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Relationship Between Milk pH and Somatic Cell Count in Dairy Cows Treated with Essential Oil-based Teat Spray

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Abstract

The present study evaluated the effect of essential oil-based teat disinfectants on milk pH and somatic cell count in dairy cows affected with subclinical mastitis. 18 lactating cows positive for subclinical mastitis were randomly assigned to three treatment groups: T₁ (2% essential oil teat dip), T₂ (2% essential oil teat spray), and T₃ (1% essential oil teat spray). The formulation contained eucalyptus, lavender, peppermint, and tea tree essential oils. The experiment was conducted for 90 days, with milk samples collected at 15-day intervals. Initially, milk pH values ranged from 7.55 to 7.65, indicating mastitis-associated alkalinity. A significant reduction in milk pH was observed in all groups, reaching near-normal values by day 90. SCC also decreased markedly, from 4.00-4.35 lakh cells/ml at the start to 1.95-2.25 lakh cells/ml at the end of the study. The results demonstrated improved udder health, with the 2% essential oil-based teat spray showing the most effective reduction in SCC and normalisation of milk pH.

Keywords: Subclinical mastitis, Essential oils, Teat spray, Somatic cell count, Milk pH.

1. Introduction

Subclinical mastitis is one of the most prevalent and economically important diseases affecting dairy cattle worldwide. Unlike clinical mastitis, it does not exhibit visible signs of inflammation, making its detection and management challenging. The condition is characterized by an elevated somatic cell count (SCC), alterations in milk composition, increased milk pH, and gradual reduction in milk yield, resulting in substantial economic losses to dairy farmers. Somatic cell count is considered a reliable indicator of udder health and intramammary infection, while milk pH reflects the physiological status of the mammary gland (Mendake *et al.*, 2026) ^[11]. During mastitis, damage to mammary epithelial cells and increased permeability of blood capillaries lead to the movement of alkaline ions into milk, causing an increase in milk pH. Therefore, both SCC and milk pH are important parameters for assessing the severity of subclinical mastitis and monitoring the effectiveness of therapeutic interventions.

In recent years, the use of herbal and plant-derived products has gained considerable attention as an alternative approach for mastitis control due to concerns regarding antimicrobial resistance and antibiotic residues in milk. Essential oils such as eucalyptus, lavender, peppermint, and tea tree oil possess antimicrobial, anti-inflammatory, antioxidant, and immunomodulatory properties, which may help improve udder health and reduce microbial load on the teat surface. Post-milking application of essential oil-based teat sprays and teat dips can provide a protective barrier against mastitis-causing pathogens and aid in the restoration of normal mammary gland function. Improvement in udder health is expected to reduce SCC, normalize milk pH, and subsequently enhance milk production (Mendake *et al.*, 2025) ^[12].

The relationship between SCC and milk pH is closely associated with the health status of the

udder. An increase in SCC due to infection is often accompanied by an increase in milk pH as a result of inflammatory changes in the mammary gland. Therefore, a reduction in SCC along with normalisation of milk pH serves as a strong indicator of recovery from subclinical mastitis. Evaluating these parameters together provides a comprehensive assessment of the efficacy of essential oil-based teat hygiene practices. Hence, the present study was undertaken to investigate the effect of different concentrations and methods of application of essential oil-based teat disinfectants on milk pH and somatic cell count in dairy cows affected with subclinical mastitis, with the objective of determining their role in improving udder health and milk quality.

Materials and Methods

Milk pH

The milk pH of each milk sample was recorded with a digital pH meter on days 0, 15, 30, 45, 60, 75, and 90. The milk samples from the individual animal were taken in a sterilised vial, and the digital pH meter was inserted into the sample to measure the pH.

Somatic Cell Count

Method

The SCC was determined by manual counting as described by (Rana *et al.*, 2023) ^[17].

Technique

Sample preparation for SCC,

For the preparation of the sample, 20 ml of milk and 10 ml of Phosphate buffer solution (PBS) were taken in a Centrifuge tube which was then subjected to a Centrifuge machine at 4000 rpm for 15 minutes at 25°C. After centrifugation, the supernatant was removed by pasture pipette, and in the remaining pallet, 10 ml PBS was added to remove fats and again centrifuge at 8000 rpm for 5 minutes. After the process of centrifugation, the supernatant was removed and 2 ml PBS was added again in a fatless pallet and left undisturbed for 5 minutes. 50 µl of methylene blue dye was added to 50 µl of fatless mixture in Eppendorf serum tubes and left undisturbed for 10 to 15 minutes. With the help of a micropipette inject 50 µl of sample in a Neubauer's chamber and observe the sample under a microscope (Rana *et al.*, 2023) ^[17].

