded by the onset of citrate secretion into colostrum and citrate was essential for milk synthesis (Peaker and Linzell, 1975).

Holt and Muir (1979) suggested that one of the functions of the citrate in milk was to sequester milk calcium.

Oshima and Fuse (1981) cited that the citric acid concentration of normal fore-milk of cows was in the range from 96 to 188 mg/dl, and that of abnormal or subclinical mastitis milk was from 63 to 166 mg/dl. The mean of the difference between normal and abnormal milk was 25.7 mg/dl. Not only in subclinical mastitis milk, but also in normal milk from different cows, the citric acid concentration changed in a parallel fashion to that of lactose and both showed a trend towards decline with advance of lactation.

Citrate regulates the rate of fat synthesis in the cytosol and high citrate concentration stimulates Fatty acid synthesis in mammary gland (Faulkner and Peaker, 1982).

Dhillon *et al.* (1995) reported that the deficiency of citric acid occurred in the mastitis cases would lead to clumping of calcium ions and would injure udder alveoli as the same has been reported for cardiac tissue. During citric acid deficiency, defective or decreased milk synthesis might result. Citrate was essential for milk synthesis and calcium ions sequestration.

Pal *et al.* (1995) reported that optimum citrate level was required for normal kreb's cycle in the milk secretary epithelium providing resistance against the genesis of mastitis.

Low percentage of citrate (91.72 ± 5.61 mg per cent) was observed in mastitis milk of cows. Following treatment with trisodium citrate the value came to the normal value of 128.7 ± 3.94 mg per cent (Singh *et al.*, 1997).

Mulkalwar *et al.* (1999) reported that the normal ewe's milk had a citric acid level of 62.55 ± 2.36 mg per cent and revealed a decreasing trend with increase in the severity of mastitis. Citric acid in ewe's milk could serve as the best indicator to assess the condition of udder health.

Percentage of citrate was lower in mastitis milk of buffaloes (64.33 ± 2.486 mg per cent) than in normal milk (Dhillon *et al.*, 2000).

Charjan *et al.* (2001) stated that the average citric acid content in normal milk was 162.79 ± 5.15,when compared to subclinical mastitis (1+) 143.39 ± 7.09, subclinical mastitis (2+) 96.38 ± 2.09, subclinical mastitis (3+) 79.21 ± 1.97 and clinical mastitis (20.05 ± 3.29 mg/100 ml milk).

2.1.2.5 Calcium

Nickerson (1960) found that the normal calcium level in milk was 130.7± 3.5 mg/dl

Holt and Muir (1979) found that a high correlation existed between soluble calcium and citrate concentration.

Mert *et al.* (1992) reported that the normal calcium level in milk was 114.36 mg per cent and it got reduced to 21.39 mg per cent during subclinical mastitis.

2.1.3 Microbial Characters

2.1.3.1 Clinical Mastitis

Rahman *et al.* (1984) reported that the incidence of mastitis was high in third lactation cows of six to eight years age group. The chief etiological agents associated with the condition were staphylococci followed by streptococci.

Wani *et al.* (1988) reported a case of clinical mastitis associated with *Corynebacterium bovis*. The isolate was found sensitive to gentamicin, ampicillin, kanamycin and cephaloridine, but resistant to chloramphenicol, streptomycin and tetracycline.

Jones and Ward (1990) reported that gram negative organism comprised 53 per cent and gram positive organisms, 39 per cent of the mastitis cases.

Sears *et al.* (1991) found that pre milking sampling sensitivity for the diagnosis of the bovine intramammary infections were 91 per cent for *Staphylococcus aureus*, 91 per cent for coagulase negative staphylococci and 97 per cent for streptococcus other than agalactiae. Post milking sampling sensitivities were 81, 45 and 58% respectively, for the same pathogens.

Gupta *et al.* (1992) isolated *Staphylococcus spp* (40.65 per cent), *Streptococcus spp.* (29.35 per cent), *E. coli* (20.02 per cent), *Corynebacterium pyogenes* (4.35 per cent), *Proteus vulgaris* (3.35 per cent), and *Pseudomonas aeruginosa,* from bovine mastitis cases and isolates were found sensitive to chloramphenicol, gentamicin, kanamycin as compared to ampicillin, oxytetracycline, norfloxacine and lincomycin.

Rai and Senani (1997) studied 28 newly calved cows with mastitis and stated that the isolation studies in mastitis milk showed *Staphylococcus aureus* (34.01 per cent), *Streptococcus uberis* (21.32 per cent), *Streptococcus agalactiae* (19.29 per cent), *Streptococcus dysgalactiae* (13.2 per cent) and *E. coli* (12.18

per cent). The isolates were highly sensitive to gentamicin, cloxacillin and oxytetracycline.

Two hundred and seventy one samples collected from the veterinary dispensaries in the Prakasam district, Andhra Pradesh were found to be highly sensitive to gentamicin, chloramphenicol and cephalexin (Anjaneyulu *et al.*, 1998).

Babu *et al.* (1998) studied 100 mastitis milk samples and found that *Staphylococcus aureus* was the chief causative agent of bovine mastitis. The other agents were *E. coli* (22 per cent), *Streptococcus spp.* (16 per cent), *Corynebacterium spp.* (2.5 per cent), *Pseudomonas spp.* (1.5 per cent), *Bacillus spp.* (one per cent) and *Aspergillus spp.* (one per cent). The antibiogram showed that the isolates were highly sensitive to chloramphenicol (92.5 per cent), ciprofloxacin (88 per cent), gentamicin (85 per cent), kanamycin (55 per cent), furazolidone (50 per cent), tetracycline (28 per cent), ampicillin (25 per cent) and streptomycin (12 per cent).

In cows with acute mastitis Bezek (1998) found no growth in 82 samples, coliforms in 54 samples, *Staphylococcus spp* in 96 samples and *Streptococcus spp* in 94 samples. He also suggested that mastitis caused by *Staphylococcus spp* or *Streptococcus spp* should be treated first with a cephalothin or penicillin-G-novobiocin preparations.

Rai *et al.* (1998) stated that the most susceptible period for the mastitis was within ten days after calving and during the initial two months. Major cause ascertained was infectious in origin with out any apparent injury.

The results of antimicrobial sensitivity tests using the isolates obtained from 15 cows diagnosed for clinical mastitis revealed that all the isolates were sensitive to gentamicin and cephaloridine (Shlke *et al.*, 1998).

Shukla *et al.* (1998) found that *Staphylococcus aureus* was a major cause of mastitis in animals, followed by streptococci (31.98 per cent). Other organisms isolated were *E. coli*, *Pseudomonas aeruginosa, Corynebacterium pyogenes, Proteus mirabilis* and *Candida albicans. Staphylococcus aureus* found to be most sensitive to aureomycin (97.31 per cent) and least to penicillin (42.95 per cent). Streptococci were found to be most sensitive to penicillin (98.73 per cent).

In 20 clinically positive peracute, acute and chronic mastitis cases, staphylococci were isolated from 11 samples, non hemolytic streptococci and gram positive rods each from three samples and coliforms, *Bacillus spp* and *E. coli* were from solitary samples. Isolates studied were found to be sensitive to gentamicin, ciprofloxacin and ceftriaxone. For non hemolytic streptococci ceftriaxone found to be the best drug of choice (Umakanthan, 1998).

Dhote *et al.* (1999) reported that majority of the mastitis milk samples contained streptococcal and staphylococcal isolates. They were found to be sensitive to ciprofloxacin and were least sensitive to amoxycillin and penicillin respectively. The gram negative bacteria isolated were highly sensitive to pefloxacin and least to amoxycillin and cloxacillin.

Mandial *et al.* (1999) isolated species of staphylococci (52.91 per cent), streptococci (30.45 per cent) and *E. coli* (13.21 per cent) from 303 milk samples of cows suspected for clinically acute or chronic mastitis. Sensitivity of isolates to cloxacillin was 81.3 per cent, gentamicin 74.52 per cent, chloromphenicol 63.12 per cent, penicillin 60.07 per cent and streptomycin 28.89 per cent.

Bacteriological examination of mastitis milk samples yielded *Staphylococcus aureus* (33.3 per cent), *Streptococcus agalactiae* (26.7 per cent), *Corynebacterium spp* (20.0 per cent), *Streptococcus dysgalactiae* (13.3 per cent) and *Streptococcus epidermidis* (6.7 per cent). Mixed infection was also recorded in 15.4 per cent samples. *In vitro*-antibiotic sensitivity examination of these

isolates revealed cephalothin, minocycline, streptomycin, tobramycin and triple sulphonamides to be the most effective followed by amoxycillin, cephotaxime, ofloxacin, penicillin and polymixin-B in decreasing order (Ghose *et al.*, 2001).

Sebastian (2001) reported that *Staphylococcus aureus* was the chief etiological agent causing mastitis followed by coagulase negative staphylococci, coliforms and *Streptococcus agalactiae.* Antibiotic sensitivity test showed that chloramphenicol and enrofloxacin were the most effective antibiotics and sulphadiazine and trimethoprim the least effective.

Kader *et al.* (2002) reported that staphylococci were found to be the major etiological agents, followed by *E. coli* and then *Bacillus spp.* as the causative agents for SCM in cows in Bangladesh. Overall sensitivity revealed that tetracycline and gentamicin were most effective, followed by streptomycin, amoxycillin, ampicillin and penicillin.

Saxena *et al.* (2002) found that teat canal infections in buffaloes were as high as 69.49 per cent. Coagulase negative staphylococci were the chief isolates, followed by *Corynebacterium spp, Streptococcus uberis, Streptococcus dysgalactiae* and *Streptococcus agalactiae.* On the basis of milk samples at the time of drying off, 36.44 per cent of buffalo quarters were found to be infected. Coagulase negative staphylococci were found to be the predominant organism followed by *Corynebacterium spp, Staphylococcus aureus, , Streptococcus dysgalactiae, Streptococcus agalactiae, Streptococcus uberis* and gram negative coccobacilli.

2.1.3.2 Subclinical Mastitis (SCM)

Ali *et al.* (1990) reported that staphylococci were the most commonly occurring pathogen for causing SCM (88.96 per cent). Other minor pathogens isolated were enterobacteria, streptococci, corynebacteria and pseudomonas. *Staphylococcus epidermidis* and *Staphylococcus-aureus* isolated from subclinical mastitis were highly sensitive to nitrofurantoin and least sensitive to

streptomycin. Tetracycline was highly effective and erythromycin, the least effective to enterobacteria. For streptococci, erythromycin and furazolidone were 100 per cent effective and streptomycin was only 20 per cent effective. For *Corynebacterium pyogenes* nitrofurantoin was highly effective where as streptomycin was least.

Buragohain and Dutta (1990) from a study on 113 milk samples, positive for SCM found that gentamicin was the most effective agent against all the isolates with an over all efficacy of 94.66 per cent.

Ramachandraiah *et al.* (1990) reported that 63.7 per cent of cows were found positive on CMT and cultural examination. *Staphylococcus spp* recorded the highest incidence (52.9 per cent) followed by *Streptococcus spp* (20.3 per cent). These isolates were found sensitive to gentamicin, chloramphenicol, co-trimoxazole and oxytetracycline and were resistant to ampicillin and penicillin.

Ruegg *et al.* (1992) isolated *Serratia marcescens* from 13 to 18 per cent of milk samples from lactating dairy cows with an outbreak of subclinical and clinical mastitis in California.

Saxena *et al.* (1993) reported that the most prevalent organism in subclinical mastitis were *Staphylococcus aureus* followed by coagulase negative staphylococci.

Saini *et al.* (1994) isolated *Staphylococcus aureus* (48.57 per cent), Coagulase negative Staphylococci (17.14 per cent), *Streptococcus spp* (11.42 per cent), *E. coli* (11.42 per cent), *Klebsiella spp* (4.28 per cent), *Corynebacterium spp* (2.85 per cent), *Proteus spp* (1.42 per cent) and *Bacillus spp.* (2.85 per cent) from subclinical mastitis cases. Antibiotic sensitivity testing indicated that gentamicin was the most sensitive antibiotic followed by chloramphenicol, co-trimoxazole, nitrofurantoin, neomycin, erythromycin, streptomycin, ampicillin, oxytetracycline, penicillin and cloxacillin.

In eight machine milked farms, staphylococci were found to be chief causative agents followed by Streptococci, *E. coli*, corynebacterium, proteus and *Klebsiella spp* responsible for subclinical mastitis in cows. Culture and sensitivity of the isolates showed gentamicin, cephalexin and chloramphenicol as the highly effective drugs and penicillin as the least effective drug (Singh *et al.*, 1994).

Biju (1996) isolated staphylococci, streptococci, corynebacterium, pseudomonas and gram positive bacilli from bovine subclinical mastitic cows.

Shlke *et al.* (1998) found that *Staphylococcus spp* was the chief causative pathogen in subclinical mastitis

Bhalerao *et al.* (2000) found that staphylococci and streptococci were the most prevalent organisms causing subclinical mastitis. Sensitivity pattern showed by the isolates were 93.18 per cent to gentamicin, 81.82 per cent to pefloxacin and 70.42 per cent to neomycin.

The major organisms isolated from 1336 quarter samples screened were *Staphylococcus spp* (61.91 per cent) and *Streptococcus spp* (25.17 per cent). Cultural examination also could isolate *E. coli, Klebsiella spp, Enterobacter spp, Proteus spp,* and *Pseudomonas spp* (Saravanan *et al.*, 2000).

Ross *et al.* (2001) isolated *Staphylococcus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae, Bacillus subtilis, Pseudomonas aeruginosa* and *E. coli* from CMT positive milk samples from cows and buffaloes. Coliform organisms were found to be sensitive to chloramphenicol, gentamicin and ciprofloxacin.

Mohinikumari and Gupta (2002) in 81 milk samples collected from cows in the periparturient phase, revealed that *Staphylococcus spp* were the most prevalent organisms followed by *Escherichia spp, Streptococcus spp, Pseudomonas spp, Corynebacterium spp* and *Klebsiella spp*. The isolates were

sensitive to gentamicin and were partially sensitive to chloramphenicol, kanamycin, nalidixic acid and erythromycin.

Staphylococcus aureus isolated from subclinical intramammary infection showed higher susceptibility to lincomycin, co-trimoxazole, gentamicin, neomycin and erythromycin. Isolates of *Streptococcus agalactiae* were sensitive to neomycin, cephaloridine, nitrofurantoin, cephalothin, gentamicin and erythromycin. *E. coli* was found sensitive to ampicillin, cephaloridine, cephalothin, gentamicin (Nauriyal and Pachauri, 2002).

