

# RELATIONSHIP BETWEEN SERUM ZINC CONCENTRATION, TEAT CANAL KERATIN THICKNESS AND SOMATIC CELL COUNT IN DAIRY COWS

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**Abstract:** The aim of this study was to evaluate the relationship between serum zinc concentration, teat canal keratin layer thickness and somatic cell count (SCC) in dairy cows. The study included 26 dairy cows excluded from production and sent to slaughter, with a total of 104 udder quarters examined. Prior to slaughter, blood samples were collected for determination of serum zinc concentration and milk samples from each udder quarter were obtained for SCC analysis. After slaughter, teats were collected for histopathological examination and the thickness of the teat canal keratin layer was measured. A significant negative correlation was observed between serum zinc concentration and SCC ( $r = -0.62$ ,  $p < 0.01$ ), indicating that higher serum zinc levels were associated with lower SCC values. Serum zinc concentration was positively correlated with teat canal keratin layer thickness ( $r = 0.58$ ,  $p < 0.01$ ), while keratin layer thickness showed a significant negative relationship with SCC ( $r = -0.54$ ,  $p < 0.05$ ). The lowest SCC values were recorded in teat canals with a keratin layer thickness of 201–300  $\mu\text{m}$ , whereas higher SCC values were found in canals with both very thin (0–100  $\mu\text{m}$ ) and excessively thick (301–400  $\mu\text{m}$ ) keratin layers. These results indicate that adequate serum zinc concentration and optimal teat canal keratin layer thickness are associated with improved udder health and lower SCC values, suggesting a potential role of zinc status and teat canal integrity in reducing the risk of subclinical mastitis in dairy cows.

**Key words:** dairy cows, zinc, teat canal, keratin layer, somatic cell count

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## Introduction

Mastitis remains one of the most significant health problems in dairy cattle, causing substantial economic losses due to reduced milk yield, impaired milk quality, increased veterinary costs and higher culling rates (Ranasinghe et al., 2021; Shafeeq et al., 2021; Stanek et al., 2024; Yu et al., 2025). Subclinical mastitis, characterized by the absence of visible clinical signs, represents a particular challenge, as it often remains undetected while negatively affecting milk production and milk quality (Hamadani et al., 2013; Cobirka et al., 2020). Somatic cell count (SCC) is widely used as a reliable indicator of udder health and the presence of subclinical intramammary infections.

The teat canal constitutes the first line of defense against the penetration of pathogenic microorganisms into the mammary gland. Its protective function is largely attributed to the keratin layer lining the teat canal, which provides both a physical barrier and bacteriostatic properties (Ezzat Alnakip et al., 2014; Cardozo et al., 2025). Structural alterations of the teat canal keratin, including thinning, excessive keratinization or irregular keratin arrangement, may impair its barrier function and facilitate bacterial invasion, leading to increased SCC and a higher risk of intramammary infection (Capuco et al., 1992; Pantoja et al., 2020).

Zinc is an essential trace element involved in numerous biological processes, including epithelial cell proliferation, differentiation and keratinization. Adequate zinc status is crucial for maintaining the integrity of epithelial and mucosal barriers, as well as for optimal immune function. Zinc deficiency has been associated with impaired keratin formation, delayed epithelial regeneration and increased susceptibility to infectious diseases (Hassan et al., 2020; Zemanova et al., 2022). Several studies have demonstrated the beneficial effects of zinc supplementation on udder health and somatic cell count in dairy cows (Pechová et al., 2006; Davidov et al., 2014; Oconitrillo et al., 2024; Li et al., 2025). But however, most available data focus either on systemic zinc status or on production parameters, while limited information is available on the relationship between serum zinc concentration, teat canal keratin morphology and SCC assessed simultaneously at the level of individual udder quarters.

Therefore, the present study was designed to test the hypothesis that serum zinc concentration is associated with the thickness of the teat canal keratin layer and that both parameters are related to somatic cell count in dairy cows. Specifically, the aim of this study was to investigate the relationship between serum zinc concentration, teat canal keratin layer thickness and SCC in dairy cows, with the objective of contributing to a better understanding of the role of zinc status and teat canal integrity in udder health and the prevention of subclinical mastitis.

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## Materials and Methods

The study was conducted on 26 dairy cows that were excluded from production and sent for slaughter. A total of 104 udder quarters were included in the analysis. Prior to slaughter, blood samples were collected from the coccygeal vein, and milk samples were collected separately from each udder quarter for SCC analysis. As all samples were obtained from animals already designated for commercial slaughter, and procedures were limited to routine blood and milk collection that did not interfere with the slaughter process or cause additional animal distress, formal approval from an ethics committee was not required according to national regulations.