Milk pH

The results of the milk pH of the dairy cows under different treatment groups are presented in Table 4.10 and graphically depicted in Fig. 4.10. There was a non-significant ($p < 0.05$) difference in the pH value of the milk at the initiation of the experiment. The pH value of the milk sample of the cows under different groups on initiation of the experiment, i.e., 0th days, was T₁ (7.61±0.08), T₂ (7.65±0.07), and T₃ (7.55±0.10). The values reveal that the pH of the milk at the initial stage was slightly alkaline for all the treatment groups. However, all three groups have shown a decrease in milk pH over the experimental period. The pH value of the milk sample recorded at the end of the experimentation, i.e., on the 90th day, was 6.68±0.05, 6.58±0.04, and 6.75±0.05 for Group T₁, Group T₂, and Group T₃, respectively. This indicated a gradual normalisation of the milk pH at the end of the experiment.

All treatments show a progressive decline over time in milk pH. Non-significant difference ($p < 0.05$) observed in group T₁ on day 0th to day 30th and a significant drop in pH was

observed on day 45th up to the end of experimentation. Group T₁ and group T₂ had sharper declines after Day 45th, and group T₂ had the lowest value at Day 90th observed. Group T₃ had shown a significant improvement from day 60th of the experiment. The values for day 0, day 15, day 30, day 45, day 60, day 75 and day 90 for Group T₁ were 7.61±0.08, 7.58±0.08, 7.46±0.09, 7.35±0.09, 7.13±0.07, 6.87±0.04, and 6.68±0.05, respectively, for Group T₂ were 7.65±0.07, 7.65±0.07, 7.56±0.05, 7.36±0.04, 7.02±0.04, 6.80±0.02, and 6.58±0.04, respectively and for Group T₃ were 7.55±0.10, 7.51±0.09, 7.48±0.09, 7.38±0.07, 7.12±0.07, 6.86±0.04, and 6.75±0.05, respectively.

The present study findings were in line with Deshmukh (2023), who observed that the pH value of the T₂ (2% EO-based teat dip) and T₁ (1% iodine-based teat dip) was improved from the 30th day of the application of the teat dip and restored to normal on 60th days. The decrease in milk pH can be attributed to the antimicrobial and anti-inflammatory activities of the essential oils (Tomanic *et al.* 2022; Hammer *et al.*, 2006; Carson *et al.*, 2006) ^[1, 3] which has led to the improvement in sub-clinical mastitis condition. Similarly, Rasool *et al.*, (2021) ^[19], found that post-milking essential oil-based teat dips containing eucalyptus and lavender oils significantly reduced the pH of milk in dairy cows suspected of having subclinical mastitis on the 4th week of treatment. Likewise, Tomar *et al.*, (2018) ^[26] reported a notable drop in pH throughout the trial of the experiment in goats. On the 100th day of using post-milking aloe vera-based teat dips in goats with subclinical mastitis, the pH of the milk returned to normal. Similarly, Waghmare *et al.*, (2013) ^[28] found that post-milking teat dip consisting of *Barberis lyceum*, *Curcuma longa*, and *Eucalyptus globus* significantly decreased the pH of milk in subclinical mastitis in cows on day 30th respectively.

Milk components that maintain pH (casein citrate, phosphate, dissolved CO₂ and bicarbonate), are regulated by udder cells' permeability to blood capillaries. Higher pH levels in the milk are caused by mastitis because of the gland's increased permeability to blood components, including bicarbonate ions (Kolte *et al.*, 2008) ^[8]. The movement of bicarbonate ions increases in milk resulting from a drop in lactose production and the addition of blood-derived alkaline salt, which has a pH above 7.0 (Bagri *et al.*, 2018) ^[2]. Increasing in pH is due to higher levels of Cl⁻ ions (Chen *et al.*, 2018) ^[4]. The decrease in the pH in present study might be due to improvement in udder condition resulting from application of essential oils in all treatment groups.

Somatic Cell Count (SCC)

The results of the somatic cell count of the cows under experimental trial are presented in Table 4.1 and the same was represented graphically in Fig. 4.1. The results show a non-significant ($p < 0.05$) value of SCC on days 0th was observed for the Groups T₁, T₂, and T₃. The values for Groups T₁, T₂, and T₃ were 4.00±0.15, 4.12±0.16, and 4.35±0.13 respectively, which reveals that all the experimental animals exhibit sub-clinical mastitis. With the progress of the treatment, there was a decrease in the somatic cell count every fortnight i.e. on the 15th, 30th, 45th, 60th, 75th, and 90th day in all the groups.

