

*Original Research***Prophylactic Potential of Tri-Sodium Citrate on Subclinical Mastitis in Cow in Different Housing System**

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Abstract

The present research work was carried out to evaluate prophylactic potential of Tri-sodium citrate powder in subclinical mastitis in milking cows. The study involved twelve HF cross dairy cows in which each diagnosed with at least one subclinical mastitis quarter. The six (n=6) cows from conventional and six (n=6) cows from loose housing system with raised SCC were administered Tri-sodium citrate powder @ 30 mg/kg B.W. orally BID for five days. The SCC values of infected quarters were reduced significantly ($P < 0.05$) on 28th day after Tri-sodium citrate therapy as compared to value on day '0' before therapy both in loose housing and conventional housing system. The percent efficacy in improvement of infected quarter after administration of Tri-sodium citrate powder in conventional and loose housing system was 63.63 % and 58.33 % respectively. Thus, results indicated that tri-sodium citrate having prophylactic potential in controlling mastitis in cows in both conventional and loose housing system.

Key words: Cow, Housing System, Prophylactic Therapy, Subclinical Mastitis, Tri-Sodium Citrate**How to cite:** Shaikh, S. R., Digraskar, S. U., Siddiqui, M. F. M. F., Syed, A. M., & Ajabe, J. S. (2019). Prophylactic Potential of Tri-sodium Citrate on Subclinical Mastitis in Cow in Different Housing System. International Journal of Livestock Research, 9(9), 198-206. doi: 10.5455/ijlr.20190414063438**Introduction**

Mastitis is one of the costliest diseases resulting in huge economic losses throughout the world including in India. Mastitis is characterized by a range of physical and chemical changes in the milk and pathological changes in the glandular tissue. The most important changes in the milk include discoloration, the presence of clots and the presence of large number of leukocytes (Constable *et al.*, 2017). Mastitis is mainly divided by two categories: clinical and subclinical mastitis on the basis of clinical signs. Sub-clinical mastitis is a type of mastitis which is characterized by the no visual changes in the quarters and milk or the existence of inflammation in the absence of gross signs (Sharma *et al.*, 2012). The economic losses due to bovine subclinical mastitis is in range of rupees 21,677/- to 88,340/- per animal per lactation (Rathod *et al.*, 2017).

Sub-clinically infected quarters can develop clinical mastitis and the rate of new infections can be high (Zdunczyk *et al.*, 2003). Therefore, subclinical mastitis is one of the costliest and is alarming disease of dairy animals throughout the world and a major obstacle in milk production.

Citrate is the main constituent, playing a buffer role in the udder. It regulates the homeostasis between Ca^{++} and H^{+} ions for maintaining the fluidity of milk through its effect on casein micelles (Shennan and Peaker, 2000). In case of citrate deficiency in udder, the clumping of Ca^{++} appears which causes injury to the parenchymatous tissues of alveoli of udder. This leads to subsequent events such as the damage of barriers between blood and milk, and inflammatory reactions in the alveolar tissue of udder (Poutrel, 1982). Generally, mastitic milk has been found to be alkaline and low in citrate (Dhillon *et al.*, 1989; Dhillon *et al.*, 1995; Prakash *et al.*, 2013). Tri-sodium citrate is safe, economical and very effective which avoid culling of animal and discard of milk. Also, it does not require withdrawal period because of residual problems in milk and meat (Dhillon and Singh, 2013). Tri sodium citrate may be useful for improving milk quality regarding somatic cell counts (Mbonwanayo *et al.*, 2016). It was hypothesized that replacement of citrate deficiency with tri-sodium citrate might play some protective role against subclinical mastitis. Therefore, the present research was carried out to evaluate the prophylactic potential of tri-sodium citrate in crossbred cows under conventional and loose housing system.

Sarfaraz *et al.* (2009) studied evaluation of tri-sodium citrate alone and in combination with levamisole HCl in the treatment of sub-clinical mastitis and found that oral administration of tri-sodium citrate and levamisole HCl are viable alternative to antibiotic therapy for sub-clinical mastitis. Dhillon and Singh (2013) studied effect of tri-sodium citrate against clinical mastitis. The animals showed good recovery and citrate content of milk returned to almost normal levels after treatment. Further, the treatment with tri-sodium citrate proved safe, economical, very effective, with no discarding of milk, no withdrawal periods and no hazard from residuals in milk and meat. Reddy *et al.* (2017) reported that supplementation of coated tri sodium citrate @ 20 gms for 10 days in cows having sub-clinical mastitis had reduced pathogenic load, increased milk fat and milk production significantly. Santoshi *et al.* (2018) observed that supplementation of tri-sodium citrate during the transition period lowered SCC, pH and EC while improved productivity of indigenous dairy cows.

