

A NEW HORIZON IN THE PATHOBIOLOGY, AETIOLOGY AND NOVEL TREATMENT OF MASTITIS IN BUFFALO

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ABSTRACT

Twenty-one dairy buffaloes suffering from clinical mastitis were the subject of this investigation. The effected quarters of the animals were clinically examined. The appearance and consistency of their milk and pH, were ascertained and recorded at the sampling site. The pH ranged from 7.00-8.50 (average 7.49). The citrate content of the milk ranged from 24.00-47.50 (average 33.71) mg /100ml. Milk from affected quarters of six buffaloes was cultured; this yielded *Staphylococci* from four quarters, *Streptococci* from one, *Escherichia coli* from two, *Bacilli* and *Klebsiella* from one each. On the basis of the lowered citrate content of milk in the affected quarters, the animals were treated with 12 gm or 30 gm of trisodium citrate in 250 ml of water orally once daily till recovery. With 12 gm doses daily, the recovery period was 7-13 days. However, with 30 gm daily, the recovery period was cut-short to 3-5 days. The pH (~6.50) and citrate content of milk (118.30 mg/100 ml) returned to almost normal levels after treatment with trisodium citrate. The bacterial colonies also reduced significantly after the treatment. The constituents of milk, e.g., total protein, fat and lactose, increased significantly after the treatment. The results of our investigation *vis-à-vis* with other workers have been discussed in detail. It has been proposed logically and

conceptually that the initial lesion in the udder is inflicted by the disturbed buffer system of the udder, i.e., lower levels of citrate and free Ca^{2++} are responsible for this injury in the udder and alkaline pH due to seepage of bicarbonate from blood into the udder providing most conducive conditions for the establishment of environmental non-contagious pathogens. Further, the treatment with trisodium citrate proved safe, economical, very effective, with no discarding of milk, no withdrawal periods and moreover no hazard from residuals in milk and meat.

Keywords: dairy buffaloes, *Bubalus bubalis*, mastitis, pathobiology, aetiology

INTRODUCTION

Mastitis is a perpetual problem of all milk producing animals (including women). The conservative estimates of economic losses from this malady have been made year after year in almost each and every state world-wide. Several groups of scientists/workers have been engaged globally to find out the exact cause and effective treatment of this most formidable disease. Though much of the work directed towards unveiling the nub of this malady has elucidated intricate biochemical interactions at the molecular level, the solution to

the problem still appears elusive.

Coming down to that versatile dairy animal, the buffalo (*Bubalis bubalis*), the 'Asian Black Gold' having a population of about 130 million globally, suffers extensively from mastitis (Fagiolo and Lai, 2007). Despite the use of best available facilities at hand to understand the pathobiology of mastitis, the problem still remain economically most important to the dairy industry throughout the world. The ideal *modus operandi* to eliminate or reduce the economic losses requires that the definite cause of mastitis be identified and then possible control measures implemented. While scanning the literature on mastitis and biosynthesis of milk in the udder, it became apparent that citrate plays a crucial role in the lactogenesis and maintain udder health through ionic equilibration (Peaker and Linzel 1975; Hyvonen *et al.*, 2010). Citrate levels are always low in mastitic milk (Dhillon and Singh 2009). It was hypothesized that replenishment of citrate deficiency with extraneous trisodium citrate might play some protective role against mastitis; hence, these studies were undertaken and the results are communicated in this paper.

MATERIALS AND METHODS

Twenty-one buffaloes affected with mastitis were included in this investigation. Milk samples from six buffaloes were cultured and identification made from ensuing colonies. The number of colonies were counted before and after the treatment of the affected buffaloes. Physical examination of the milk and udders was made and the degree of mastitis was graded on the basis of the following scale:

+ ---- Presence of flakes (See Figure 1)

++---- Serosanguineous milk with admixture of flakes (See Figure 2.)

+++-- Curdeled milk with admixture of blood clots (Figure 3.)

++++- Frank blood with whitish tinge of milk (Figure 4.)

Grading of milk was compared with the pH of milk from the affected quarters to qualitatively identify the severity of mastitis. Milk citrate content was determined by the method of White and Davies (1963) quantitatively before and after the treatment. The appearance and consistency, the pH and the citrate content of milk were the main criteria in treatment with trisodium citrate.

The treatment consisted of 12 gm or 30 gm of trisodium citrate in 250 ml of water daily as a drench till recovery. No other treatment, such as antibiotic, was given.

RESULTS AND DISCUSSION

Table 1 presents the data on the effect of mastitis on various parameters of milk before and after treatment with trisodium citrate in buffaloes. It was observed that lowered citrate content was restored to normal levels after recovery. There was a relative consistent lowering of udder milk pH of the affected quarters it came down to ~6.50 and the consistency of milk was also restored to normal at recovery, which occurred within 4-7 days after the treatment.