All treatment groups T₁, T₂, and T₃, showed statistically significant ($p < 0.05$) reductions in somatic cell count from day 0th to day 90th within the rows. The values of group T₁ for day 0, 15, 30, 45, 60, 75, and 90 days being 4.00±0.15, 3.62±

0.18, 3.40 ± 0.18 , 2.77 ± 0.12 , 2.10 ± 0.1 , 2.05 ± 0.05 , and 1.97 ± 0.04 , respectively.

Group T₁ had shown non-significant ($p < 0.05$) difference within the treatment group till day 15th. however, a significant difference within group T₁ was observed between day 30th and day 45th. Also, a significant difference ($p < 0.05$) was observed between day 45th and day 60th. However, no significant difference ($p < 0.05$) was observed between day 60th and day 75th for group T₁. Further, a significantly lower SCC ($p < 0.05$) was also observed for group T₁ between day 75th and day 90th.

For group T₂, a non-significant ($p < 0.05$) difference within the treatment group was observed between day 0th and day 15th. However, a significant difference ($p < 0.05$) within group T₂ was observed between day 15th and day 30th. Also, a significant difference ($p < 0.05$) was observed between day 30th and day 45th. Similarly, a significant difference ($p < 0.05$) was also observed for group T₂ between day 75th and day 90th. However, no significant ($p < 0.05$) difference in SCC in group T₂ was observed between day 45th and day 60th; further, no significant ($p < 0.05$) difference in SCC in group T₂ was observed between day 75th and day 90th. The values for Group T₂ for day 0, 15, 30, 45, 60, 75 and 90 being 4.12 ± 0.16 , 4.12 ± 0.16 , 3.65 ± 0.15 , 2.90 ± 0.16 , 2.47 ± 0.1 , 2.22 ± 0.08 and 1.95 ± 0.07 , respectively. For group T₃ a non-significant ($p < 0.05$) difference within the treatment group was observed between day 0 and day 15. However, significantly lower SCC values ($p < 0.05$) within group T₂ were observed between day 15 and day 30. Also, significantly lower values for SCC ($p < 0.05$) were observed between day 30 and day 45. Further, with the advancement of the experiment, there is a reduction in the SCC on days 60, 75, and 90. However, no significant ($p < 0.05$) difference in SCC in group T₃ was observed between day 45, day 60, day 75, and day 90. The values for Group T₃ for day 0, 15, 30, 45, 60, 75, and 90 days being 4.35 ± 0.13 , 4.17 ± 0.12 , 3.85 ± 0.10 , 3.45 ± 0.09 , 3.02 ± 0.05 , 2.55 ± 0.07 , and 2.25 ± 0.07 , respectively.

Significant difference ($p < 0.05$) in Group T₁ (2.77 ± 0.12) and T₂ (2.9 ± 0.16) and Group T₁ (2.77 ± 0.12) and T₃ (3.45 ± 0.09) was observed from day 45th. A significant difference ($p < 0.05$) in Group T₁ and T₂ and Group T₁ and T₃ continues up to day 90th with values as 1.97 ± 0.04 , 1.95 ± 0.07 , and 1.97 ± 0.04 ,

2.25 ± 0.07 in Group T₁ and T₂ and Group T₁ and T₃ respectively, whereas, the non-significant difference was observed in values of Group T₁ (1.97 ± 0.04) and T₂ (1.95 ± 0.07). The present findings were in line with the findings of Deshmukh (2023) who reported a decrease in SCC in groups treated with Essential Oil (EO)-based teat dip and Iodine-based teat dip. The readings of SCC in Groups T₁ and T₂ show that the animals have recovered from subclinical mastitis on the 90th day of experimentation. The results of in line with the findings of Rasool *et al.*, (2021) [19] who reported a decrease in SCC of the treatment group in dairy cows on the application of EO based post milking teat dips (Eucalyptus oil and lavender oil with dimethyl sulphoxide). Rathaur *et al.*, (2020) [20] reported a significant decrease in SCC on the 10th day of treatment with the application of herbal paste (turmeric, castor oil, lemon, and aloe vera) after milking in cows with subclinical mastitis. Similarly, Waghmare *et al.*, (2013) [28], and Vala *et al.*, (2013) [27] also reported a significant reduction in the SCC in cows on the application of Mastidip (*Berberis lyceum*, *Curcuma longa*, *Eucalyptus globulus*) post-milking teat dip. Biradar *et al.*, (2022) also reported a significant decrease in somatic cell count on the 60th day with the application of Aloe vera-based teat dip in HF crossbred cows positive for subclinical mastitis.