Blood samples were obtained from the caudal vein, following standard principles of asepsis and antisepsis while minimizing animal distress. Samples were collected into additive-free vacutainer tubes (BD Vacutainer Systems, Preanalytical Solutions, UK) to obtain serum. Each vacutainer was labeled with the cow identification number and kept at room temperature until further processing.

Blood samples were centrifuged to obtain serum, which was stored under appropriate conditions until analysis. Serum zinc concentration was determined by flame atomic absorption spectrophotometry using a (PerkinElmer PinAAcle 900) spectrometer. To minimize matrix interference, serum samples were diluted 1:5 with 0.1 M deionized nitric acid (HNO<sub>3</sub>). The analysis was performed at a wavelength of 213.9 nm, using a zinc hollow cathode lamp as the radiation source and an air-acetylene flame. A standard calibration curve (0.1–2.0 mg/L) was prepared using a certified reference zinc solution. The results were expressed in  $\mu\text{mol/L}$ .

Milk samples were collected from all four udder quarters. Prior to sampling, teat ends were thoroughly disinfected. Each milk sample bottle was labeled with the cow identification number. Somatic cell count was determined using the fluoro-opto-electronic method with a Fossomatic analyzer (Foss Electric, Hillerød, Denmark). Subclinical mastitis was defined as an SCC exceeding  $400 \times 10^3$  cells/mL (Pyörälä, 2003).

After slaughter, teats were collected from each udder quarter. Tissue samples were fixed in 10% neutral buffered formalin for 48 h, routinely processed, and embedded in paraffin. Sections of 5  $\mu\text{m}$  thickness were cut and stained with hematoxylin and eosin (H&E) according to the methods described by Senthilkumar et al. (2020). Morphometric analysis of the teat canal was performed using a light microscope (Olympus BX51) equipped with a digital camera. The thickness of the keratin layer was measured at five random points per section using ImageJ software (National Institutes of Health, Bethesda, MD, USA). Prior to measurement, the software was calibrated using a stage micrometer to convert pixels into micrometers ( $\mu\text{m}$ ).

Statistical analysis was performed using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics, including mean, standard deviation, and frequency distributions, were calculated for all examined parameters. The normality of the data distribution was assessed using the Shapiro-Wilk test. Differences in SCC and zinc levels among teat canals grouped by keratin layer thickness were analyzed using one-way analysis of variance (ANOVA). Furthermore, the strength and direction of the relationships between serum zinc, keratin layer thickness, and SCC were evaluated using Pearson's correlation coefficient ( $r$ ). Statistical significance was set at  $p < 0.05$  and  $p < 0.01$ . Due to the hierarchical structure of the data (multiple quarters per cow), results were interpreted with caution regarding the independence of observations.

## Results and Discussion

The results of the present study indicate a clear association between serum zinc concentration, keratin layer thickness of the teat canal and somatic cell count (SCC), suggesting an important role of zinc status and teat canal integrity in udder health.

The distribution of serum zinc concentrations and SCC values among the examined cows is shown in Table 1.

**Table 1.** Descriptive statistics of serum zinc concentration and SCC in the examined cows (n=26)

Parameter	Zn conc. ( $\mu\text{mol/L}$ )	SCC $\times 10^3/\text{mL}$
Cow 1	11.66	440
Cow 2	13.62	450
Cow 3	11.79	440
Cow 4	20.47	290
Cow 5	12.56	400
Cow 6	19.28	310
Cow 7	14.07	390
Cow 8	13.28	490
Cow 9	6.04	460
Cow 10	1.75	420
Cow 11	3.12	470
Cow 12	7.88	400
Cow 13	4.56	430
Cow 14	15.07	410
Cow 15	16.41	390
Cow 16	22.64	310
Cow 17	15.76	410
Cow 18	15.05	450
Cow 19	16.76	430

**Table 1. continue**

Parameter	Zn conc. ( $\mu\text{mol/L}$ )	SCC $\times 10^3/\text{mL}$
Cow 20	16.08	440
Cow 21	20.35	290
Cow 22	17.51	310
Cow 23	13.23	470
Cow 24	18.12	380
Cow 25	18.22	350
Cow 26	17.59	370
Mean $\pm$ SD	13.91 $\pm$ 5.37	398.08 $\pm$ 56.50
Median	15.06	410
Minimum	1.75	490
Maximum	22.64	290
Range	20.89	200

Considerable individual variability in serum zinc concentration and SCC was observed among the examined cows (Table 1), which is consistent with previous studies reporting differences in mineral status and susceptibility to subclinical mastitis in dairy cattle (Davidov et al., 2012; Li et al., 2025). Cows with higher serum zinc concentrations generally exhibited lower SCC values.

