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Effect of novel teat dips on milk biochemical parameters in dairy cattle

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Abstract

The study was conducted on bovine mastitis with an objective to prevent its occurrence. The attempt was envisaged through use of copper sulphate and zinc sulphate, used as post milking teat dips and were selected after evaluating their antimicrobial potential against major mastitis causing bacteria *in vitro* using agar well diffusion method. Different concentrations of these compounds in distilled water were prepared (10%, 7.5%, 5% and 2.5%). *In vitro* cytotoxicity studies were also carried out using these concentrations. The *in vitro* antimicrobial and cytotoxicity studies revealed 2.5% solution of copper sulphate and zinc sulphate to be most effective in terms of preventing bacterial growth and being safe. For the prophylactic study 40 healthy cows, maintained under similar management and found negative for mastitis through CMT, pH, EC, SCC and culture of milk samples were included in the study. Cows were randomly divided into 4 groups, viz., group I, group II, group III and group IV with ten cows in each. Group I, served as control, in which no teat dip was used. In group II animals conventional teat dip (0.5% Povidone Iodine) was used. In group III and group IV Copper Sulphate (2.5% aqueous solution) and Zinc Sulphate (2.5% aqueous solution) teat dips were used respectively. Post-milking teat dipping was done twice a day for a period of two months. Milk samples were collected aseptically from all the quarters on Day 0, 15, 30, 45 and 60 to see the effect of post-milking teat dipping with copper sulphate and zinc sulphate on biochemical properties of milk (Fat, SNF, Protein and lactose) and compare them with povidone iodine and control groups. The biochemical properties of milk did not change using copper sulphate and zinc sulphate as post milking teat dips. It is concluded that the use of 2.5% each of copper sulphate and zinc sulphate solutions as post-milking teat dips can prove beneficial in prophylaxis of bovine mastitis.

Keywords: Bovine mastitis, copper sulphate, zinc sulphate, teat dipping, milk fat, milk SNF, milk lactose, milk protein

1. Introduction

Mastitis is a parenchymal inflammation of the mammary gland characterized by a range of physical and chemical changes in the milk and pathological changes in the glandular tissue [1]. Mastitis is still recognized as Major problem in dairy animals as it is one of the most prevalent and problematic disease of dairy animals, causing heavy economic losses in terms of quality and quantity of milk [2]. Mastitis is a difficult problem to comprehend because it is a disease caused by many etiological agents. Its incidence depends on the microorganisms, the surrounding environment and the defence mechanisms in the udder tissues and blood. Bovine udder remains in constant touch with mud, manure and urine and is therefore continuously exposed to environmental contaminants including microbial pathogens. These factors make the udder highly susceptible to pathogenic invasions. Finding successful strategies for the control of bovine mastitis is a challenge for dairy producers. Currently the programs are based on hygiene and include teat disinfection, antibiotic therapy and culling of chronically infected cows. The National Institute for Research in Dairying (NIRD) program includes different strategies for mastitis control management such as dry cow treatment, milking techniques, teat disinfection with topical antiseptic substances (pre-dipping and post-dipping) and antibiotic treatment of clinical mastitis cases [3]. However, the use of antibiotics, confined to selected severe cases, requires bacterial isolation and antibiotic selection [4]. In addition, the routine use of antibiotics is questionable because they can generate unwanted residues not accepted in milk, and because they can spread the emergence of antimicrobial-resistant strains [5]. Thus,

proper control of mastitis in dairy herd is considered an indispensable process to ensure both animal health and food (milk) safety. For this, numerous control programs have been developed over the last few decades and despite the massive development in mastitis control techniques, mastitis still constitutes the main problem of dairy production [6]. Among these controlling regimes, teat dipping has acquired great importance as an essential mastitis preventive tool [7]. While pre-milking teat dipping is necessary to reduce the microbial population and minimize new intramammary infections, post-milking teat dips have been used mainly in highly infected herds and has been revealed as a very effective tool to prevent mastitis incidence [8]. Teat dips can function by providing a physical barrier to bacterial entry through the teat orifice. Additionally, bacteria that may be present can be killed by antibacterial ingredients of some teat dips. Although well-known and commercially available teat dips do have a beneficial effect on preventing the spread of mastitis, there is still a continuing need for improved compositions and systems for treating and preventing mastitis because recent researches have revealed that not all types of mastitis causing pathogens are responding the same to teat dipping [9].