2.1.3.3 California Mastitis Test (CMT)

Carroll and Schalm (1962) suggested that polymerized DNA originating from cells of the inflammatory exudates in mastitis milk was the component responsible for positive CMT reactions.

Schneider and Jasper (1964) described a method for standardisation of the scoring of CMT reactions.

Ashworth *et al.* (1967) summarized that the CMT reaction was a sensitive test for subclinical mastitis. All composition tests commonly used to show the presence of mastitis,agree with the CMT test.

Luedecke *et al.* (1967) showed that as the severity of CMT reaction increased, the leukocyte count, catalase activity and A-esterase activity also increased.

Schalm (1977) reported that somatic cell numbers in foremilk from healthy bovine quarters were commonly less than 100,000/ml.

Out of 125 cows which were found positive (2+ and 3+) on CMT, 75 animals were found culturally positive (Rindsig *et al.*, 1979).

Obara and Komatsu (1984) reported that NAGase activity in milk was a better marker for grading the severity of inflammation than for estimating cell count in milk.

The sensitivity of CMT, Bromothymolblue indicator card test and white side test was 78.5 per cent, 75.27 per cent and 52.67 per cent respectively, where as the specificity was 97.02 per cent, 96.48 per cent and 94.85 per cent (Pal and Verma, 1988).

Vianni and Nader (1989) cited that out of 254 quarter milk samples, two of the CMT positive samples were negative to the coulter counter (below 5 x 10^5 cells/ml) while 20 of the negative samples were positive to the coulter counter, giving an agreement of 91.34 per cent.

Kothe *et al.* (1993) reported that Modified California mastitis test and sodium lauryl sulfate teepol test were equally effective and of use in indirectly assessing the degree of mastitis and the corresponding increase in somatic cell count.

The mean values of SCC (x 10^4 cells ml) obtained for CMT Scores were 1 (79.9), 2 (333.5), 3 (670.3), 4 (1354.0) and 5.(4455.6) (Brito *et al.*, 1997).

Chanda *et al.* (1997) reported that CMT was having rapid and highest sensitivity to SCM followed by Bromothymol blue test and modified white side test.

2.2 TREATMENT OF MASTITIS

2.2.1 Antibiotics

Ziv (1980a) opined that antibiotics which were lipophillic weak bases (eg:erythromycin, tylosin, lincomycin, clindamycin and spiramycin), the more lipophilic tetracyclines (doxycycline and minocycline) and penethamate hydriodide, which were easily accessible to milk have certain advantages in

systemic anti-mastitis therapy over antibiotics like penicillin G, streptomycin and oxytetracycline.

Ziv (1980b) reported that problems regarding the intramammary therapy could be solved by using bases that will permit quicker excretion of antibiotic, but higher dose have to be used and at more frequent treatment intervals to compensate for intrinsic shorter periods of action. Among penicillins, the absorption rate of penethamate was twice as that of ampicillin. Cephalexin was most preferred than other cephalosporins. All aminoglycosides were poorly absorbed.

In bovine acute mastitis, streptococci, staphylococci and *E. coli* were isolated and ampicillin (250 mg) and prednisolone (10 mg) in 10 ml distilled water were infused intramammarily in each of the affected quarter. The treatment was repeated after 12 hr and continued for four to six days. A cure rate of 73.07 per cent was obtained (Varshney and Joshy, 1986).

Owens *et al.* (1988) suggested that the combination of amoxycillin intramammary plus procaine penicillin G intramuscularly resulted in bacteriologic cure of 51.4 per cent of quarters and 48 per cent of cows compared to 25 per cent of quarters and 30.4 per cent of cows for intramammary infusion alone.

Jarp *et al.* (1989) suggested that the most efficient regimen for all bacteria was five days systemic treatment with procaine penicillin intramuscularly (53.1 per cent cured). One intramuscular injection of a combination of procaine penicillin and dihydrostreptomycin followed by intramammary treatment daily per infected quarter for four days was the second best (46.7 per cent cured). The least efficient regimen consisted of systemic therapy with procaine penicillin for three days (36.9 per cent cured).

Pal *et al.* (1989) concluded that the treatment with five milliliter of five per cent aqueous solution of chloramphenicol sodium succinate in each of the

affected quarters in every 24 hr for five days was effective for treating both clinical and subclinical bovine mastitis caused by gram positive or gram negative bacteria. It gave better results with little adverse effects.

Uppal *et al.* (1989) found that Tylosin @ 10 mg/kg body weight intramuscularly daily for six days or one gram of Tylotrate containing 50 per cent tylosin tartarate powder, dissolved in 20 ml of distilled water was infused intramammary @ 20 ml in each affected quarter for five days was effective against staphylococcus and streptococcus spp showing resistance to the most commonly used antibiotics.

Bansal *et al.* (1990) cited that using cefuroxime, the mastitis cure rates were more in cows (78.95 per cent animalwise and 80.00 quarter wise) than in buffaloes (42.86 per cent animal wise and 49.44 quarter wise).

Buragohain and Dutta (1990) compared the efficacies of four regimens of treatment for subclinical mastitis in cows and concluded that 80 mg gentamicin sulphate diluted in six milliliter sterile distilled water infused intracisternally at 12 hour interval for five days gave 100 per cent cure rates followed by nitrofurazone of 91.2 per cent cure rate.

In a treatment trial using several intramammary preparations, Binodkumari and Supekar (1992) found that Furazolidone was the most efficacious (84.62 per cent) and penicillin (15.38 per cent) the least sensitive to udder microflora. The over all cure rate was 76.34 per cent after the treatment.

In experimentally induced *E. coli* mastitis, Pyorala *et al.* (1994) revealed that bacteria were eliminated from the quarters within seven days in cows received trimethoprim-sulphadiazine @ 48 mg/kg first intravenously and then intramuscularly, three times at 12 hr interval (six challenges) and in cows which received colistin sulfate at six million units intramammarily three times at 12 hr interval (six challenges).

Forty cows with SCM were treated either with two or four intramuscular injections of penicillinG sodium (10,000 IU/kg) every 12 hour or with a single intramuscular injection of sulfadiazine-trimethoprim combination (15 mg/kg) or were left untreated. One week after the treatment, 10 per cent of the sulfadiazine-trimethoprim treated cows, 30 per cent of the penicillin (two injections) and 60 per cent of the penicillin (four injections) treated cows were recovered (Pedraza *et al.*, 1995).

Bagherwal and Shukla (1996) treated successfully the mastitis caused by staphylococcus, streptococcus and *E. coli* with pefloxacin @ two milligram per kilogram body weight daily intravenously for three consecutive days. Which were resistant to ampicillin, cloxacillin, streptomycin, oxytetracycline, chlortetracycline, furazolidone, erythromycin, neomycin and nitrofurazone.

Rai and Senani (1997) concluded that therapeutic trial with the antibiotic showing 3+ or 4+ *in vitro* sensitivity pattern was not fully satisfactory and the antibiogram might not be fully effective for devising treatment for mastitis.

Cure rate of gentamicin (80 mg mixed with four milliliter distilled water infused intramammary) for treating subclinical mastitis was found to be 87.8per cent followed by chloramphenicol sodium succinate (150 mg mixed with four milliliter of distilled water) 85.71per cent and Floclox-L 84 per cent (Mandial *et al.*, 1999).

Bhalerao *et al.* (2000) proved that pefloxacin infusion, 200 mg in 50 ml distilled water per quarter per day for three consecutive days was highly effective in treating subclinical mastitis. Amoxycillin – cloxacillin combination (250 mg) or neomycin (250 mg) were also found to be effective.

Preez (2000) stated that failure of mastitis therapy was due to pathological changes that occur in the udder parenchyma as a result of inflammatory reaction to mastitogenic bacteria, pharmacokinetic properties of antimicrobial mastitis

drug, mastitogenic bacterial and related factors, poor animal husbandry and veterinary interventions.

Shephard *et al.* (2000) conducted a study in cows which were recently calved and had an individual cell count of milk was 500,000 cells/ml or greater. They concluded that antibiotic treatment during lactation of cows with high somatic cell counts in milk was ineffective in reducing bacterial infections and in reducing somatic cell counts to acceptable numbers.

Sebastian (2001) concluded that oxytetracycline @ 10 mg/kg for five days was more effective followed by enrofloxacin and amoxycillin – cloxacillin combination in cows with clinical and subclinical mastitis

Hillerton and Kliem (2002) reported that in *Streptococcus uberis* mastitis, aggressive intramammary antibiotic therapy at every milking achieved 100 per cent clinical cure in six days. Parenteral antibiotic treatment alone had 91 per cent cure in six days, whereas combination of these two had 100 per cent cure in six days. Use of oxytocin alone for three days failed to achieve any clinical cure and oxytocin with labeled use of intramammary antibiotic (once a day for three days) had 10 per cent cure in six days.

The efficacy of enrofloxacin in *Staphylococcus aureus* mastitis using intramammary (250mg made to 10ml in sterile distilled water infused to teat during three days) and systemic treatments (five milligram per kilogram body weight intramuscularly once a day for three days) were 72 and 75.6 per cent respectively (Rantala *et al.*, 2002).

2.2.2 Trisodium Citrate

Kalorey *et al.* (1993) studied 18 lactating cows positive for subclinical mastitis and observed a favourable reduction in pH of the milk after treatment with tri-sodium citrate @ 30 mg/kg orally for five days. Post treatment cultural examination revealed only 13.33 per cent quarters culturally positive when

compared to pre treatment value (66.66 per cent quarters). The isolates identified were *Staphylococcus aureus* and *Staphylococcus epidermidis*.

Dhillon *et al.* (1995) reported that there was decrease in bacterial count and replenishment of milk citric acid after administration of 12 g of trisodium citrate in 250 ml of water orally till recovery from the mastitis. Milk pH was dropped to 6.5, which was unfavorable for bacterial growth.

Pal *et al.* (1995) studied 17 cows with mastitis and proved that effective management of mastitis was achieved (55.55 per cent cows) by the oral administration of trisodium citrate alone @ 30 mg/kg body weight for six days. Along with tri-sodium citrate, intramammary administration of cloxacillin sodium one tube, 48 hourly for three occasions or chloramphenicol @ 3 mg/kg body weight intramuscularly daily for five consecutive days or gentamicin sulphate @ 100 mg reconstituted with 2.5 ml of distilled water and infused intramammary twice daily for four days, were required for cent per cent recovery from mastitis. Failure of citrate therapy in some cases might be due to presence of heavy bacterial population in mastitic quarters.

Following oral treatment with 15 g of tri-sodium citrate in 250 ml water daily for six to eight days, milk consistency, pH and percentage of citrate, total proteins and lactose in mastitis milk in cows were restored to near normalcy (Singh *et al.*, 1997)

Rai *et al.* (1998) reported a significant reduction in the incidence of infectious mastitis after giving trisodium citrate orally for five days just after calving.

Triple therapy with trisodium citrate, ampicillin and gentamicin appeared to be the ideal therapy for subclinical mastitis in 80 buffaloes compared to triple drug therapy with trisodium citrate, tilox and streptopenicillin (Dicrysticin-S) or solitary administration of tri-sodium citrate (Reddy *et al.*, 1999).

Restoration of milk consistency, pH , percentage of total proteins, lactose, citrate and fat was found after treatment with 15 g of tri-sodium citrate in 250 ml water orally once daily till recovery in ten buffaloes suffering from mastitis (Dhillon *et al.*, 2000).

2.2.3 Other Methods

Dudko (1994) suggested a new method for treating subclinical mastitis using Biomast. Biomast was a new polish preparation containing killed cultures of *Corynebacterium uberis* Strain 22 that was injected subcutaneously in the region of right and left supramammary lymphnodes. Six weeks after the injection, shedding of *Staphylococcus aureus* with milk stopped in eight cows and another eight had negative results of CMT.

Tyler *et al.* (1994) reported that cows receiving hypertonic saline intravenously (7.5 per cent sodium chloride 5 ml/kg body weight) had some beneficial effect for treating endotoxin induced shock associated with coliform mastitis. The hypertonic saline caused rapid redistribution of body fluid from the intracellular to intravascular and extracellular fluid compartments, which enhanced circulating blood volume and tissue perfusion. Relative plasma volume was greater in cows received hypertonic sodium chloride than those received isotonic sodium chloride.

Arita (1998) treated 30 cases of peracute mastitis, due to gram negative bacteria with oxytocin intra arterialy, antibiotics and steroids. The patients were milked out immediately after the arterial injection and antibiotics and steroids were also infused intramammarily. Within 24 hrs of treatment, clinical signs and lactation were restored to normalcy. Oxytocin was expected to eject the residual milk with bacteria, endotoxin and cytokine.

Eighty one cows having clinical mastitis were treated with lysosubtilin (Broad spectrum preparation of lytic enzymes from *Bacillus subtilis)* 3.5 x 10.6 U dissolved in 100 ml distilled water given intramammary through the teat canal.

Lysosubtilin significantly decreased both the time for clinical recovery and percentage of animals that suffered relapses within two months (Biziulevichius and Lukauskas, 1998).

Zanetti *et al.* (1998) examined the effects of vitamin E and selenium in dairy cows. The cows were given five milligram selenium plus 500 IU Vitamin E orally and it was noted that supplementation increased serum selenium levels and reduced the incidence of subclinical mastitis.

Dietary supplements of selenium and vitamin E in greater amounts than were required for nutritional adequacy could have complementary functions in reducing somatic cell counts and both the severity and duration of clinical mastitis (Hemingway, 1999).

Hoeben *et al.* (1999) found that the groups of cows which were infected with *Streptococcus uberis* mastitis regained the milk yield to near normalcy after the treatment with 500 mg recombinant bovine somatotropin for seven days before and after infection, but in the control group total yield and yield of the infected quarter remained lower than preinfection yields. Somatotropin protected the mammary gland from excessive production losses and compositional changes occurring during the mastitis period.

Sato *et al.* (1999) opined that the somatic cell count in quarter milk was significantly decreased at three, seven and 14 days after initial injection of three days treatment of vitamin B_2 (2.5 mg/kg at two days intervals).

Ogata and Nagahata (2000) infused ozone into the inflamed quarter of cows via teat canal using ozone gas generating equipment and found that 60 per cent of cows with acute clinical mastitis treated with ozone therapy did not require any antibiotics for recovery. Thus this ozone therapy method was proven to be effective, safe and cost effective and carried no risk of drug residues in milk.

Costa *et al.* (2002) reported that mastitic cows treated with gentamicin and cefacetril separately had a cure rate of 80.77 per cent and 83.93 per cent respectively. However, cows treated with intramammary infusion of proteolytic enzymes with corticosteroids had a cure rate of 48 per cent and cows treated with intramammary infusion of proteolytic enzymes with vitamin A, E and mannitol had a cure rate of 68 per cent.