The somatic cells in milk include Macrophages, Neutrophils, Epithelial cells, and Mononuclear cells whereas Neutrophils and PMN Leukocytes act as the second line of defense in the mammary gland and they act as both morphological and chemical barriers for pathogens. Macrophages are found abundant (66-88%) in the mammary gland, they identify the pathogens and signal the leukocytes and immune cells resulting in the accumulation of leucocytes and immune cells in mammary glands and thereby increasing the count of somatic cells Alhussien & Dang (2018) [1], Orlandini & Bijgaart, (2011), and Harmon, (1994) [7].

On the 90th day, all the groups showed a decrease in SCC, however, the lowest values were observed in Group T₂ (1.95 ± 0.04) followed by Group T₁ (1.97 ± 0.04) and Group T₃ (2.25 ± 0.07) indicating better efficacy of 2% EO-based Teat spray over 2% EO-based teat dip and 1% EO-based Teat spray.

Table 1: Mean $\pm$ SE values of dairy cows' milk pH under different treatment groups

Groups	0 th Day	15 th Day	30 th Day	45 th Day	60 th Day	75 th Day	90 th Day	CD
T ₁	$7.61^d \pm 0.08$	$7.58^d \pm 0.08$	$7.46^{dc} \pm 0.09$	$7.35^{bc} \pm 0.09$	$7.13^b \pm 0.07$	$6.87^a \pm 0.04$	$6.68^a \pm 0.05$	0.22
T ₂	$7.65^e \pm 0.07$	$7.65^e \pm 0.07$	$7.56^e \pm 0.05$	$7.36^d \pm 0.04$	$7.02^c \pm 0.04$	$6.80^b \pm 0.02$	$6.58^a \pm 0.04$	0.22
T ₃	$7.55^c \pm 0.10$	$7.51^c \pm 0.09$	$7.48^c \pm 0.09$	$7.38^c \pm 0.07$	$7.12^b \pm 0.07$	$6.86^a \pm 0.04$	$6.75^a \pm 0.05$	0.22

The mean bearing different superscript (a,b,c,d, & e) within the row differ significantly ($p < 0.05$)

Table 2: Mean $\pm$ SE values of dairy cows' somatic cell count ($\times 10^5$ cells/ml) under different treatment groups

Groups	0 th Day	15 th Day	30 th Day	45 th Day	60 th Day	75 th Day	90 th Day	CD
T ₁	$4.00^e \pm 0.15$	$3.62^{de} \pm 0.18$	$3.40^d \pm 0.18$	$2.77^{bc} \pm 0.12$	$2.10^{Cb} \pm 0.1$	$2.05^{Bb} \pm 0.05$	$1.97^{Ba} \pm 0.04$	0.291
T ₂	$4.12^e \pm 0.16$	$4.12^{cd} \pm 0.16$	$3.65^c \pm 0.15$	$2.90^{Bb} \pm 0.16$	$2.47^{Bb} \pm 0.1$	$2.22^{Ba} \pm 0.08$	$1.95^{Ba} \pm 0.07$	0.312
T ₃	$4.35^e \pm 0.13$	$4.17^c \pm 0.12$	$3.85^b \pm 0.10$	$3.45^{Aa} \pm 0.09$	$3.02^{Aa} \pm 0.05$	$2.55^{Aa} \pm 0.07$	$2.25^{Aa} \pm 0.07$	0.205
CD				0.381	0.323	0.212	0.192	

The mean bearing different superscripts (A, B, & C) within the column differ significantly ($p < 0.05$)

The mean bearing different superscripts (a,b,c,d,e,&f) within the row differ significantly ($p < 0.05$)

Conclusion

The present study demonstrated that the application of essential oil-based teat spray and teat dip formulations effectively improved udder health in dairy cows affected with subclinical mastitis, as evidenced by a significant reduction in somatic cell count (SCC) and normalization of milk pH. All

treatment groups showed a progressive decline in SCC and milk pH throughout the 90-day experimental period, indicating recovery from subclinical mastitis. The reduction in SCC may be attributed to the antimicrobial and anti-inflammatory properties of the essential oils, which helped control intramammary infections and reduce inflammation of

the mammary gland. Simultaneously, the decrease in milk pH towards the normal physiological range reflected restoration of mammary gland integrity and reduced leakage of alkaline blood constituents into milk.

Among the treatments evaluated, the 2% essential oil-based teat spray (T₂) showed the greatest efficacy, recording the lowest SCC and milk pH values at the end of the experiment, closely followed by the 2% essential oil-based teat dip (T₁). The findings indicate a strong relationship between decreasing SCC and normalisation of milk pH, confirming that both parameters can serve as reliable indicators of udder health improvement. Therefore, the use of essential oil-based teat spray, particularly at a 2% concentration, can be recommended as an effective natural strategy for the management of subclinical mastitis and for improving milk quality in dairy cows.

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