One alternative to be used as effective teat disinfection may be a copper-based product. The antimicrobial properties of copper have been recognized for several years and applying these properties to the prevention of diseases such as bovine mastitis is a new area of research. The use of antimicrobial copper was accepted for the first time in 2008 by the United States Environmental Protection Agency [10]. Copper antibacterial functionality is associated with various mechanisms, including damage to the microbial DNA, altering bacterial protein synthesis and membrane integrity [11]. In addition, the antibacterial effect of copper is already proved for *E. coli* and *S. aureus*, two of main bacterial species involved in mastitis [12]. Since copper inhibits bacterial multiplication of different species isolated from bovine mastitis, it may be an attractive alternate for applying as a teat dip to control bovine mastitis in milk farms [13] and its use as an alternative to prevent bovine mastitis appears as a novel and promising idea.

Another common preventive measure is application of Zinc containing teat dip to the teats after milking to maintain hygiene and to prevent mammary infections. Zinc is required for keratin production which lines the interior of the teat canal and acts as a plug to trap bacteria and prevent their entry into the udder [14]. Three distinct mechanisms of action of zinc have been put forward (i) the production of reactive oxygen species (ROS) because of the semi-conductive properties (ii) the destabilization of microbial membranes upon direct contact of zinc particles to the cell walls and (iii) the intrinsic antimicrobial properties of Zn^{2+} ions released by zinc salts in aqueous medium [15].

In view of the existence of many protocols of teat dipping as pre-milking and post-milking, and the existence of many reports concerning the efficacy of various teat dips in mastitis control protocol, the present study was conducted to evaluate the efficacy of copper sulphate and zinc sulphate as post-milking teat dips to prevent dairy cows from mastitis and evaluate the effect of these teat dips on milk biochemical parameters (Fat, SNF, Lactose and protein).

2. Materials and methods

The trial was conducted on normal healthy mastitis free cows (Jersey and Holstein Frisean) in different stages of lactation (two to six) at Mountain Livestock Research Institute,

Manasbal. The animals selected for trial were maintained under identical feeding and management practices. The milking of all cows was carried out by hand milking twice a day. Forty healthy (mastitis free) cows were selected for trial. These cows were randomly divided into four equal groups having ten cows in each group (Table 1). Group I served as control, in which no teat dip was applied. In group II conventional teat dip (0.5% Povidone iodine) was used. In group III Copper Sulphate teat dip (2.5% aqueous solution) and in group IV Zinc Sulphate teat dip (2.5% aqueous solution) was used. Post-milking teat dipping was done twice a day for a period of two months.

Table 1: Prophylactic Trial Design

Groups	Post- milking teat dip
Group I	No teat dip
Group II	Povidone iodine (0.5%)
Group III	Copper sulphate (2.5%)
Group IV	Zinc sulphate (2.5%)

2.1 Effect of teat dipping on composition of Milk (Milk Biochemistry)

Composition of freshly drawn milk samples after every 15 days in control group and in groups where Copper Sulphate and Zinc Sulphate was used as post milking teat dip was determined by milkoscan- the automatic milk analyser to notice any change in fat, SNF, protein and lactose. The results obtained in groups where Copper Sulphate and Zinc Sulphate teat dip was used were compared with the control group in which no teat dip was used.

Procedure

The MilkoScan was turned ON and the option of cattle milk was selected. Milk sample to be tested was brought below the suction pipe of the milkoScan and the instrument was run by pressing enter key. Results were displayed within 1 minute. Then the exit key was pressed to remove residual milk from milkoScan and at last thorough cleaning of milkoScan with acidic and alkaline solution was done.