Kolodziejczyk *et al.* (2002) observed a faster regression of clinical signs, much higher percentage of recoveries, and lower percentage of defective and dead animals in sows which received hydium KLP @ 0.02 mg/kg bodyweight applied over the udder once immediately showing the signs of coliform mastitis along with the appropriate antibiotic parenterally.

Matsuda *et al.* (2002) reported that the cows affected with mastitis which received ice pack therapy (applied twice a day for two hour for one to three days) showed more rapid decreases in body temperature, mammary swelling and mammary temperature. Also excessive inflammation of the gland disappeared rapidly. It was concluded that ice pack therapy was useful in addition to current standard bovine mastitis treatment.

Naresh *et al.* (2002) stated that the recovery rate was faster in cases of clinical mastitis treated with ascorbic acid @ 25 mg/kg body weight subcutaneously along with intramammary infusions for five consecutive days, than the control group which received only intramammary infusions. Subclinical mastitic cows treated with ascorbic acid @ 25 mg/kg body weight subcutaneously for five consecutive days showed 83.33 per cent recovery. Ascorbic acid was administered in clinical and subclinical cases even after cure considering its immunostimulatory and healing inducing effects.

Materials and Methods

3. MATERIALS AND METHODS

The study was carried out in the Department of Clinical Medicine, College of Veterinary and Animal Sciences, Mannuthy, for a period of three semesters during the years 2002 and 2003.

3.1 OUT LINE OF STUDY

Cows presented to the Veterinary College Hospital, Mannuthy, with the history of reduction in milk yield, were screened for subclinical mastitis using California Mastitis Test and 20 animals with subclinical mastitis were selected for treatment trials. Fifteen cases of clinical mastitis presented to the hospital were also selected for therapeutic trials. The selected cases of both sub clinical and clinical cases of mastitis were subjected to detailed clinical examination. Milk samples were collected from the affected and non affected quarters of all the cases for the estimation of pH, antibiotic sensitivity test, estimation of citric acid, calcium, lactose and chloride in milk on zero, third and sixth day of treatment. Treatment trails were carried out by using tri-sodium citrate orally and suitable antibacterials parentrally.

3.2 PARAMETERS STUDIED

3.2.1 Detailed Clinical Examination and Signalment

Detailed clinical examination was carried out as per the procedure described by Boddie (1969). Also the udder was examined in detail for pathological changes in udder tissue and milk.

3.2.2 California Mastitis Test (CMT)

The CMT was conducted, as per the procedure described by Schalm *et al.* (1971).

3.2.2.1 Reagents

Sodium lauryle sulphate	-	40 g
Teepol	-	150 ml
Bromocresole purple	-	10 mg
Distilled water (up to)	-	1000 ml

3.2.2.2 Interpretation

Symbol	Suggested meaning	Description of visible reaction	Interpretation (cells/ml)
-	Negative	Mixture remains liquid	0-200,000
T	Trace	A slight slime forms and tend to disappear with continued movement of the fluid	150,000-500,000
1	Weak	A distinct slime but no tendency toward slime formation. With continued movement of the paddle the precipitate may disappear	400,000-1,500,000
2	Distinct positive	The mixture thickens immediately with gel formation. As the mixture is caused to swirl, it tends to move as a mass around the periphery of the cup, leaving the bottom of the cup exposed. When the motion is stopped, the mixture levels out again	800,000-5,000,000
3	Strong positive	A gel is formed which causes the surface of the mixture to become convex, with a central peak. Viscosity is greatly increased with a tendency for the mass to adhere to the bottom of the cup	Over 3,000,000
+	Alkaline milk	The reaction is distinctly alkaline, indicated by a deeper purple colour	Depression of secretory activity as a result of *inflammation or drying* – off of the gland
Y	Acidic milk	The mixture is yellow indicating acid milk	Fermentation of lactose by bacterial action within the gland

3.2.3 pH of Milk

pH of the milk samples were noted while striping the quarter during clinical examination using pH-indicator strips-non bleeding (Spezialindikator pH 6.5-10.0, Merck) and later on the pH was checked using pH meter in the laboratory.

3.2.4 Culture and Sensitivity

Bacteriological Culture of milk samples was carried out as per the method described by Barrow and Feltham (1993). *In vitro* antibiotic sensitivity of the organisms were studied using disc diffusion technique (Baur *et al* .,1966).

3.2.4.1 Culture Media

1. Triptone soya agar
2. Mueller Hinton agar
3. Peptone water
4. Antibiotic disc (Himedia)

- Ampicillin (A) - 10 mcg/disc
- Chloramphenicol (C) - 10 mcg/disc
- Cloxacillin (Cx) - 10 mcg/disc
- Enrofloxacin (Ex) - 5 mcg/disc
- Gentamicin (G) - 10 mcg/disc
- Oxytetracycline (O) - 30 mcg/disc
- Sulphadiazine (Sz) - 300 mcg/disc
- Trimethoprime (Tr) - 5 mcg/disc
- Ciprofloxacin (Cf) - 5 mcg/disc
- Cefotaxime (Ce) - 10 mcg/disc
- Streptomycin (S) - 10 mcg/disc

3.2.4.2 Interpretation

The zone of inhibition of bacterial growth around each disc was measured and interpreted as sensitive or resistant by comparing the ranges given by the manufactures.

3.2.5 Estimation of Citric Acid

Estimation of citric acid was carried out as per the method described by White and Davis (1963).

3.2.5.1 Reagents

1. 24% (w/v) Trichloro acetic acid (TCA) solution
2. Whatmann No.40 paper
3. Sodium citrate ($Na_3C_6H_5O_7.\ 2H_2O$)
4. Pyridine
5. Acetic anhydride

3.2.5.2 Preparation of Standard Citrate Solutions

0.1913 g of sodium citrate was dissolved in water and diluted to 200 ml in a volumetric flask. Zero, two, three, four and five milliliters of this solution was pipetted out in to 25 ml volumetric flask, diluted to 12.5 ml with water and then to volume with 24% (w/v) TCA solution. A volume of one milliliter of each of these solutions contains the equivalent of 0 (blank), 0.050, 0.075, 0.100 and 0.125 mg citric acid respectively (Figure 1).

3.2.5.3 Preparation of the Sample

3.2.5.3.1 Procedure

One milliliter of blank solution, standard citrate solutions and TCA filtrates were pipetted out into a series of test tubes and the tubes were placed in

random order; the maximum tubes in one group should be about twenty. 1.3 ml of pyridine was added to each tube and each tubes were swirled to mix its contents. 5.7 ml of acetic anhydride was added to the first tube, stoppered, swirled the tubes to mix its contents and immediately it was placed in a large, thermostatically controlled water bath at 32°C. The same was done with each tube and the tubes were left in the water bath for 30 minutes. The optical density of the standard and filtrate solutions were measured relative to the blank using a wave length of 428 nm and a light path of one centimeter. The ambient temperature should not be less than 20°C.

3.2.6 Estimation of Calcium

Estimation of calcium was done using atomic absorption spectrophotometer. (Perkin – Elmer model, - AAS 3110)

3.2.7 Estimation of Lactose

Estimation was done using the procedure described by Feitosateles *et al.* (1978) (Figure 2)

3.2.8 Estimation of Chloride

The chloride content of the milk was estimated according to Jenness (1988) by titrating milk with 0.1 N silver nitrate solution with potassium chromate as indicator. Titer value was multiplied by 0.0355 to obtain chloride per cent of the sample.

3.3 THERAPEUTIC TRIALS

The clinical and subclinical mastitis cases selected for therapeutic trials were divided into five groups. Suitable antibiotics were selected depending on the clinical signs and antibiotic sensitivity tests wherever possible.

3.3.1 Sub Clinical Mastitis

Group 1 (seven animals) - Treated with Tri-sodium citrate @ 30 mg/kg body weight orally once daily for five days

Group 2 (six animals) - Treated with antibiotic parenterally for three to five days

Group 3 (seven animals) - Treated with Tri-sodium citrate @ 30 mg/kg body weight orally once daily for five days and antibiotic parenterally for three to five days.

3.3.2 Clinical Mastitis

Group 1 (seven animals) - Treated with Tri-sodium citrate @ 30 mg/kg body weight orally once daily for five days and antibiotic parentarally for three to five days.

Group 2 (eight animals) - Treated with antibiotic parenterally for three to five days.

Animals were allotted to each group randomly. The milk samples were collected, from the affected and non affected quarters of all the cases for estimation of pH, citric acid, calcium, lactose and chloride on zero, third and sixth day of treatment.

3.4 STATISTICAL ANALYSIS

Data were analysed as per the method described by Snedecor and Cochran (1994).