The organisms isolated from different milk samples were: *Staphylococci*, *Streptococci*, *E. coli*, *Bacilli* and *Klebsiella*. The treatment also reduced the number of colonies in the culture at different dilutions of mastitic milk. The treatment with trisodium citrate proved very effective in



Figure 1. Grade +, pH 7.0, Citrate 45.60 mg/100 ml milk.



Figure 2. Grade ++, pH 7.5, Citrate 30.06 mg/100 ml milk.



Figure 3. Grade +++, pH 8.0, Citrate 30.90 mg/100 ml milk.



Figure 4. Tube 1- Grade +++, pH 8.5, Citrate 24.00 mg/100 ml milk Tube No. 6 containing clear milk after treatment.

controlling clinical mastitis in affected quarters. The treated animals did not show any side effects.

MOST PROBABLE CAUSE(S) OF MASTITIS

Thus far, the most common causes of mastitis in dairy animals have been primarily imputed to infectious agents (Zhao and Lacasse 2007). On the basis of infectious causes of mastitis a procession of drugs purported to be effective against these culprits emerged on the scene for controlling ailment in dairy animals. In the beginning these drugs appeared specious. However, continual use of these chemicals proved palliative and presented enormous problem of drug resistance and milk and meat residue dangers for humans (Costa *et al.*, 1997). Moreover, the effectiveness of these antimicrobials in controlling mastitis in dairy animals was rarely more than 50% (Deluyker *et al.*, 2005). Different management practices, e.g. dry-cow therapy, teat dipping, and hygienic measures, were evolved to alleviate effects of this formidable problem, but the devil of mastitis is still rampant and unrelenting. Nevertheless, delving into milk synthesis, the mechanisms of injury to the parenchymatous tissue

of the udder appears to be becoming a bit clearer.

It has been widely demonstrated that citrate is the ‘harbinger of lactogenesis’ (Peaker and Linzel, 1975). The same authors further reported that the level of citrate in udder of cow, goat and women shoots up 46 times around parturition. These findings enthuse one to speculate that as citrate plays a pivotal role in milk synthesis, it might possibly be associated with mastitis in dairy animals.

It has been reported extensively that mastitic milk is significantly low in citrate (Oshima and Fuse, 1981; Dhillon and Singh, 2009). Our investigations have also revealed that citrate levels are very low in milk of quarters affected with mastitis (30.90 to 36.53 mg/ 100 ml). As stated above, a certain minimum concentration of citrate is essential for the normal synthesis of milk in the alveoli in the udder. Therefore, any drop in the citrate content would result in faulty synthesis of milk in a particular quarter(s) of the udder. We have observed that the affected quarters had very low concentrations of citrate as compared with healthy quarters of the same animal (Dhillon *et al.*, 1989, 1991). The deficiency of citrate in a particular quarter may be due to nutritional, metabolic or some other intrinsic unknown factors which need

Table 1. Effect of mastitis on different parameters of milk from buffaloes before and after Treatment with trisodium citrate given daily 12 gm in 250 ml of water as drench.

	Citrate mg/100 ml	pH	No. of Bacterial colonies (dilutions)			Doses of trisodium citrate
			1:10	1:100	1:1000	
Before	33.71	7.49	111.50	64.33	43.33	8 (7-13)
(Range)	24.00-47.50	7.00-8.50	91-141	47-83	33-58	
SE	2.10	0.10	8.08	5.84	4.77	
After	118.30	6.50	90.00	50.83	28.00	
(Range)	96-146	--	76-102	34-65	23-35	
SE	5.82	--	4.89	4.71	2.03	

further investigation.

The literature extant on mastitis have clearly revealed that mastitic milk is alkaline (pH 7.0 and above). The normal pH of milk in udder is ~6.50, a level which does not appear to be congenial for the growth of commonly isolated organisms from mastitic milk (Cruickshank *et al.*, 1970). Moreover, the philosophical postulation of invasion by environmental (non-contagious) organisms through the teat canal and establishment of infections in udder seems untenable due to the presence of mechanical, chemical and immunological defense barriers throughout this route (Sordillo and Streicher 2002). Also, it has been demonstrated histologically that 3.1% of samples collected from the udders of slaughtered cows from which microorganisms were isolated did not show any histological changes (Benites *et al.*, 2002). Frost *et al.* (1980) reported minimal damage to alveolar tissue after 'moderate' cases of mastitis induced experimentally with *E coli*. Furthermore, the notion of infection as a cause of mastitis gets eclipsed by the studies of Newbould and Neave (1965) who could not establish 100% infections in udder through deliberate intra-mammary infusions with *Staphylococcus aureus* cultures. Several cases of clinical mastitis in bovines from which no infectious organism was isolated are on record (Wanasinghe and Frost, 1979; Bramley *et al.*, 1981; Gonz'alez *et al.*, 1988).