3. Statistical analysis

Data generated was statistically evaluated and subjected to statistical analysis as per the standard procedures described by Snedecor and Cochran (1994). The data was presented as $M \pm SE$ and significance of mean difference was tested by using one way ANOVA followed by Duncan's New Multiple Range Test (DNMRT) using the Statistical Package for the Social Sciences, Base 20.0 (SPSS Software products, Marketing Department, SPSS Inc. Chicago, USA)

4. Results and discussion

4.1 Effect of teat dipping with povidone iodine, copper sulphate and zinc sulphate on milk fat percentage

In the present study no significant ($p < 0.05$) change in milk fat % was observed in group I (Control), group II, group III and group IV from day 0 to day 60, indicating no effect of post-milking teat dipping with povidone iodine, copper sulphate and zinc sulphate on milk fat %. However non-significant ($p < 0.05$) decrease in milk fat was observed in group I as compared to group II, III and IV. The decrease in milk fat % in group I (Control) was because of sub-clinical infection in this group as no control measure (teat dip) was adopted in this group. Our findings are in agreement with Auld and Hubbell (1998) [16] who reported a decrease in fat concentration in mastitis.

4.2 Effect of teat dipping with povidone iodine, copper sulphate and zinc sulphate on milk SNF

In the present study no significant ($p < 0.05$) change in milk SNF % was observed in group I (Control), group II, group III and group IV from day 0 to day 60, indicating no effect of post-milking teat dipping with povidone iodine, copper sulphate and zinc sulphate on milk SNF %, however non-significant ($p < 0.05$) decrease in milk SNF was observed in group I as compared to group II, III and IV. The decrease in milk SNF % in group I (Control) was because of sub-clinical infection in this group which occurs as no control measure (teat dip) was adopted in this group. Our findings of decreased SNF content of milk in group I are in corroboration with Bansal *et al.* (2005) [17] and Reis *et al.* (2013) [18] who also reported a decrease in SNF content of milk in mastitis.

4.3 Effect of teat dipping with povidone iodine, copper sulphate and zinc sulphate on milk Protein

In the present study no significant ($p < 0.05$) change in milk protein % was observed in group I (Control), group II, group III and group IV from day 0 to day 60 indicating no effect of post-milking teat dipping with povidone iodine, copper sulphate and zinc sulphate on milk protein %, however non-significant ($p < 0.05$) decrease in milk protein was observed in group I as compared to group II, III and IV. The non-significant ($p < 0.05$) decrease in milk protein (Casein) in group I (Control) was because of sub-clinical infection in this group which occurs as no control measure (teat dip) was adopted in this group. Our findings are in corroboration with

Auldrist and Hubble (1998) [16] who reported that the decrease in casein concentrations during mastitis is largely due to post-secretory degradation of casein by proteinases originating from mastitis-causing organisms, leucocytes or the blood and in part to a reduction in the synthesis and secretion of casein as a result of physical damage to the mammary epithelial cells by microbial toxins during mastitis.

4.4 Effect of teat dipping with povidone iodine, copper sulphate and zinc sulphate on milk lactose

In the present study no significant ($p < 0.05$) change in milk lactose % was observed in group I (Control), group II, group III and group IV from day 0 to day 60, indicating no effect of post-milking teat dipping with povidone iodine, copper sulphate and zinc sulphate on milk lactose %, however more non-significant ($p < 0.05$) decrease in milk lactose was observed in group I as compared to group II, III and IV (Table 2). The non-significant ($p < 0.05$) decrease in milk lactose in group I (Control) was because of sub-clinical infection in this group which occurs as no control measure (teat dip) was adopted in this group. Our findings of decrease in the concentration of milk lactose are in agreement with Bruckmaier *et al.* (2004) [19] and Bansal *et al.* (2005) [17]. The decline in milk lactose in mastitis is in due to damage to the alveolar epithelial cells. Furthermore, according to Auldrist *et al.* (1995) [20], many of the common mastitis-causing organisms are capable of fermenting lactose. The lower concentrations of lactose in mastitic milk may be partly due to the activities of these organisms.

Table 2: Effect of teat dipping with povidone iodine, copper sulphate and zinc sulphate on milk biochemical parameters (fat, SNF, protein and lactose) (Mean \pm SE)