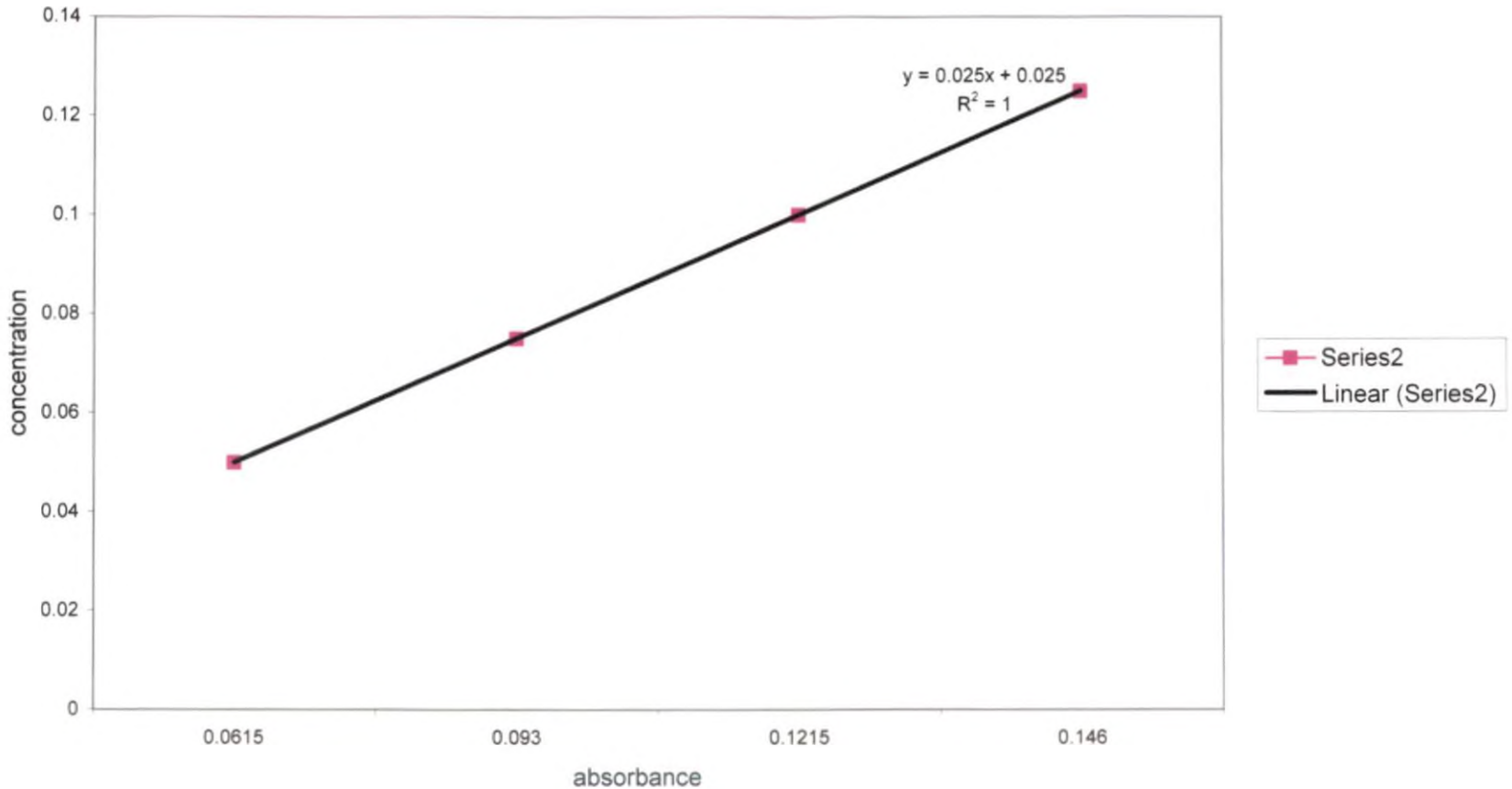

Fig1: Standard curve for citric acid estimation

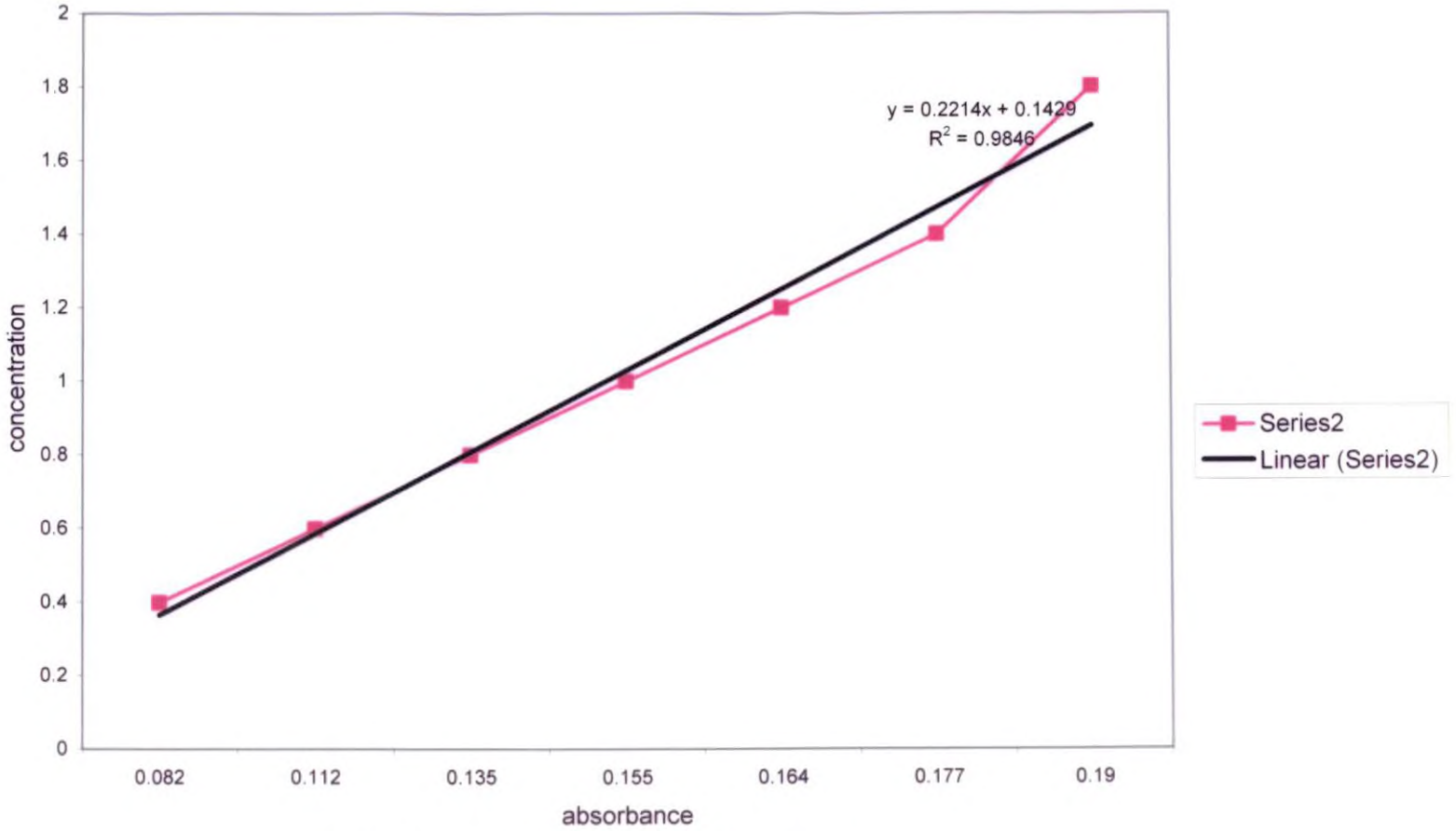

Fig2: Standard curve for lactose estimation

Results

4. RESULTS

Twenty cases of subclinical mastitis and fifteen cases of clinical mastitis were utilized for the experiment. Subclinical mastitis cases were divided into three groups and clinical mastitis cases were divided into two groups based on the therapeutic trial adopted. Data obtained were analysed statistically wherever required. Efficacy of the treatment was assessed by clinical improvement and changes in the physical, microbiological and biochemical characters of milk.

4.1 CLINICAL OBSERVATION

On clinical examination of the subclinical mastitis group, no obvious abnormality could be detected. The temperature, pulse and respiratory rate were found to be normal. No abnormality in the gross appearance of the udder and milk was observed in this group.

In clinical mastitis group, an elevated rectal temperature (102.8°F-103.8°F) was observed in three cases. Marked reduction of feed intake , elevated respiratory rate and congested mucous membrane were observed in one of these cases. In all the other cases the clinical parameters were within the normal range.

Physical characters of the milk and udder were observed and presented in the Table 1.In clinical mastitis the affected quarters were hard , hot and painfull on palpation in 13 cases and in two cases the affected quarters were found to be fibrosed also. Milk from the affected quarters changed to abnormal colour and consistency. Milk was thin , watery, yellowish, straw colored , with clots and flakes.

In group 1 animals (treated with trisodium citrate and antibiotic) clinical cure was observed in all the seven animals. These animals started secreting normal milk from fifth day of treatments (five cases showed clinical cure by third day itself).

In group 2 (treated with antibiotic alone) milk came to the normal level in three cases by the 3rd day. In all other cases normal milk was observed from sixth day onwards.

California Mastitis Test

Milk samples from 38 cases suspected for subclinical mastitis were tested using CMT reagent. Twenty four cases were found to be positive for CMT and 14 cases were negative. On cultural examination of CMT positive milk samples, 20 samples yielded bacterial growth .

CMT was conducted during and after treatment in all the 20 positive cases. In animals treated with tri-sodium citrate alone (Group 1) CMT was found to be negative in all the seven cases by fifth day of treatment. In group 2 animals (treated with antibiotic) CMT was found to be negative in all the six cases by fourth day of treatment, whereas CMT was found negative in all seven cases by third day of treatment in group 3 animals which were treated with antibiotic and tri-sodium citrate.

4.2 MICROBIAL CHARACTERS

4.2.1 Bacteriological Findings

4.2.1.1 Subclinical Mastitis

Twenty four milk samples found to be positive for CMT were subjected to cultural examination. Twenty samples yielded growth on agar plate. No growth was noticed in four samples. From 16 milk samples gram positive bacteria was isolated. From three samples gram negative coccobacillary organisms were isolated.One sample yielded combined infection of both gram positive cocci and gram negative cocco bacilli .

Cultural examination of milk on sixth day after treatment, three cases in group 1 yielded no growth and in others number of bacterial colonies found to be reduced. All cases in groups 2 and 3 yielded no growth by sixth day of treatment.

4.2.1.2 Clinical Mastitis

Fifteen milk samples were subjected to cultural examination. Gram positive cocci were isolated from nine cases and gram negative coccobacilli were isolated from four cases. A combined infection of both these organisms observed in the remaining two cases .

Bacteriological cure was observed in all the seven cases in group 1 by sixth day of treatment whereas the same was observed only in six cases in group 2.

4.2.2 *In Vitro* Antibacterial Susceptibility Tests on the Isolates

The isolates obtained, on cultural examination, was found to be sensitive to various chemotherapeutic agents (Table 2 and Table 3). The treatment was adopted in due consideration to the antimicrobial susceptibility testing wherever possible.

4.2.2.1 Subclinical Mastitis

All seventeen gram positive isolates obtained were found to be hundred percentage sensitive to enrofloxacin, cefotaxime and ciprofloxacin. Among these, 82.4 per cent of the isolates were found sensitive to ampicillin, 76.5 per cent sensitive to trimethoprim, gentamicin and oxytetracycline, 70.6 per cent were found to be sensitive to chloramphenicol, 47.1 per cent sensitive to cloxacillin and 41.2 per cent sensitive to sulphadiazine and streptomycin. These gram-positive coccal organisms showed 58.8 per cent resistant to sulphadiazine and streptomycin and 52.9 per cent to cloxacillin.

All of the four gram negative bacteria isolated were found sensitive to chloramphenicol, trimethoprim and gentamicin. Three of them were found sensitive to enrofloxacin and ciprofloxacin (75 percent). Two isolates were found sensitive to ampicillin, cloxacillin and cefotaxime (50 percent). One among the isolates were found sensitive to sulphadiazine, streptomycin and oxytetracycline (25 percent) (Table 2). Seventy five per cent of the isolates showed resistance to Sulphadiazine , streptomycin and oxytetracycline. And 50 per cent of the isolates showed resistance to ampicillin, cloxacillin and cefotaxime.

4.2.2.2 Clinical Mastitis

Eleven gram positive isolates were obtained and all the isolates were sensitive to enrofloxacin and ciprofloxacin. 90.9 percentage were sensitive to chloramphenicol, oxytetracycline and cefotaxime, 72.7 percentage to trimethoprim and gentamicin, 63.6 percentage to ampicillin and cloxacillin, 36.4 percentage to sulphadiazine, and 9.1 percentage to streptomycin.

Among the 17 isolates, six were gram negative coccobacilli and 100 percentage of the isolates were sensitive to chloramphenicol, enrofloxacin, cefotaxime, ciprofloxacin and gentamicin. Fifty per cent were sensitive to cloxacillin and 33.3 per cent to ampicillin and sulphadiazine (Table 3).All the isolates were found resistant to streptomycin and 66.7 per cent found resistant to ampicillin and sulphadiazine.

4.3 BIOCHEMICAL CHARACTERS

Biochemical parameters of the milk such as pH, citric acid, lactose, calcium and chloride levels showed significant difference between the milk from normal quarter and that of affected quarters in both clinical and subclinical mastitis. The various parameters in different groups were presented in Tables 4, 5, 6, 7 and 8.

4.3.1 Biochemical Characters of Milk from Normal and Affected Quarters in Mastitis

4.3.1.1 pH

4.3.1.1.1 Subclinical Mastitis

The mean values of pH of milk in cows affected with subclinical mastitis before treatment were 6.848 ± 0.16, 6.835 ± 0.19 and 6.85 ± 0.12 in group 1, 2 and 3 respectively. These values showed significant increase (P≤ 0.01) when compared to the mean value of the pH of milk (6.508 ± 0.06) of normal quarter (Table 9).

The mean values of pH of milk of affected quarters significantly decreased (P≤0.01) to 6.48 ± 0.09, 6.645 ± 0.18 and 6.567 ± 0.08 in groups 1, 2 and 3 respectively after the treatment (Table 9).

4.3.1.1.2 Clinical mastitis

The mean value of pH of the milk in clinical mastitis were found to be 7.483 ± 0.12 and 7.533 ± 0.38 in the group 1 and 2 respectively. Significant increase (P≤ 0.01) in the pH of milk when compared to the milk of non affected quarter (6.6 ± 0.15) was observed (Table 10).

After treatment, the mean values of pH of milk of affected quarters decreased significantly (P≤0.01) to 6.55 ± 0.12, 6.683 ± 0.15 and became normal in groups 1 and 2 respectively after the treatment (Table 10).

4.3.1.2 Citric Acid

4.3.1.2.1 Subclinical mastitis

The mean value of citric acid in milk of cows with subclinical mastitis before treatment were 104.833 ± 10.85, 92.5 ± 16.28 and 116.167 ± 10.09 mg/dl in groups 1, 2 and 3 respectively. A significant decrease (P≤0.01) was noticed

when compared to the milk of non affected quarter having mean citric acid value of 152.9 ± 12.279 mg/dl (Table 9).

After treatment, the mean values of citric acid level of mastitis milk was found to be increased significantly (P≤0.01) to 146.66 ± 17.97, 126.167 ± 5.6 and 141.833 ± 9.93 mg/dl and became normal in groups 1, 2 and 3 respectively (Table 9).

4.3.1.2.2 Clinical mastitis

The mean citric acid value in the milk of normal quarter in clinical mastitis groups was 145 ± 21.55 mg/dl. A significant decrease (P≤0.01) of mean citric acid value of milk of affected quarter was observed. The values were 60.167 ± 7.55 and 60.183 ± 6.85 mg/dl in groups 1 and 2 respectively (Table 10).

After treatment, the mean values of citric acid in milk of affected quarters were found to be increased significantly (P≤0.01) to 145.5 ± 5.15, 128.833 ± 16.86 mg/dl and became normal in groups 1 and 2 respectively (Table 10).

4.3.1.3 Lactose

4.3.1.3.1 Subclinical mastitis

The mean lactose concentrations in the subclinical mastitis milk before the treatment were 2.483 ± 0.50, 2.583 ± 1.04 and 3.783 ± 0.21 g/dl in the groups 1, 2 and 3 respectively. It showed significant decrease (P≤0.01) from the mean level of lactose in the milk of non affected quarter having a value of 4.28 ± 0.38 g/dl (Table 9).

A significant increase (P≤0.01) in the mean lactose level of mastitis milk was observed after the treatment. The values were 4.133 ± 0.58, 3.917 ± 0.50 and 4.417 ± 0.15 g/dl in groups 1, 2 and 3 respectively (Table 9).

4.3.1.3.2 Clinical mastitis

In clinical mastitis, the mean value of lactose in mastitis milk before treatment was found to be 1.3 ± 0.36 and 1.6 ± 0.53 g/dl in groups 1 and 2 respectively. Significant decrease (P≤0.01) when compared to the milk of non affected quarters having a mean lactose value of 4.36 ± 0.57 g/dl was observed (Table 10).

After the treatment, the mean values of lactose of mastitis milk was found to be increased significantly (P≤0.01) to 4.25 ± 0.25 and 4.233 ± 0.43 g/dl in groups 1 and 2 respectively (Table 10).

4.3.1.4 Chloride

4.3.1.4.1 Subclinical mastitis

The mean chloride value in the milk of subclinical mastitis before the treatment were 0.17 ± 0.03, 0.158 ± 0.04 and 0.153 ± 0.01 g/dl in groups 1, 2 and 3 respectively. These values showed significant increase (P≤0.01) when compared with the level of chloride (0.104 ± 0.0125 g/dl) in the milk of nonaffected quarters (Table 9).

The mean chloride levels after the treatment were 0.11 ± 0.01, 0.124 ± 0.02 and 0.109 ± 0.01 g/dl in groups 1, 2 and 3 respectively. These values showed significant decrease (P≤0.01) from the pretreatment values (Table 9).

4.3.1.4.2 Clinical mastitis

The mean chloride values of milk of affected quarters in clinical mastitis before the treatment were found to be 0.275 ± 0.04 and 0.289 ± 0.04 g/dl in groups 1 and 2 respectively. These values showed significant increase (P≤0.01) when compared to the chloride level (0.1139 ± 0.006 g/dl) in the milk of non affected quarter (Table 10).

The mean levels of chloride in the milk of affected quarter after the treatment were 0.116 ± 0.01 and 0.123 ± 0.01 g/dl in groups 1 and 2 respectively. These values showed significant decrease (P≤0.01) when compared to the mean chloride levels before the treatment (Table 10).

4.3.1.5 Calcium

4.3.1.5.1 Sub clinical mastitis

The pretreatment mean values of calcium in the milk of subclinical mastitis were 101.667 ± 2.94, 103.333 ± 4.68 and 107.667 ± 5.28 mg/dl in groups 1, 2 and 3 respectively. A significant decrease was observed (P≤0.01) when compared to the milk of non affected quarters having a mean value of 123.3 ± 6.66 mg/dl (Table 9).

After the treatment, the mean levels of calcium were found to be increased to 117.667 ± 5.2, 112.5 ± 2.51 and 116.5 ± 5.58 mg/dl in groups 1, 2 and 3 respectively. These values showed significant increase (P≤0.01) from the pre treatment mean values (Table 9).

4.3.1.5.2 Clinical mastitis

The mean value of calcium in the milk of normal quarters were 123 ± 7.11 mg/dl. A significant decrease was observed (P≤0.01), in the mean value of calcium in the mastitis milk. The values were 74.0 ± 25.81 and 71.0 ± 28.95 mg/dl in the groups 1 and 2 respectively (Tables 10).

After the treatment, the mean levels of calcium were 119.0 ± 7.29 and 113.333 ± 7.12 mg/dl in groups 1 and 2 respectively. These values showed significant increase (P≤0.01) from the pretreatment mean values (Table 10).

4.4 EFFICACY OF TREATMENT

4.4.1 Subclinical Mastitis

The post treatment values of different parameters of group 1 and group 3 cases were compared with those values of group 2 cows.

Significant difference was observed ($P \leq 0.05$) between the post treatment citric acid levels and calcium levels in group 1 cows compared to that of group 2 cows (Table 9).