Material and Methods

Selection of Animals

In the present research project twenty-four HF crossbred lactating cows from conventional (n=12) and loose (n=12) housing systems in early to middle lactation having average weight around 400 kg were selected. In the selected cows six cows each from conventional and loose housing system were healthy while six cows from conventional and six cows from loose housing system were having at least one quarter positive for

subclinical mastitis which were diagnosed by somatic cell count (SCC), modified california mastitis test (MCMT), pH and electrical conductivity (EC). The selected cows were randomly divided into four groups each having six cows (n=6). Group I and group III comprise of cows from conventional housing healthy control and loose housing healthy control, respectively which were maintained on regular diet; Group II and group IV were made up of cows from conventional housing system and loose housing system, respectively which were administered tri-sodium citrate powder @ 30 mg/kg B.W., orally BID for five days

Sampling and Diagnosis

About 5-10 ml of milk sample was collected from individual quarter of each cow in sterilized bottles and was subjected to various diagnostic tests viz. MCMT, pH and EC. The SCC test was performed in the laboratory within 1-2 hours after collection. The SSC has more accuracy to discriminate healthy milk from sub-clinical mastitic milk (Tiwari *et al.*, 2018). The milk samples were tested to assess the quarter health status and milk samples were tested on day '0' before institution of prophylactic regimen and on day 7, 14 and 28 after therapy in each group, for comparative evaluation, during the routine morning milking hours.

Statistical Methods

The data obtained in the current research work were statistically analyzed and compared by employing mean, standard error and CRD using WASP (Web Agri Stats Package) version 2.0.

Results and Discussion

The efficacy was recorded after observing the results of somatic cell count (SCC) modified California mastitis test (MCMT), pH and electric conductivity (EC) tests as shown in Table 1, 2 & 3. Therapy of tri-sodium citrate powder showed a significant ($P < 0.05$) reduction in SCC, MCMT, pH and EC, in both conventional and loose housing system as compared with respective control and on day '0' pre-treatment to day '28' post-treatment.

Citrate is one of the main constituents of milk which plays a major role in buffering in the udder. Deficiency of citrate in udder is result into the clumping of Ca^{++} which results into injury to parenchymatous tissues in the udder alveoli. It means subsequent events such as the damage of barriers between blood and milk and inflammatory reactions would be established (Poutrel, 1982). Tri-sodium citrate has buffering and antimicrobial properties which can maintain normal pH of milk and subsequently prevent the formation of calcium ion crystals leads to cure from mastitis and improvement in milk quality (Shennan and Peaker, 2000; Yousaf *et al.*, 2010). Tri sodium citrate may be useful for improving milk quality regarding somatic cell counts (Mbonwanayo *et al.*, 2016).

Table 1: Prophylactic effect of Tri-sodium citrate on reduction in SCC

Gr oup No.	Prophylactic medication group	Reduction in SCC (X10 ³)																CD (5%)
		0 Day				7 Day				14 Day				28 Day				
		LF	LH	RF	RH	LF	LH	RF	RH	LF	LH	RF	RH	LF	LH	RF	RH	
I	Conventional	163.395	174.298	163.395	177.923	174.296	170.665	156.133	185.185	177.926	167.026	163.396	199.703	181.554	156.131	159.763	185.181	
	Control	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	
II	Tri-sodium citrate	236.021	312.273	206.973	279.593	210.606	275.962	192.441	254.173	185.185	232.381	177.928	232.389	170.666	203.343	167.026	214.238	51.809
	Conventional	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	
III	Loose	174.296	196.073	181.554	188.816	177.926	185.188	159.763	192.441	188.811	192.441	170.668	206.975	185.185	181.556	159.763	210.608	
	Control	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	
IV	Tri-sodium citrate	221.496	312.273	214.235	286.853	199.701	268.700	210.605	239.653	192.447	232.381	203.345	206.976	185.181	206.975	181.554	196.073	44.832
	Loose	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	

Values having at least one common superscript do not differ significantly at $P < 0.05$.