Citrate, indeed, is the main constituent of the buffer system responsible for the maintenance of pH (6.5) in the udder; it regulates the homeostasis between Ca²⁺ and H⁺ ions and is the mainstay for the fluidity of milk through its effect on casein micelles (Faulkner and Peaker, 1982; Shennan and Peaker 2000). Citrate in the udder also ensures the sequestration of soluble Ca²⁺ in milk (Kon and Cowie, 1961) and there is

significant synchronization between the two (Holt and Muir, 1979). Hence, deficiency of citrate in the udder would lead to the 'clumping' of Ca²⁺, which manifests as flakes in the mastitic milk. These flakes of Ca²⁺ probably injure the parenchymatous tissue in the udder alveoli due to reduced moderator effect of citrate. Due to this injury the impermeable barrier to citrate in both directions between blood and milk is disrupted and the inflammatory reaction sets in leading to an array of subsequent events. Such injuries due to free Ca²⁺ have been reported in myocardium (Fleckenstein *et al.*, 1974 ; Singal *et al.*, 1979). It has also been recorded that a calcium-dependant endonuclease is associated with necrotic type changes in tissues (Arends *et al.*, 1990). Furthermore, another important ion bicarbonate which transudates from blood into milk during mastitis due to permeability of barrier changes the pH of udder towards alkalinity i.e., 7.0 or more. When such lesions in the udder are created and most conducive environments become available, the udder is subsequently invaded by environmental pathogens culminating in clinical/subclinical 'infectious mastitis'.

We have also reported that lactose, total proteins and fat are substantially lowered in mastitic milk (Singh *et al.*, 1997; Dhillon *et al.*, 2000; Singh *et al.*, 2007). However, these constituents in milk increased markedly on recovery affected by trisodium citrate therapy. The increment in fat was spectacular (190%) because citrate plays an indirect role through NADPH in *de novo* synthesis of fatty acids in the mammary gland (Garnsworthy *et al.*, 2006). Reduction in the number of bacterial colonies after treatment with trisodium citrate has also been observed by other workers (Dhillon *et al.*, 1995). These observations along with our studies substantiate that this treatment is radical and works at the root cause of mastitis resulting in

remarkable cure of the malady without producing any side effects.

Other contributory factors which further exacerbate the pathogenesis of mastitis are the involvement of neutrophils, infectious agents, plasma proteins, cytokines, free radicals etc., which need exhaustive investigations (Zhao and Lacasse, 2007).

Taking all the findings of the above investigations together, it can be concluded that the initial lesion in the pathogenesis of mastitis is caused by the disturbed homeostasis of citrate and Ca²⁺ in the udder. On the basis of this hypothesis, we treated the clinical cases of mastitis in buffaloes by administering 12 gm to 30 gm of trisodium citrate in 250 ml of water daily as a drench. Similarly several workers have treated acute and/or sub-acute cases of mastitis in buffaloes with excellent results. They also compared it with other antimicrobials and reported that trisodium citrate was superior as far as the restoration of normal pH and other constituents of milk in the udder was concerned (Yousaf *et al.*, 2010; Prakash *et al.*, 2010).

The treatment of mastitis with this salt has been further standardized by enhanced doses to cut-short the recovery period. The oral dose has been raised to 30 gm in 250 ml of water daily as a drench and the recovery period cut-short to 3-5 days depending upon the severity of mastitis. The disruption of the impermeable barrier between blood and milk in udder, as stated above, formed the basis of intravenous administration of trisodium citrate, which directly reaches the site of injury and normalizes the pH (6.5) in the udder and the infectious agents are scavenged off, thus, restoring ionic equilibrium. The intravenous administration of trisodium citrate in sterilized normal saline as 5% given morning and evening in 50 ml doses

and the recovery period shortened to 1-3 days (Singh *et al.*, 2007; Dhillon and Singh, 2009). This treatment was safe, economical, and very effective and avoided culling and discarding of milk with the minimal pain to the animal. Moreover, there were no withdrawal periods or hazards from residual problems in milk and meat.

Based upon our above observations some pharmaceuticals have come up with the formulations containing trisodium citrate as the major content recommended for the treatment or prevention of mastitis in dairy animals and are used extensively with remarkable degree of success in the field.

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