No much significant difference was observed between the post treatment values for the other parameters.

Among group 1 animals, in four cases, the level of citric acid returned to the normal range by third day of treatment. By sixth day of treatment the level was within the normal range in all the cases. Among group 2 animals, only in three cases the level came to normal range by sixth day of treatment. Among group3 animals in all cases the level of citric acid returned to normal range by sixth day of treatment.

All the cases in group 1 and 3 the pH level came to normal level by sixth day of treatment. Among group 2 animals in four cases the pH level came to normal by sixth day of treatment (Table 11).

Complete bacteriological cure was noticed in three cases among group 1 animals (tri-sodium citrate alone) on cultural examination by sixth day of treatment. In the remaining four cases the number of bacterial colonies found to be reduced. In the other two groups, for all the cases, complete bacteriological cure was observed by sixth day (Table 12).

In group 1 animals by sixth day of treatment, an increase in milk production by 0.382 ± 0.06 l/day/animal was observed. But in group 2 animals

0.033 ± 0.002 l/day/animal increase in milk production was only observed (Table 13).

4.4.2 Clinical Mastitis

In group1 animals (oral tri-sodium citrate along with parenteral antibiotic) clinical cure observed in all the seven animals by fifth day of treatment. Among this in five cases the secretions from the affected glands became normal in appearance (clots disappeared) by third day of treatment itself. But in group 2 animals (antibiotic alone) only in three cases milk became normal by third day.

The post treatment biochemical values in milk did not differ significantly between the treatment groups.

The levels of citric acid and pH came to normal level by sixth day of treatment in all cases among group1 animals. Whereas in group 2, only in 4 cases the levels came to the normal range (Table14).

Bacteriological cure observed in all the cases among group 1 animals by sixth day of treatment. Whereas bacteriological cure observed in 6 cases out of 8 cases by sixth day of treatment in group 2 (Table 14).

In two cases under group 1, the animal regained the normal milk yield. But in all other cases under group 1 and 2, milk yield found to be reduced than initial level.

Table 1. Physical character of milk and udder in clinical mastitis group

No. of cases	Udder	Milk	Organism isolated
2	Hard, hot and painful	White milk with fine clots	Gram positive cocci
2	Hot and painful	Slight yellow tint in milk with fine clots	"
3	Hard, hot and painful	Cream coloured milk with flakes	"
2	Hard, hot and painful	Yellow watery milk with fine clots	"
1	Hard, hot and painful	Yellow coloured watery milk	Gram negative coccobacilli + Gram positive cocci
2	Hard, fibrosed and painful	Yellow watery milk with clots	Gram negative coccobacilli
2	Hard, hot and painful	Straw coloured watery milk	Gram negative coccobacilli
1	Hard and hot	Yellow curd like milk	Gram positive cocci

Table 2. *In vitro* antimicrobial sensitivity of the isolates – subclinical mastitis

Antibiotic	Sensitive		Resistant	
	Gram positive cocci	Gram negative bacilli	Gram positive cocci	Gram negative bacilli
1. Chloramphenicol	12 (70.6)	4 (100.0)	5 (29.4)	0 (Nil)
2. Ampicillin	14 (82.4)	2 (50.0)	3 (17.6)	2 (50.0)
3. Trimethoprim	13 (76.5)	4 (100.0)	4 (23.5)	0 (Nil)
4. Enrofloxacin	17 (100.0)	3 (75.0)	0 (Nil)	1 (25.0)
5. Ciprofloxacin	17 (100.0)	3 (75.0)	0 (Nil)	1 (25.0)
6. Sulphadiazine	7 (41.2)	1 (25.0)	10 (58.8)	3 (75.0)
7. Gentamicin	13 (76.5)	4 (100.0)	4 (23.5)	0 (Nil)
8. Cloxacillin	8 (47.1)	2 (50.0)	9 (52.9)	2 (50.0)
9. Streptomycin	7 (41.2)	1 (25.0)	10 (58.8)	3 (75.0)
10. Oxytetracycline	13 (76.5)	1 (25.0)	4 (23.5)	3 (75.0)
11. Cefotaxime	17 (100.0)	2 (50.0)	0 (Nil)	2 (50.0)

Values in the parenthesis denotes percentage

Table 3. *In vitro* antimicrobial sensitivity of the isolates – clinical mastitis

Antibiotic	Sensitive		Resistant	
	Gram positive cocci	Gram negative bacilli	Gram positive cocci	Gram negative bacilli
1. Chloramphenicol	10 (90.9)	6 (100.0)	1 (9.1)	0 (Nil)
2. Ampicillin	7 (63.6)	2 (33.3)	4 (36.4)	4 (66.7)
3. Trimethoprim	8 (72.7)	5 (83.3)	3 (27.3)	1 (16.7)
4. Enrofloxacin	11 (100.0)	6 (100.0)	0 (Nil)	0 (Nil)
5. Ciprofloxacin	11 (100.0)	6 (100.0)	0 (Nil)	0 (Nil)
6. Sulphadiazine	4 (36.4)	2 (33.3)	7 (63.6)	4 (66.7)
7. Gentamicin	8 (72.7)	6 (100.0)	3 (27.3)	0 (Nil)
8. Cloxacillin	7 (63.6)	3 (50.0)	4 (36.4)	3 (50.0)
9. Streptomycin	1 (9.1)	0 (Nil)	10 (90.9)	6 (100.0)
10. Oxytetracycline	10 (90.9)	5 (83.3)	1 (9.1)	1 (16.7)
11. Cefotaxime	10 (90.9)	6 (100.0)	1 (9.1)	0 (Nil)

Values in the parenthesis denotes percentage

Table 4. Biochemical changes in milk - subclinical mastitis – Group 1 (Treated with tri-sodium citrate)(Mean±SE)

No. of animals	Parameters	Zero day	Third day	Sixth day	Normal milk from non-affected quarter
7	pH	6.848 ± 0.16	6.595 ± 0.12	6.48 ± 0.09	6.508 ± 0.06
	Citric acid (mg/dl)	104.833 ± 10.85	131.0 ± 20.44	146.66 ± 17.97	152.9 ± 12.279
	Lactose (g/dl)	2.483 ± 0.50	3.633 ± 1.07	4.133 ± 0.58	4.28 ± 0.38
	Chloride (g/dl)	0.17 0 ± 0.03	0.145 ± 0.02	0.110 ± 0.01	0.104 ± 0.0125
	Calcium (mg/dl)	101.667 ± 2.94	109.50 ± 3.33	117.667 ± 5.2 0	123.3 ± 6.66

Table 5. Biochemical changes in milk subclinical mastitis – Group 2 (Treated with antibiotic alone) (Mean±SE)

No. of animals	Parameters	Zero day	Third day	Sixth day	Normal milk from non-affected quarter
6	pH	6.835 ± 0.19	6.7 ± 0.24	6.645 ± 0.18	6.508 ± 0.06
	Citric acid (mg/dl)	92.5 ± 16.28	111.333 ± 11.86	126.167 ± 5.6	152.9 ± 12.279
	Lactose (g/dl)	2.583 ± 1.04	3.433 ± 0.62	3.917 ± 0.50	4.28 ± 0.38
	Chloride (g/dl)	0.158 ± 0.04	0.139 ± 0.02	0.124 ± 0.02	0.104 ± 0.0125
	Calcium (mg/dl)	103.333 ± 4.68	108.333 ± 3.88	112.5 ± 2.51	123.3 ± 6.66

Table 6. Biochemical changes in milk Subclinical mastitis – Group 3 (Treated with Antibiotic and trisodium citrate) (Mean±SE)

No. of animals	Parameters	Zero day	Third day	Sixth day	Normal milk from non-affected quarter
7	pH	6.85 ± 0.12	6.611 ± 0.03	6.567 ± 0.08	6.508 ± 0.06
	Citric acid (mg/dl)	116.167 ± 10.09	130.5 ± 3.62	141.833 ± 9.93	152.9 ± 12.279
	Lactose (g/dl)	3.783 ± 0.21	4.150 ± 0.24	4.417 ± 0.15	4.28 ± 0.38
	Chloride (g/dl)	0.153 ± 0.01	0.131 ± 0.01	0.109 ± 0.01	0.104 ± 0.0125
	Calcium (mg/dl)	107.667 ± 5.28	112.333 ± 11.15	116.5 ± 5.58	123.3 ± 6.66

Table 7. Biochemical changes in milk - Clinical Mastitis – Group 1 (Treated with Antibiotic and trisodium citrate) (Mean±SE)

No. of animals	Parameters	Zero day	Third day	Sixth day	Normal milk from non-affected quarter
7	pH	7.483 ± 0.12	6.85 ± 0.23	6.55 ± 0.12	6.6 ± 0.15
	Citric acid (mg/dl)	60.167 ± 7.55	127.167 ± 14.26	145.5 ± 5.15	145.0 ± 21.55
	Lactose (g/dl)	1.3 ± 0.36	3.083 ± 0.29	4.25 ± 0.25	4.36 ± 0.57
	Chloride (g/dl)	0.275 ± 0.04	0.159 ± 0.03	0.116 ± 0.01	0.1139 ± 0.006
	Calcium (mg/dl)	74.0 ± 25.81	115.333 ± 6.06	119 ± 7.29	123.0 ± 7.11

Table 8. Biochemical changes in milk - Clinical Mastitis – Group 2 (Treated with Antibiotic alone) (Mean±SE)

No. of animals	Parameters	Zero day	Third day	Sixth day	Normal milk from non-affected quarter
8	PH	7.533 ± 0.38	7.0 ± 0.24	6.683 ± 0.15	6.6 ± 0.15
	Citric acid (mg/dl)	60.183 ± 6.85	92.3 ± 40.34	128.833 ± 16.86	145.0 ± 21.55
	Lactose (g/dl)	1.6 ± 0.53	3.25 ± 0.65	4.233 ± 0.43	4.36 ± 0.57
	Chloride (g/dl)	0.289 ± 0.04	0.181 ± 0.03	0.123 ± 0.01	0.1139 ± 0.006
	Calcium (mg/dl)	71.0 ± 28.95	94.0 ± 16.92	113.333 ± 7.12	123.0 ± 7.11

Table 9. Biochemical characters of milk in subclinical mastitic cows (Mean±SE)

Animal	Treatment		pH	Citric acid (mg/dl)	Lactose (g/dl)	Chloride (g/dl)	Calcium (mg/dl)
Normal milk non affected quarter			6.508 ± 0.06	152.9 ± 12.279	4.28 ± 0.38	0.104 ± 0.0125	123.3 ± 6.66
Group 1 (n = 7)	Tri-sodium citrate alone	Pretreatment	6.848 ± 0.16	104.833 ± 10.85	2.483 ± 0.50	0.17 ± 0.03	101.667 ± 2.94
		Post treatment	6.48 ± 0.09^{S}	146.66 ± 17.97^{S}	4.133 ± 0.58^{S}	0.110 ± 0.01^{S}	117.667 ± 5.2^{S}
Group 2 (n = 6)	Antibiotic Alone	Pretreatment	6.835 ± 0.19	92.5 ± 16.28	2.583 ± 1.04	0.158 ± 0.04	103.333 ± 4.68
		Post treatment	6.645 ± 0.18^{S}	126.167 ± 5.6^{S}	3.917 ± 0.50^{S}	0.124 ± 0.02^{S}	112.5 ± 2.51^{S}
Group 3 (n = 7)	Tri-sodium citrate and antibiotic	Pretreatment	6.85 ± 0.12	116.167 ± 10.09	3.783 ± 0.21	0.153 ± 0.01	107.667 ± 5.28
		Post treatment	6.567 ± 0.08^{S}	141.833 ± 9.93^{S}	4.417 ± 0.15^{S}	0.109 ± 0.01^{S}	116.5 ± 5.58^{S}

S – Significant (P≤0.01)

Table 10. Biochemical characters of milk in clinical mastitic cows (Mean±SE)

Animal	Treatment		pH	Citric acid (mg/dl)	Lactose (g/dl)	Chloride (g/dl)	Calcium (mg/dl)
Normal milk from non affected quarter			6.6 ± 0.15	145.0 ± 21.55	4.36 ± 0.57	0.1139 ± 0.006	123 ± 7.11
Group 1 (n = 7)	Antibiotic and Tri-sodium citrate	Pretreatment	7.483 ± 0.12	60.167 ± 7.55	1.30 ± 0.36	0.275 ± 0.04	74.0 ± 25.81
		Post treatment	6.55 ± 0.12^{S}	145.5 ± 5.15^{S}	4.25 ± 0.25^{S}	0.116 ± 0.01^{S}	119.0 ± 7.29^{S}
Group 2 (n = 8)	Antibiotic Alone	Pretreatment	7.533 ± 0.38	60.183 ± 6.85	1.6 ± 0.53	0.289 ± 0.04	71.0 ± 28.95
		Post treatment	6.683 ± 0.15^{S}	128.833 ± 16.86^{S}	4.233 ± 0.43^{S}	0.123 ± 0.01^{S}	113.333 ± 7.12^{S}

S – Significant (P≤0.01)

Table 11. Efficacy of treatment with respect to Biochemical characters – subclinical mastitis

Group	Citric acid level in Milk				pH of milk			
	No. of animal							
	Third day		Sixth day		Third day		Sixth day	
	Normal	Below normal	Normal	Below normal	Normal	Below normal	Normal	Below normal
Group 1 (n=7) (Tri-Sodium citrate alone)	4 (57.14)	3 (42.86)	7 (100)	Nil	5 (71.43)	2 (28.57)	7 (100)	Nil
Group 2 (n=6) (Antibiotic alone)	1 (16.67)	5 (83.33)	3 (50)	3 (50)	2 (33.33)	4 (66.67)	4 (66.67)	2 (33.33)
Group 3 (n=7) (Antibiotic + Tri-Sodium citrate)	4 (57.14)	3 (42.86)	7 (100)	Nil	5 (71.43)	2 (28.57)	7 (100)	Nil

Values in the parenthesis denotes percentage

Table 12. Efficacy of treatment with respect to microbial characters – Subclinical mastitis

	Bacteriological cure
Group 1 (n=7) (Tri-Sodium citrate alone)	3 (42.86)
Group 2 (n=6) (Antibiotic alone)	6 (100)
Group 3 (n=7) (Antibiotic + Tri-Sodium citrate)	7 (100)

Values in the parenthesis denotes percentage

Table 13. Efficacy of treatment with respect to milk yield – Subclinical mastitis

	Milk yield in litres/day/animal
Group 1	Increased by 0.382 ± 0.06 l
Group 2	Increased by 0.033 ± 0.002 l
Group 3	Increased by 0.213 ± 0.03 l