Table 2: Prophylactic effect of Tri-sodium Citrate on reduction in pH

Group No.	Prophylactic medication group	Reduction in pH																CD (5%)
		0 Day				7 Day				14 Day				28 Day				
		LF	LH	RF	RH	LF	LH	RF	RH	LF	LH	RF	RH	LF	LH	RF	RH	
I	Conventional	6.533	6.550	6.533	6.550	6.550	6.533	6.517	6.567	6.55	6.517	6.517	6.583	6.550	6.517	6.517	6.600	0.125
	Control	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	
II	Tri-sodium citrate	bcd	a	bcdef	ab	cdef	ab	def	def	abc	ef	bcde	f	bcd	f	def	f	0.116
	Conventional	6.683	6.817	6.633	6.750	6.617	6.750	6.583	6.733	6.550	6.667	6.533	6.683	6.533	6.600	6.533	6.650	
III	Loose	6.533	6.583	6.550	6.567	6.567	6.567	6.517	6.583	6.550	6.567	6.517	6.617	6.567	6.567	6.517	6.650	0.116
	Control	+	+	+	+	+	+	+	+	+	+	+	±	+	+	+	+	
IV	Tri-sodium citrate	def	a	def	ab	def	abc	def	bcd	ef	cde	def	def	ef	cdef	f	def	0.116
	Loose	6.633	6.833	6.633	6.800	6.617	6.750	6.617	6.700	6.583	6.667	6.600	6.617	6.567	6.650	6.550	6.633	
		+	0.05	0.03	0.04	0.02	0.05	0.04	0.04	0.02	0.04	0.04	0.05	0.05	0.05	0.02	0.05	

Values having at least one common superscript do not differ significantly at $P < 0.05$.



Table 3: Prophylactic effect of Tri-sodium Citrate on reduction in EC

Group No.	Prophylactic medication group	Reduction in EC (mS/cm)																CD (5 %)
		0 Day				7 Day				14 Day				28 Day				
		LF	LH	RF	RH	LF	LH	RF	RH	LF	LH	RF	RH	LF	LH	RF	RH	
I	Conventional	4.473	4.480	4.430	4.515	4.527	4.429	4.368	4.492	4.537	4.440	4.423	4.632	4.543	4.385	4.398	4.618	0.425
	Control	± 0.09	± 0.05	± 0.06	± 0.06	± 0.09	± 0.05	± 0.04	± 0.01	± 0.08	± 0.02	± 0.03	± 0.09	± 0.07	± 0.04	± 0.04	± 0.16	
II	Tri-sodium citrate	bcde	a	defgh	abc	defgh	ab	efgh	bcd	fgh	bcdef	gh	cdefg	h	defgh	h	bcde	0.425
	Conventional	4.995	5.492	4.715	5.258	4.735	5.323	4.595	5.035	4.537	4.945	4.498	4.875	4.425	4.693	4.448	5.000	
III	Loose	4.458	4.625	4.522	4.572	4.467	4.563	4.447	4.582	4.600	4.632	4.428	4.757	4.560	4.473	4.365	4.855	0.419
	Control	+ 0.05	+ 0.09	+ 0.08	+ 0.07	+ 0.05	+ 0.08	+ 0.10	+ 0.08	+ 0.11	± 0.10	+ 0.04	± 0.15	+ 0.14	+ 0.04	+ 0.04	+ 0.18	
IV	Tri-sodium citrate	bcde	a	bcde	a	bcde	bcd	bc	ab	cde	bcde	cde	bcde	e	cde	cde	de	0.419
	Control	4.877	5.543	4.788	5.402	4.715	4.950	4.960	5.133	4.583	4.892	4.555	4.717	4.487	4.640	4.562	4.535	
		± 0.16	+ 0.16	± 0.13	+ 0.11	± 0.19	± 0.19	+ 0.18	+ 0.18	± 0.15	± 0.20	± 0.09	± 0.14	+ 0.10	+ 0.14	+ 0.12	+ 0.11	

Values having at least one common superscript do not differ significantly at $P < 0.05$.