Groups	Days	Fat	SNF	Protein	Lactose
Group I	Day 0	6.23 \pm 0.295 ^{aA}	7.12 \pm 0.150 ^{aA}	2.66 \pm 0.044 ^{aA}	3.68 \pm 0.085 ^{aA}
	Day 15	5.97 \pm 0.362 ^{aA}	6.95 \pm 0.180 ^{aA}	2.58 \pm 0.053 ^{aA}	3.58 \pm 0.086 ^{aA}
	Day 30	5.87 \pm 0.339 ^{aA}	6.90 \pm 0.135 ^{aA}	2.58 \pm 0.045 ^{aA}	3.52 \pm 0.087 ^{aA}
	Day 45	5.77 \pm 0.333 ^{aA}	6.87 \pm 0.132 ^{aA}	2.55 \pm 0.046 ^{aA}	3.53 \pm 0.074 ^{aA}
	Day 60	5.75 \pm 0.327 ^{aA}	7.05 \pm 0.160 ^{aA}	2.58 \pm 0.040 ^{aA}	3.55 \pm 0.068 ^{aA}
Group II	Day 0	6.22 \pm 0.326 ^{aA}	7.38 \pm 0.115 ^{aA}	2.72 \pm 0.043 ^{aA}	3.79 \pm 0.075 ^{aA}
	Day 15	6.15 \pm 0.333 ^{aA}	7.31 \pm 0.093 ^{abA}	2.68 \pm 0.054 ^{aA}	3.76 \pm 0.068 ^{abA}
	Day 30	6.07 \pm 0.308 ^{aA}	7.27 \pm 0.126 ^{abA}	2.69 \pm 0.074 ^{aA}	3.68 \pm 0.100 ^{aA}
	Day 45	5.98 \pm 0.305 ^{aA}	7.25 \pm 0.136 ^{abA}	2.71 \pm 0.110 ^{abA}	3.71 \pm 0.087 ^{abA}
	Day 60	5.95 \pm 0.298 ^{aA}	7.23 \pm 0.126 ^{aA}	2.71 \pm 0.109 ^{abA}	3.71 \pm 0.080 ^{abA}
Group III	Day 0	6.10 \pm 0.323 ^{aA}	7.45 \pm 0.143 ^{aA}	2.82 \pm 0.092 ^{aA}	3.82 \pm 0.098 ^{aA}
	Day 15	6.11 \pm 0.327 ^{aA}	7.46 \pm 0.136 ^{bA}	2.88 \pm 0.110 ^{aA}	3.85 \pm 0.091 ^{bA}
	Day 30	6.12 \pm 0.309 ^{aA}	7.46 \pm 0.157 ^{aA}	2.87 \pm 0.099 ^{aA}	3.81 \pm 0.119 ^{aA}
	Day 45	5.94 \pm 0.317 ^{aA}	7.28 \pm 0.157 ^{abA}	2.78 \pm 0.127 ^{abA}	3.75 \pm 0.094 ^{abA}
	Day 60	5.92 \pm 0.321 ^{aA}	7.30 \pm 0.150 ^{aA}	2.79 \pm 0.127 ^{abA}	3.74 \pm 0.090 ^{abA}
Group IV	Day 0	5.54 \pm 0.333 ^{aA}	7.26 \pm 0.120 ^{aA}	2.82 \pm 0.147 ^{aA}	3.78 \pm 0.089 ^{aA}
	Day 15	5.46 \pm 0.375 ^{aA}	7.26 \pm 0.105 ^{abA}	2.82 \pm 0.148 ^{aA}	3.79 \pm 0.067 ^{abA}
	Day 30	5.25 \pm 0.403 ^{aA}	7.29 \pm 0.109 ^{abA}	2.85 \pm 0.151 ^{aA}	3.80 \pm 0.076 ^{aA}
	Day 45	5.10 \pm 0.378 ^{aA}	7.52 \pm 0.166 ^{bA}	2.97 \pm 0.120 ^{bA}	3.91 \pm 0.070 ^{bA}
	Day 60	5.21 \pm 0.360 ^{aA}	7.37 \pm 0.125 ^{aA}	2.91 \pm 0.114 ^{bA}	3.86 \pm 0.054 ^{bA}

Values with different superscript differ significantly ($P < 0.05$); capital alphabets represent column-wise and small alphabets represent row-wise

5. Conclusion

Since the use of copper sulphate and zinc sulphate solutions as post milking teat dips at a concentration of 2.5% did not produce any adverse effect on milk biochemical properties, these solutions are recommended for use as post milking teat dips to prevent the occurrence of bovine mastitis. These solutions being more efficacious, cost effective and easily

available could prove a major breakthrough in prevention of bovine mastitis.

6. Conflict of interest

The authors declare no conflict of interest.

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