Table 14. Efficacy of treatment with respect to Biochemical characters – Clinical Mastitis

	Citric acid and pH level in milk			
	No. of animal			
	Third day		Sixth day	
	Normal	Below normal	Normal	Below normal
Group 1 (7 animals	2 (28.57)	5 (71.43)	7 (100)	Nil
Group 2 (8 animals)	Nil	8(100)	4(50)	4(50)

Values in the parenthesis denotes percentage

Table 15. Efficacy of treatment with respect to microbial characters – Clinical Mastitis

	No. of animals	No. of animal	
		Clinical cure	Bacteriological cure
Group 1 (Antibiotic + Trisodium citrate)	7	7 (100)	7 (100)
Group 2 (Antibiotic Alone)	8	8 (100)	6 (75)

Values in the parenthesis denotes percentage

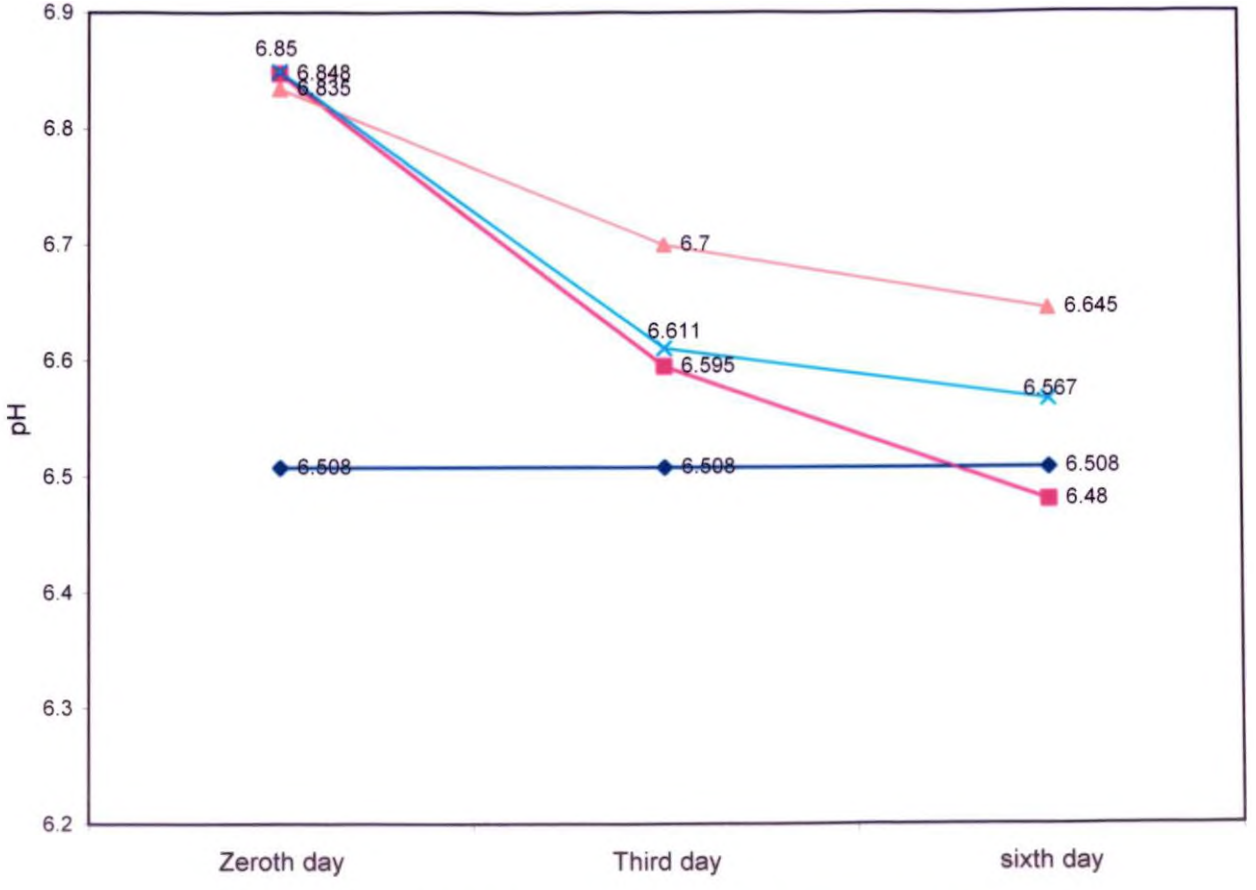

Fig3:pH of milk in sub clinical mastitis

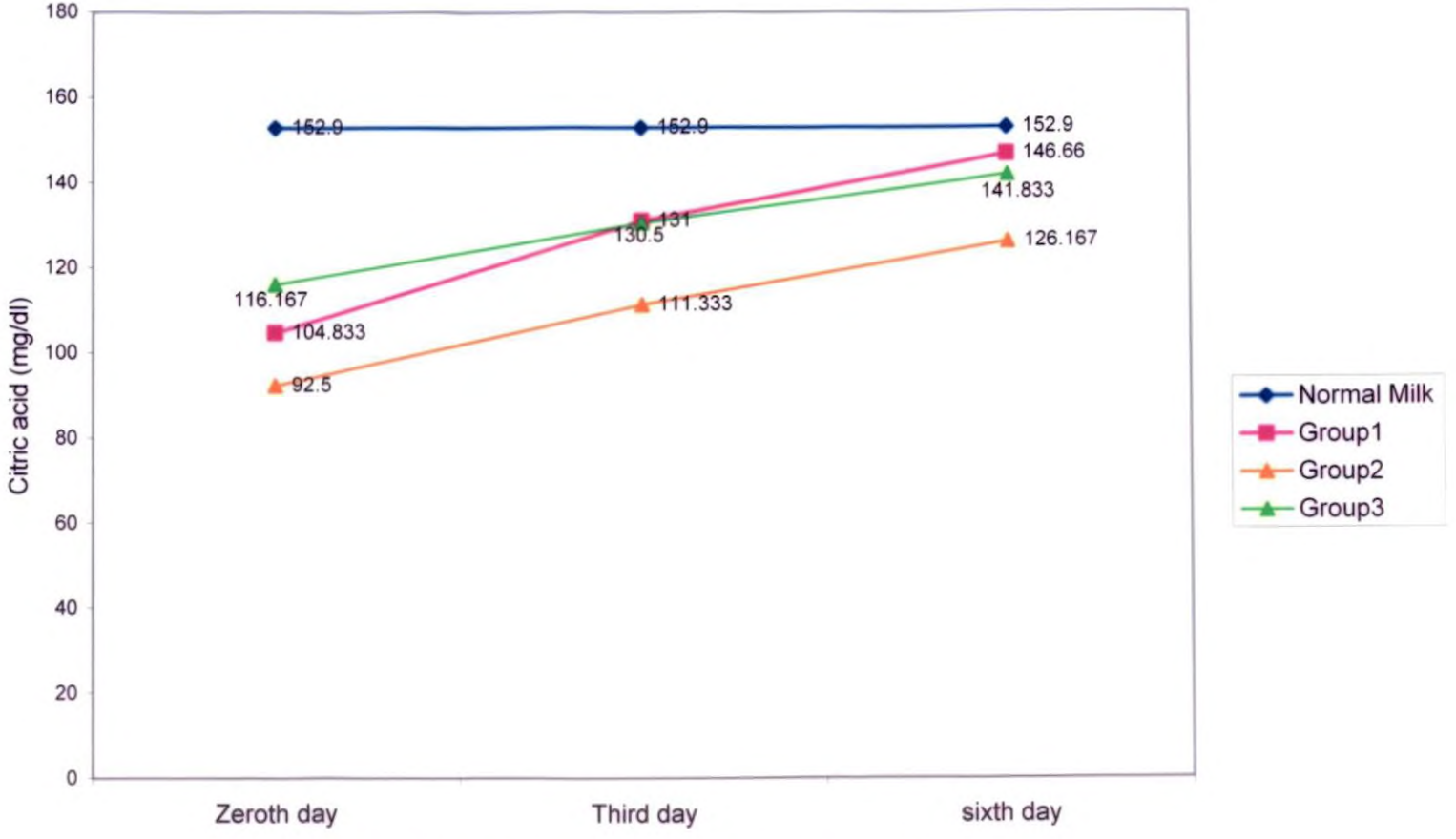

Fig4: Citric acid level of milk in Subclinical Mastitis

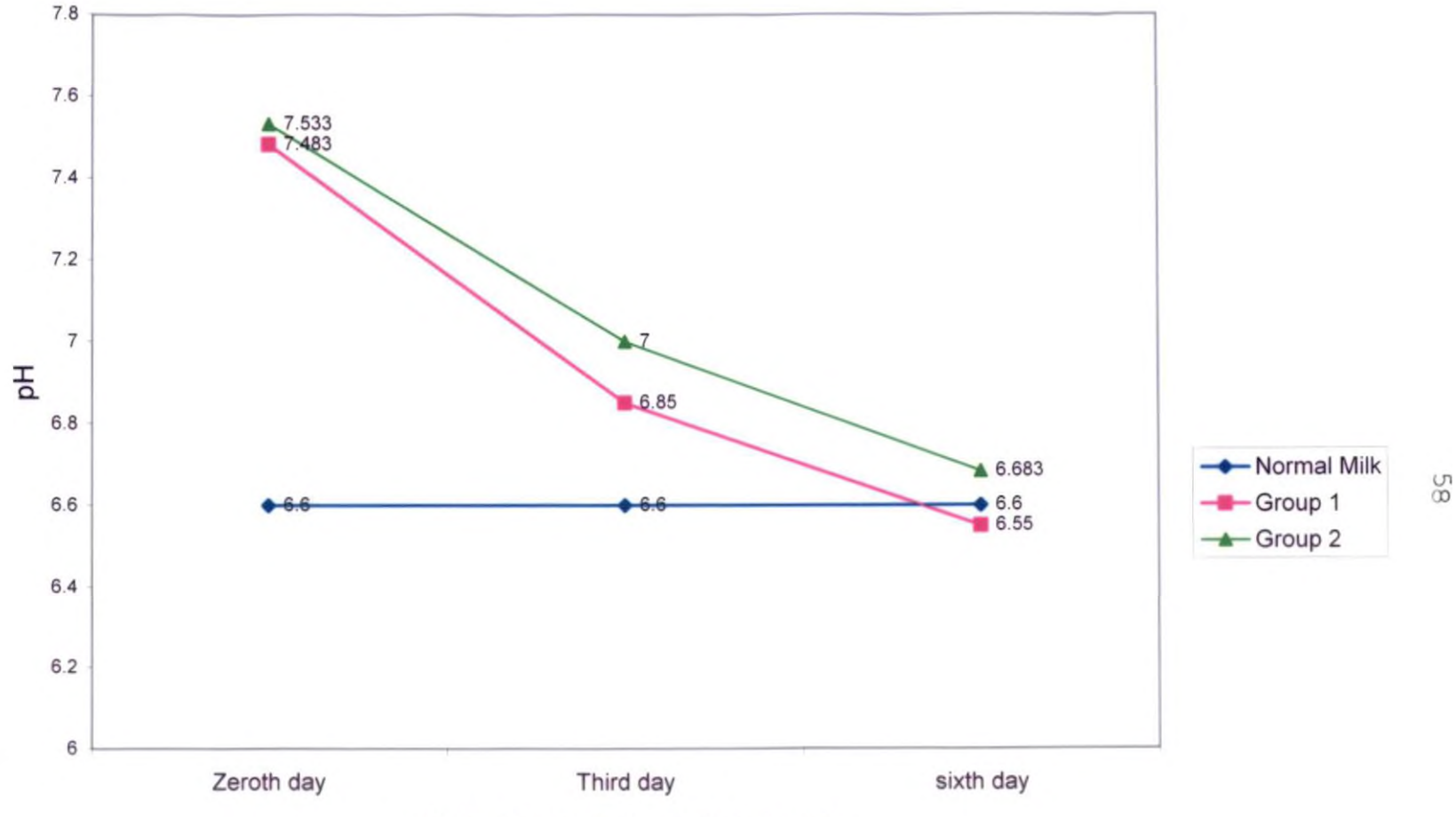

Fig5: pH of milk in clinical mastitis

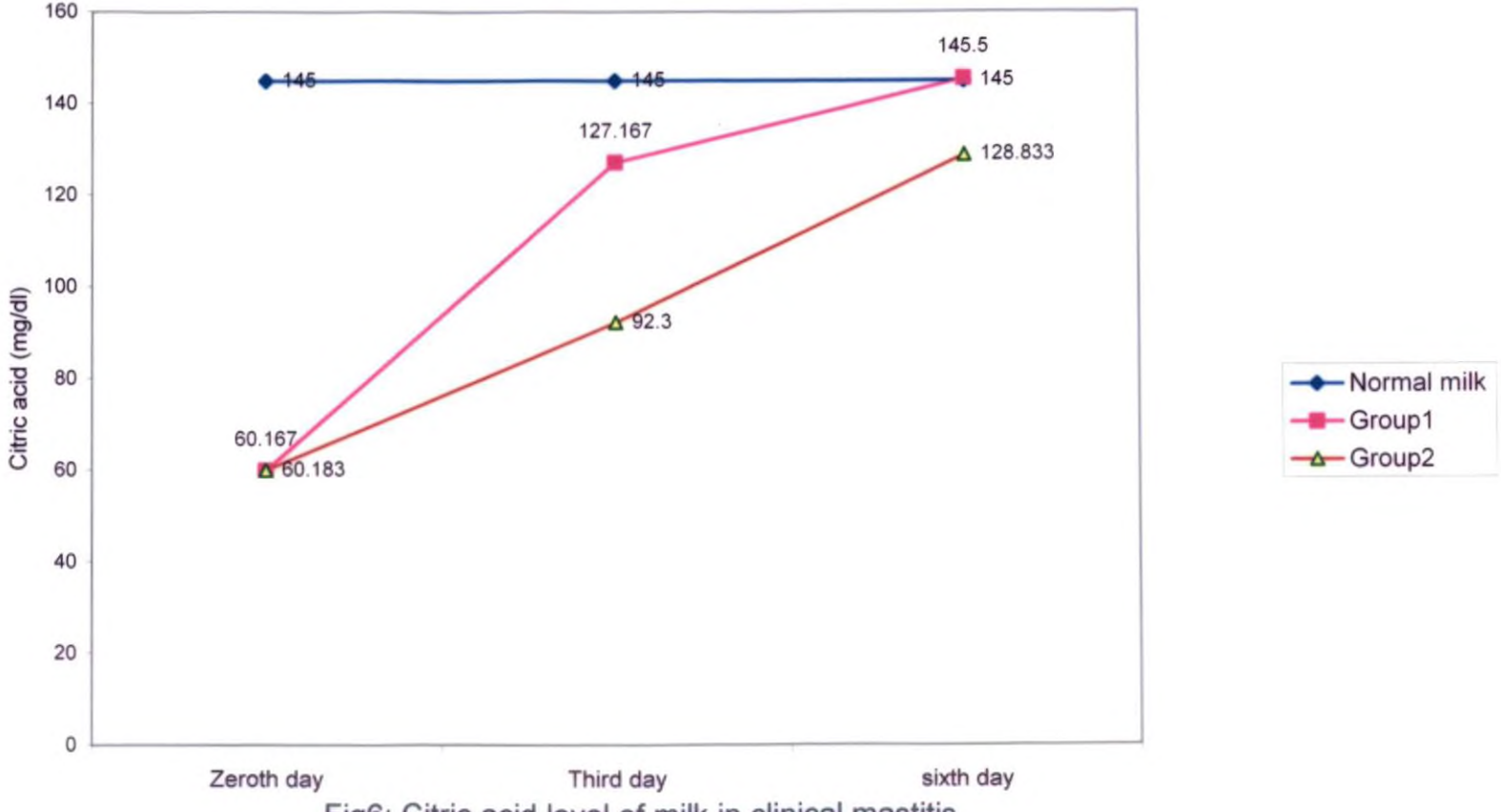

Fig6: Citric acid level of milk in clinical mastitis

Discussion

5. DISCUSSION

Mastitis is a major problem affecting the milk production in dairy cattle. In most of the cases milk production will not be restored after the treatment with antibiotic due to various reasons. Efficacy of tri-sodium citrate in the treatment of both subclinical and clinical mastitis in cattle is discussed.