In current research work, the treatment group showed lower milk SCC, pH and EC levels as compared to the respective control group. This may be due to the citrate which plays a buffering role inside the mammary glands, it regulates the homeostasis between Ca⁺⁺ and H⁺ ions and supports the fluidity of milk through its effect on casein micelles (Shennan and Peaker, 2000). Moreover, restoration of tri-sodium citrate in animals suffering from mastitis decreases pH of milk and perhaps acts against Ca⁺⁺ injury and reduces the inflammatory process in the quarters of the udder, which in turn makes mammary secretary cells more efficient and ultimately the milk yield is increased (Prakash *et al.*, 2013). Similar findings were reported by Sarfaraz *et al.* (2009), Rai *et al.* (2013), Dhillon and Singh (2013), Reddy *et al.* (2017), Prakash *et al.* (2010), Prakash *et al.* (2013), Santoshi *et al.* (2018), Pal *et al.* (1995) and Dhillon *et al.* (1995) for use of tri-sodium citrate for prevention and treatment of bovine mastitis.

The prophylactic effect on reduction of udder inflammation and percent efficacy of treatment in various groups is depicted in Table 4 and Fig. 1.

Table 4: Efficacy of therapy of tri-sodium citrate in conventional and loose housing system

Group	Housing System	Prophylactic Therapy	Infected Quarters				Percent Efficacy
			0 Day	7 Day	14 Day	28 Day	
II	Conventional	Tri-sodium citrate	11	10	5	4	63.63
IV	Loose	Tri-sodium citrate	12	8	4	5	58.33

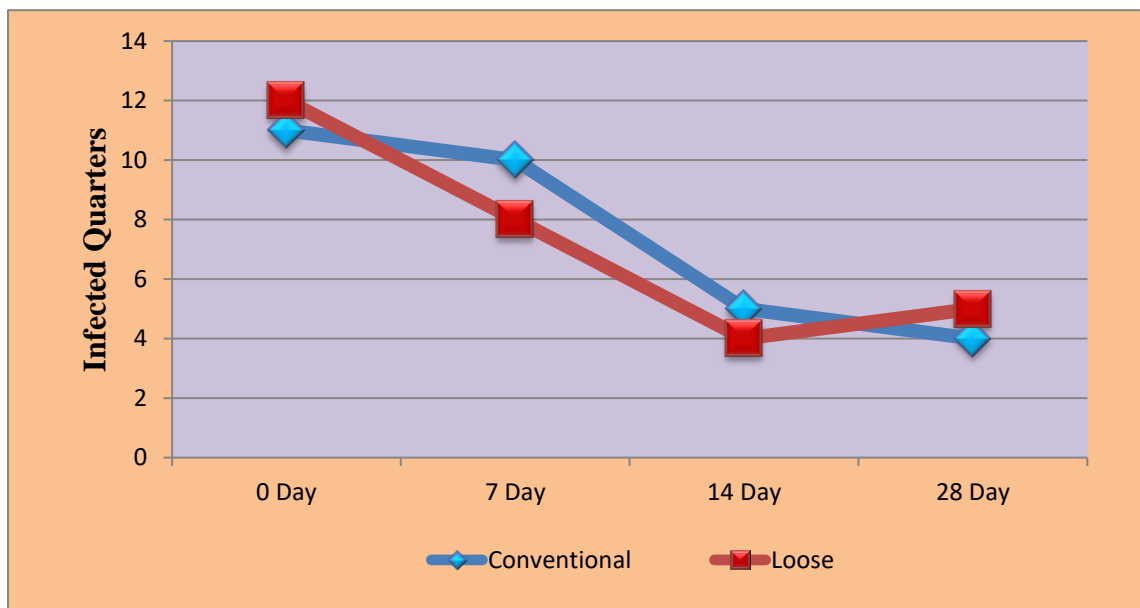


Fig. 1: Therapeutic efficacy of tri-sodium citrate in conventional and loose housing system

The per cent efficacy of group II and IV is 63.63 and 58.33, respectively. This indicates that therapy of tri-sodium citrate could eliminate intramammary infection effectively. Therefore, it is clear that effect of tri-sodium citrate is slightly better in conventional housing system than in loose housing system.

Conclusion

Therapy of tri-sodium citrate powder @ 30 mg/kg B.W. orally twice a day for five days was found more efficacious in conventional housing system (63.63 %) than in loose housing system (58.33 %) in the prevention of subclinical mastitis in cows

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