5.1 CLINICAL OBSERVATION

In subclinical mastitis, the clinical parameters were normal in all animals and there were no visible abnormalities in the milk and udder. The findings were in agreement with the reports of Schalm *et al.* (1971), Dhote *et al.* (1999) and Radostits *et al.* (2000).

In clinical mastitis group, elevated temperature and other systemic signs were observed in one case and the organism isolated from this case was gram-negative coccobacilli. These findings were in agreement with the reports of Radostits *et al.* (2000).

Gram positive cocci were isolated from cases where the secretions from the affected quarters appeared white, slight yellow tinted, cream coloured or yellow coloured with flakes or fine clots. These findings were in agreement with Rai *et al.* (1998) and Radostits *et al.* (2000).

Gram negative coccobacilli were isolated from cases where milk appeared straw or slight yellow tinted having watery consistency. This was in agreement with Lohuis *et al.* (1990), Pyorala *et al.* (1994) and Morin *et al.* (1998). Where as Orbitzhauser *et al.* (1995) reported that cows were more likely to have watery milk when clinical mastitis was caused by streptococci rather than Enterobacteriaceae.

California mastitis test

The polymerized DNA originating from cells of the inflammatory exudates in mastitic milk is the component responsible for positive CMT reaction (Carrol and Schalm,1962). Among the cases suspected for subclinical mastitis 63.16 percentage of the samples were found to be positive on CMT. On cultural examination, 83.33 percentage of CMT positive samples were found to yield growth. The findings were in agreement with reports of Pal and Verma (1988), while Rindsig et al. (1979) observed only 60 percentages of samples culturally positive which were found positive on CMT.

After the treatment, CMT was found to be negative in all the cases by fifth, fourth and third day of treatment in groups 1, 2 and 3 respectively.

5.2 MICROBIAL CHARACTERS

5.2.1 Bacteriological Findings

5.2.1.1 Subclinical Mastitis

In the present study, from 85 percentage of the samples gram positive bacteria and from remaining 15 percentage of the samples, gram negative coccobacillary organisms were isolated. These findings were in full agreement with the reports of Ali *et al.* (1990), Saxena *et al.* (1993), Saini *et al.* (1994), Shlke *et al.* (1998) and Ross *et al.* (2001). The higher incidence of gram positive cocci may be due to the fact that staphylococci are tissue invaders and are resistant to most of the antibiotics due to their indiscriminate use. Moreover some staphylococcus strains can destroy penicillins through the production of enzyme penicillinase. These organisms harbour the udder and teat and are major pathogens of subclinical mastitis which may flare up as clinical episodes subsequently (Prabhakar *et al.*, 1989).

After treatment, in 42.86 percentage of the cases under group 1 (treated with tri-sodium citrate alone) bacteriological cure was observed on sixth day of

treatment and in the remaining cases, number of bacterial colonies were found to be reduced. This finding was in agreement with the finding of Dhillon *et al.* (1995) that the tri-sodium citrate administration raised the milk citric acid level leading to reciprocal fall in milk pH to 6.5 which created unfavourable conditions for bacterial growth, thereby resulting in fall in bacterial count. In other treatment groups, complete bacteriological cure was observed by sixth day of treatment.

5.2.1.2 Clinical Mastitis

From 60 percentage of clinical mastitis, gram positive cocci organisms and from 26.67 percentage of cases gram negative cocco bacillary organisms were isolated and 13.33 percentage of cases yielded a mixed infection. This was in accordance with the earlier observations of Sears *et al.* (1991), Gupta *et al.* (1992), Rai and Senani (1997), Mandial *et al.* (1999) and Ghose *et al.* (2001). Where as Jones and Ward (1990) reported a higher incidence of gram negative organism (53 per cent) than the gram positive organisms.

In group 1 animals complete bacteriological cure was observed in all cases on sixth day which was treated with antibiotic plus tri-sodium citrate. Where as in group 2 animals bacteriological cure was observed only in 75 percentage of cases. These findings were in agreement with the results obtained by Pal *et al.* (1995).

5.2.2 *In Vitro* Antibacterial Susceptibility Testing

5.2.2.1 Subclinical Mastitis

In vitro antibiotic sensitivity studies on 17 gram positive isolates revealed that enrofloxacin, cefotaxime and ciprofloxacin were the most sensitive antibacterials followed by ampicillin, trimethoprim, gentamicin and oxytetracycline. Streptomycin and sulphadiazine were the least sensitive

antibacterial agent. These results were comparable with the observations of Ali *et al.* (1990), Saini *et al.* (1994) and Singh *et al.* (1994).

The sensitivity pattern of gram negative bacteria showed that chloramphenicol, trimethoprim and gentamicin were highly sensitive followed by enrofloxacin and ciprofloxacin. Sulphadiazine, streptomycin and oxytetracycline were the least sensitive. The present findings were comparable with the reports of Ross *et al.* (2001) and Nauriyal and Pachauri (2002).

5.2.2.2 Clinical Mastitis

Eleven gram positive isolates were subjected to *in vitro* antimicrobial susceptibility testing. Enrofloxacin and ciprofloxacin were found to be highly sensitive followed by chloramphenicol, oxytetracycline and cefotaxime. Streptomycin was the least sensitive antibiotic. These findings were in agreement with the observations made by Babu *et al.* (1998), Dhote *et al.* (1999) and Sebastian (2001); but Ghose *et al.* (2001) observed that gram positive bacteria was highly sensitive to streptomycin. Low sensitivity of streptomycin in this area may be due to development of resistance by bacteria due to indiscriminate use of this antibiotic earlier.

Gram negative cocco bacillary organisms showed high sensitivity to chloramphenicol, enrofloxacin, cefotaxime, ciprofloxacin and gentamicin followed by cloxacillin. They showed least sensitivity to sulphadiazine and streptomycin and the same was observed by Dhote *et al.* (1999) and Sebastian (2001).

When considering a potential antibiotic for the treatment of non-lactating cows or cows with clinical mastitis, quantified data relevant to the common pathogens of the population under study are important. Bacterial isolates can be tested *in vitro*, using any antibiotic impregnated disk available, however, cows must be treated with a drug approved for such use in cattle (Bezek, 1998). In field conditions, it is not practically possible to identify the

causative organisms of mastitis and to select the suitable antibiotic. So it is always essential to have periodic culture and antibiotic sensitivity studies for better and effective mastitis treatment.

5.3 BIOCHEMICAL CHARACTERS OF MILK FROM NORMAL AND AFFECTED QUARTERS IN MASTITIS

Colonisation of bovine mammary gland by pathogenic bacteria results in a series of events which lead to major alterations in the composition of milk. Compositional changes in the mastitic milk may be slight or great according to the severity and the extent of inflammation of mammary gland.

5.3.1. pH

pH of milk in subclinical mastitis was (6.848 ± 0.16, 6.835 ± 0.19 and 6.85 ± 0.12 in groups 1, 2 and 3 respectively) and it was significantly higher than the pH of normal quarter milk (6.508 ± 0.06) and was in agreement with Dhillon *et al.* (1989) and Kalorey *et al.* (1993). Yadav *et al.* (1991) reported that in most of the crossbred cows the pH of normal milk was 6.62.

In clinical mastitis it was 7.483 ± 0.12 and 7.533 ± 0.38 in groups 1 and 2 respectively and was significantly higher than the normal quarter milk pH (6.6 ± 0.15). An increase in the pH of milk has been also reported by Singh *et al.* (1997) and Mulkalwar *et al.* (1999) in case of clinical mastitis.

The acidity of milk is due to free acidic groups of casein, citrate and phosphate as well as to the presence of dissolved CO_2 .The pH of the mastitis milk may be increased from the normal acidic range towards the alkaline range due to the diffusion of blood components in to the milk as a result of inflammation and subsequent increase in the permeability of the mammary epithelium (Schalm *et al.*, 1971).He also stated that the rise in pH was due to the leakage of blood bicarbonate into the milk.

During the treatment the pH of the milk in subclinical mastitis gradually redueed to 6.595 ± 0.12 and 6.611 ± 0.03 on third day which was treated with tri- sodium citrate alone (group 1) and tri- sodium citrate along with antibiotic (group 3) respectively . And on sixth day of treatment the pH of milk of the above groups of animals became normal (6.48 ± 0.09 and 6.567 ± 0.08 respectively) and was in agreement with Dhillon *et al.* (1995). Where as in animals treated with antibiotic alone , decrease in pH was not so appreciable when compared with groups 1 and 3 (6.7 ± 0.24 on third day and 6.645 ± 0.18 on sixth day) (Figure 3). In clinical mastitis also there was gradual reduction in pH on third day and it became almost normal in animals treated with tri-sodium citrate and antibiotic (6.85 ± 0.23 on third day and 6.55 ± 0.12 on sixth day) (Figure 5) and it was in agreement with the reports made by Reddy *et al.* (1999) and Dhillon *et al.* (2000).

One of the factors which influence the pH of the milk is the secretion of the citrate in to the milk (Schalm *et al* ., 1971) . So in the present study it can be presumed that oral administration of the tri- sodium citrate increases the concentration of the citric acid in milk which inturn corrected the pH of the milk. Where as in other two groups , which were treated with antibiotic alone , the reduction in pH was very slow and not appreciable as that of animals treated with tri- sodium citrate.

5.3.2 Citric Acid

The citric acid level in the milk of subclinical mastitis were found to be decreased to 104.833 ± 10.85, 92.5 ± 16.28 and 116.167 ± 10.09 mg/dl in groups 1,2 and 3 respectively which was significantly lesser than the normal quarter milk (152.9 ± 12.279 mg/dl). These findings were consistent with that of Schalm *et al.* (1971), Oshima and Fuse (1981) and Charjan *et al.* (2001).

The citric acid level of the milk in clinical mastitis were also found to be drastically decreased to 60.167 ± 7.55 and 60.183 ± 6.85 mg/dl in groups 1 and 2

respectively before the treatment compared to the subclinical mastitis group and it was significantly lesser than the citric acid content of normal quarter milk (145 ± 21.55 mg/dl) and was in agreement with Singh *et al.* (1997), Mulkalwar *et al.* (1999) and Charjan *et al.* (2001).

During treatment the citric acid level in milk in subclinical mastitis gradually increased to 131.0 ± 20.44 and 130.5 ± 3.62 mg/dl on third day which was treated with tri- sodium citrate alone (group 1) and tri- sodium citrate along with antibiotic (group 3) respectively . And on sixth day of treatment the citric acid level in milk of the above groups of animals became normal (146.66 ± 17.97 and 141.833 ± 9.93mg/dl respectively). But in animals treated with antibiotic alone, increase in citric acid was not so considerable when compared with groups 1 and 3 (111.333 ± 11.86 on third day and 126.167 ± 5.6 mg/dl on sixth day in group 2) (Figure 4). In clinical mastitis also there was gradual increase in citric acid on third day and it became almost normal in animals treated with tri-sodium citrate and antibiotic (127.167 ± 14.26 on third day and 145.5 ± 5.15 mg/dl on sixth day) (Figure 6) and was in agreement with the findings of Dhillon *et al.* (2000).

The acidity of milk is due to free acidic groups of casein, citrate and phosphate as well as to the presence of dissolved CO_2 (Schalm *et al.*, 1971). A low level of citric acid was observed in mastitis milk. Extent of reduction in the level of citric acid may depend on the severity of inflammation. Citric acid deficiency causes retention of calcium in the secretory alveoli adversely affecting the alveoli by causing necrosis of the secretory epithelium and thereby leading to cessation of milk (Pal *et al.*, 1995). It was noted that the citric acid level increased drastically in groups 1 and 3 in subclinical mastitis animals from third day onwards which was treated with oral tri-sodium citrate. Administration of tri-sodium citrate raised the citric acid level of the milk. The level of citric acid was found to be higher in group 1 under clinical mastitis (treated with tri-sodium citrate plus antibiotic) ie, a radical increase to127.167 ±14.26 mg/dl on third day and it became normal on the sixth day than the group 2 (treated with antibiotic

alone). Tri-sodium citrate raised the citric acid in milk to a much satisfactory level.

5.3.3 Lactose

An average of 0.77 percentage reduction in lactose was noted between one sample ranked negative in CMT and one ranked three in CMT (Ashworth *et al.*, 1967).

The lactose level in subclinical mastitic milk (2.483 ± 0.50, 2.583 ± 1.04 and 3.783 ± 0.21 g/dl in groups 1, 2 and 3 respectively) was significantly lower than the level of lactose in normal quarter milk (4.28 ± 0.38 g/dl) and was in agreement with Fernando *et al.* (1985), Mert *et al.* (1992), Dhakkal and Kapur (1993), Singh *et al.* (1998) and Coulon *et al.* (2002). The value of normal milk in the present investigation, was lower than the values reported by Hirpurkar *et al.* (1987), Yadav *et al.* (1991) and Sathian (2001) and in agreement with the reports of Dhakkal and Kapur (1993), Mert *et al.* (1992) and Charjan *et al.* (2001). The difference in these observations could be ascribed to seasonal variation and the stage of lactation.

The milk lactose level of clinical mastitis was 1.3 ± 0.36 and 1.6 ± 0.53 g/dl in groups 1 and 2 respectively and it was significantly lower than the normal quarter milk lactose level (4.36 ± 0.57 g/dl) reported by Singh *et al.* (1997), Singh *et al.* (1998), Charjan *et al.* (2001) and Coulon *et al.* (2002).

During treatment, the lactose level in milk in subclinical mastitis gradually increased to 3.633 ± 1.07 and 4.15 ± 0.24g/dl on third day which was treated with tri- sodium citrate alone (group 1) and tri- sodium citrate along with antibiotic (group 3) respectively . And on sixth day of treatment the lactose level in milk of the above groups of animals became normal (4.133 ± 0.58 and 4.417 ± 0.15 g/dl respectively). Where as in animals treated with antibiotic alone, increase in lactose was not so considerable when compared with groups 1 and 3 (3.433 ± 0.62 on third day and 3.917 ± 0.50 g/dl on sixth day in group 2)

and was in agreement with Dhillon *et al.* (2000). In clinical mastitis also there was gradual increase in lactose on third day and it became almost normal in animals treated with tri-sodium citrate and antibiotic (3.083 ± 0.29 on third day and 4.25 ± 0.25g/dl on sixth day) and was in agreement with the findings of Dhillon *et al.* (2000). Not much difference observed between the two groups in clinical mastitis.

Lactose is a disaccharide formed in the udder. It is involved in osmotic pressure regulation along with chlorides. Any factor bringing about increased intramammary pressure decreases lactose synthesis. The lactose level becomes normal when the infection subsides and intramammary pressure decreases (Schalm *et al.*, 1971).

5.3.4 Chloride

The chloride levels of subclinical mastitic milk before treatment were 0.17 ± 0.03, 0.158 ± 0.04 and 0.153 ± 0.01 g/dl in groups 1, 2 and 3 respectively and they were significantly higher when compared to the level of chloride of normal quarter milk (0.104 ± 0.0125 g/dl). These findings were consistent with the reports of Ashworth *et al.* (1967) and Singh *et al.* (1998). Chloride is involved in the osmotic pressure regulation along with lactose. So when intramammary pressure increases along with reduction in lactose there will be increased concentration of chloride (Schalm *et al.*, 1971). Chloride showed significant increase along with the pH of the milk as CMT reaction became more severe (Ashworth *et al.*, 1967).

The chloride level of milk from the quarters affected with clinical mastitis were 0.275 ± 0.04 and 0.289 ± 0.04 g/dl in groups 1 and 2 respectively before the treatment which was significantly higher when compared to the chloride level in the milk (0.1139 ± 0.006 g/dl) of normal quarter and it was in agreement with Singh *et al.* (1998) and Charjan *et al.* (2001).

During treatment the chloride level in milk in subclinical mastitis gradually decreased to 0.145 ± 0.02 and 0.131 ± 0.01g/dl on third day which was treated with tri- sodium citrate alone (group 1) and tri- sodium citrate along with antibiotic (group 3) respectively . And on sixth day of treatment the chloride level in milk of the above groups of animals became normal (0.11 ± 0.01 and 0.109 ± 0.01g/dl respectively). Where as in animals treated with antibiotic alone, decrease in chloride was not so considerable when compared with groups 1 and 3 (0.139± 0.02 on third day and 0.124± 0.02g/dl on sixth day in group 2). In clinical mastitis also there was gradual decrease in chloride on third day and it became almost normal in animals treated with tri-sodium citrate and antibiotic (0.159 ± 0.03 on third day and 0.116 ± 0.01 on sixth day) when compared with the group 2 animals.

5.3.5 Calcium

It was noted that there was 4 percentage less calcium in mastitic milk compared to normal milk (Tallamy and Randolf, 1970).

Calcium level of the milk from the quarter affected with subclinical mastitis before the treatment were 101.667 ± 2.94, 103.333 ± 4.68 and 107.667 ± 5.28 mg/dl in groups 1, 2 and 3 respectively and they were significantly lower than the normal quarter milk calcium level (123.3 ± 6.66 mg/dl) i.e. there was about 10% reduction in the calcium level compared to the normal milk calcium level. Where as a much higher reduction in the calcium level (21.39 mg/dl) was observed by Mert *et al.* (1992). Present study showed more agreement (4 percentage less calcium than the normal milk) with the findings of Tallamy and Randolf (1970). Normal level of calcium of milk obtained in this study was in agreement with the reports of Nickerson (1960). But Mert *et al.* (1992) observed a little lower level of calcium (114.36 mg/dl) in normal milk.

In the present study, the calcium level of the milk from the quarter affected with mastitis before the treatment were 74.0 ± 25.81 and 71.0 ± 28.95

mg/dl in groups 1 and 2 respectively and they were significantly lower than the normal quarter milk calcium level (123 ± 7.11 mg/dl). These findings were consistent with the report of Tallamy and Randolf (1970).

During treatment the calcium level in milk in subclinical mastitis gradually increased to 109.5 ± 3.33 and 112.333 ± 11.15mg/dl on third day which was treated with tri- sodium citrate alone (group 1) and tri- sodium citrate along with antibiotic (group 3) respectively . And on sixth day of treatment the calcium level in milk of the above groups of animals became normal (117.667 ± 5.2 and 116.5 ± 5.58mg/dl respectively). Where as in animals treated with antibiotic alone , increase in calcium was not so considerable when compared with groups 1 and 3 (108.33 ± 3.88 on third day and 112.5 ± 2.51 mg/dl on sixth day in group 2). In clinical mastitis also there was gradual increase in calcium on third day and it became almost normal in animals treated with tri-sodium citrate and antibiotic (115.333 ± 6.06 on third day and 119.0 ± 7.29 on sixth day) when compared with the group 2.

It was noted that the levels of calcium after treatment in group 1 and3 under the subclinical mastitis group and group 1 under the clinical mastitis group were higher compared to the other groups. Oral administration of tri-sodium citrate maintains the citrate levels in the milk to near normalcy. A high correlation exist between the soluble calcium and citrate concentration (Holt and Muir, 1979). This citrate is essential for the sequestration of calcium in to the milk (Schalm *et al.*, 1971).

5.4 EFFICACY OF TREATMENT

After treatment with tri-sodium citrate, the level of citric acid and other parameters came to normalcy in groups 1 and 3 affected with subclinical, mastitis. Administration of tri-sodium citrate orally, replenished the citrate deficiency in animal system and restored the milk contents to normalcy. Tri-sodium citrate was found to be good in reducing the pH of milk from alkalinity to

6.5 (Dhillon *et al.*, 1989, Kalorey *et al.*, 1993, Dhillon *et al.*, 1995, Singh *et al.*, 1997 and Dhillon *et al.*, 2000).

The present study revealed that the levels of citric acid and pH came to normalcy at an earlier period (by the third day in four subclinical mastitis cases and by sixth day, in all the cases in group 1 and group 3) than the group 2 which was treated with antibiotic alone. So in the present study it can be presumed that oral administration of the tri- sodium citrate increased the concentration of the citric acid in milk which inturn corrected the pH of the milk. The recovery from subclinical mastitis occur due to restoration of citrate ratio to other interacting ions as suggested by Faulkner and Peaker (1982).

Complete bacteriological cure was observed in 42.86 percentage of cases in group 1 (tri-sodium citrate alone). In the rest of the cases number of bacterial colonies were found to be reduced. Tri-sodium citrate decrease the pH of milk and thus made it unfavourable for the growth of bacteria (Dhillon *et al.*, 1995). Kalorey *et al.* (1993) observed almost similar findings i.e. 53.34 percentage of subclinical mastitis cases were found to be culturally negative on day sixth after tri-sodium citrate oral therapy and also found 80.67 percentage of cases complete bacteriological cure on day 21 after the treatment. These findings were quite similar to those of Dhillon *et al.* (1995) which also indicated reduction in bacterial count after the above treatment in clinical mastitis cases.

In subclinical mastitis cases, there was slight increase in the milk yield of recoverd animals in group 1 (0.382 ± 0.06 l/day/animal) when compared to the other 2 groups. It can be presumed that oral administration of tri- sodium citrate restored the normal citric acid level in the milk. Citrate was essential for the milk synthesis and it can be regarded as a harbinger for lactogenesis (Peaker and Linzell, 1975).

In clinical mastitis, among group 1 animals (treated with antibiotic plus tri-sodium citrate) 100 percentage clinical cure was observed by fifth day of

treatment. In 71.4 percentage of cases milk became normal in colour, consistency and without any clots by third day of treatment itself. Where as normal appearance of the milk noted only in 37.5 percentage of cases on third day of treatment in group 2 (antibiotic alone) animals.

Hundred percent bacteriological cure was observed in group 1 by sixth day of treatment. While only 75 per cent bacteriological cure was observed in the other groups. This was in agreement with Pal *et al.* (1995). Reddy *et al.* (1999) observed only 85 percentage of cure rate in animals treated with tri-sodium citrate orally plus intramammary antibiotic for five days. Failure of citrate therapy in some cases might be due to the presence of heavy bacterial population in mastitic quarter. The citric acid and pH level came to normalcy by sixth day of treatment in group 1 where as adequate normal level was not achieved in group 2.

In clinical mastitis group milk yield was not restored to the expected level in group 1. Even though, 28.57 percentage of cases level has been reached to the pre infection level.

Summary

6. SUMMARY

The project was under taken to assess the effect of tri-sodium citrate for the treatment of mastitis in cattle. The objectives were to study the changes in pH of milk in mastitis, to evaluate the levels of citric acid, calcium, lactose and chloride in the mastitis milk and to study the efficacy of oral administration of tri-sodium citrate as therapeutic agent in subclinical mastitis and as a supportive therapy along with antibiotic in clinical mastitis.

Twenty cases of subclinical mastitis and fifteen cases of clinical mastitis were utilized for the study. Subclinical mastitis cases were divided in to three groups and clinical mastitis cases were divided in to two groups, based on the therapeutic trial adopted.

Milk was apparently normal in all cows affected with subclinical mastitis. Where as milk was having varying consistency, colour and abnormal constituents in clinical mastitis. Gram positive cocci were isolated from cases in which the milk appeared white, cream, slightly yellow or yellow coloured with fine clots or flakes. Gram negative coccobacilli were isolated from cases in which the milk appeared straw or yellow coloured watery secretion with or without clots.

Gram positive cocci were isolated from majority of the subclinical and clinical mastitis cases than the gram negative coccobacilli.

In vitro antibacterial susceptibility testing of isolates from subclinical and clinical mastitis showed that gram positive cocci were highly sensitive to enrofloxacin, cefotaxime and ciprofloxacin followed by chloramphenicol, ampicillin, trimethoprim, gentamicin and oxytetracycline. Gram negative cocco bacilli were highly sensitive to chloramphenicol, trimethoprim, gentamicin, enrofloxacin, cefotaxime and ciprofloxacin followed by cloxacillin and ampicillin.

The mean values of pH and chloride levels in the milk from affected quarter in subclinical and clinical mastitis cases before the treatment showed significant increase compared to the milk from non affected quarter. These values decreased significantly to normal level after treatment.

The mean values of citric acid, lactose and calcium levels in the milk from affected quarter in subclinical and clinical mastitis cases before the treatment showed significant decrease compared to the milk from non affected quarter. These values increased significantly to normal levels on post treatment.

In subclinical mastitis, the post treatment citric acid and calcium levels, showed significant differences between the tri-sodium citrate and antibiotic treated groups.

Oral administration of tri-sodium citrate was found to be effective in managing subclinical mastitis even though complete bacteriological cure was observed only in 42.85 percentage of cases. The other biochemical characters of the milk came to normal level in all cases in this group by sixth day of treatment. Increase in milk production after the treatment was also found to be slightly better in animals treated with tri-sodium citrate.

Oral administration of tri-sodium citrate along with antibiotic found to be effective in managing clinical mastitis cases. Hundred percentage bacteriological cure was noticed in this group on sixth day of treatment whereas only 75 per cent bacteriological cure observed in the other group. In 50 per cent of the cases treated with antibiotic alone, the citric acid and pH level came to the normal range on post treatment. But no significant difference observed between the post treatment values in these two groups.

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EFFECT OF TRISODIUM CITRATE IN THE TREATMENT OF MASTITIS IN CATTLE

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ABSTRACT

A study was conducted in the Department of Clinical Medicine, College of Veterinary and Animal Sciences, Mannuthy, for a period of three semesters during the year 2002 and 2003, to assess the efficacy of oral administration of tri-sodium citrate as therapeutic agent in subclinical mastitis of cattle and as a supportive treatment along with antibiotic in clinical mastitis of cattle and to evaluate the levels of pH, citric acid, calcium, lactose and chloride in milk of *cattle affected with mastitis.*

Twenty cases of subclinical mastitis and fifteen cases of clinical mastitis were utilized for the study. Subclinical mastitis cases were divided in to three groups and clinical mastitis cases divided into two groups based on the therapeutic trial adopted.

Gram positive cocci were isolated from cases in which the milk appeared white, cream, slight yellow or yellow coloured with fine clots or flakes. Gram negative coccobacilli were isolated from cases in which the milk appeared straw or yellow coloured watery milk with clots or without clots. Gram positive cocci were isolated from majority of the subclinical and clinical mastitis cases than the gram negative coccobacilli.

Gram positive cocci were highly sensitive to enrofloxacin, cephotaxime and ciprofloxacin. Gram negative coccobacilli were highly sensitive to chloramphenicol, trimethoprim, gentamicin, Enrofloxacin, Cefotaxime and ciprofloxacin.

The pH and chloride levels of the affected quarter milk in subclinical and clinical mastitis cases before the treatment showed significant increase compared to the nonaffected quarter milk values. These values showed significant decrease to normal level on post treatment.

The mean values of citric acid, lactose and calcium levels of the affected quarter milk in subclinical and clinical mastitis cases before the treatment, showed significant decrease before treatment when compared to the nonaffected quarter milk values. These values significantly increased and reached normal levels after treatment.

Oral administration of tri-sodium citrate was found to be effective in treating subclinical mastitis cases. All the biochemical parameters of milk came to normal level in all cases in the trisodium citrate alone treated group by sixth day of treatment and also the increase in milk production after the treatment was slightly better in this group.

Oral administration of tri-sodium citrate along with antibiotic was found to be effective in managing clinical mastitis cases. 100 percent bacteriological cure was observed in this group whereas only 75per cent bacteriological cure observed in the other group.