Histopathological examination of 104 teat canals showed substantial variability in keratin layer thickness. The highest proportion of teat canals exhibited a keratin layer thickness of 201–300  $\mu\text{m}$  (37.50%), followed by canals with a thickness of 301–400  $\mu\text{m}$  (32.69%). Thinner keratin layers ( $\leq 200$   $\mu\text{m}$ ) were observed less frequently. Such variability in keratin layer thickness has also been reported by other authors and may reflect differences in teat canal exposure, milking conditions and epithelial turnover (Pantoja et al., 2020).

Differences in keratin layer thickness were associated with variations in somatic cell count (Table 2). The lowest mean SCC ( $339 \times 10^3$  cells/mL) was observed in teat canals with a keratin layer thickness of 201–300  $\mu\text{m}$ . Slightly higher SCC values were recorded in canals with a thickness of 101–200  $\mu\text{m}$  ( $408 \times 10^3$  cells/mL). In contrast, markedly higher SCC values were detected in teat canals with very thin keratin layers (0–100  $\mu\text{m}$ ;  $465 \times 10^3$  cells/mL) as well as in canals with excessive keratinization (301–400  $\mu\text{m}$ ;  $424 \times 10^3$  cells/mL). These findings suggest that both insufficient and excessive keratin layer thickness may compromise the protective function of the teat canal and increase susceptibility to subclinical mastitis (Pantoja et al., 2020; Cardozo et al., 2025).

These findings suggest that not only an underdeveloped but also an excessively thick keratin layer may compromise the protective function of the teat canal. Excessive keratinization may lead to irregular keratin structure and fissures, potentially favoring bacterial colonization (Cardozo et al., 2025), while a thin

keratin layer may provide insufficient mechanical and antimicrobial protection (Paduch et al., 2012).

**Table 2.** Serum zinc concentration, keratin layer thickness and SCC

No. of teat canals	Mean Zn conc. ( $\mu\text{mol/L}$ )	Keratin layer thickness ( $\mu\text{m}$ )	Mean SCC $\times 10^3/\text{mL}$
8	10.72	0-100	465
23	11.65	101-200	408
39	18.47	201-300	339
34	11.26	301-400	424

The results of this research indicate that optimal serum zinc concentration and adequate keratin layer thickness are associated with improved udder health, as reflected by lower SCC values. In our study, the mean serum zinc concentration was  $13.91 \pm 5.37 \mu\text{mol/L}$ . This is in agreement with Zadnik (2010), who reported that cows with healthy udders typically maintain serum zinc levels within the physiological range of 12–18  $\mu\text{mol/L}$ . Furthermore, our observation of a strong negative correlation between zinc and SCC ( $r = -0.62$ ) supports the findings of Zadnik (2010), who established that serum zinc concentrations below 9.64  $\mu\text{mol/L}$  are significantly associated with increased SCC and a higher prevalence of subclinical mastitis. These findings underline the importance of appropriate zinc nutrition and the structural integrity of the teat canal in the prevention of intramammary infections (Davidov et al., 2013; Nudda et al., 2023).

Correlation analysis revealed a significant negative relationship between serum zinc concentration and somatic cell count (SCC) ( $r = -0.62$ ,  $p < 0.01$ ), indicating that higher serum zinc levels were associated with lower SCC values. A significant positive correlation was observed between serum zinc concentration and keratin layer thickness of the teat canal ( $r = 0.58$ ,  $p < 0.01$ ). In addition, keratin layer thickness was negatively correlated with SCC ( $r = -0.54$ ,  $p < 0.05$ ), suggesting a protective role of an adequately developed keratin layer against increased somatic cell counts (Table 3).

**Table 3.** Relationship between serum zinc concentration, keratin layer thickness and SCC

Compared variables	Correlation coefficient ( $r$ )	p-value
Serum Zn – SCC	-0.62	< 0.01
Serum Zn – keratin layer thickness	0.58	< 0.01
Keratin layer thickness – SCC	-0.54	< 0.05

The observed inverse relationship between serum zinc and SCC ( $r = -0.62$ ;  $p < 0.01$ ) reinforces the critical role of this microelement in udder defense mechanisms. By promoting keratin synthesis and cellular regeneration, zinc maintains the structural integrity of the teat canal, which serves as the primary barrier against bacterial entry (Zemanova et al., 2022). Consequently, the severe zinc deficiency recorded in some of our subjects (minimum  $1.75 \mu\text{mol/L}$ ) likely impaired this barrier function, explaining the higher inflammatory responses and SCC levels observed (Prasad, 2008; Zadnik, 2010).

In the present study, serum zinc concentration was positively correlated with the thickness of the keratin layer of the teat canal. This relationship suggests that adequate zinc status may contribute to the development and maintenance of an optimal keratin layer, which represents the first physical and chemical barrier against pathogen entry (Nickerson, 1985). The observed negative correlation between keratin layer thickness and SCC further emphasizes the protective role of the teat canal keratin in preventing inflammatory processes within the udder (Zigo et al., 2021).

## Conclusion

This study demonstrates a significant relationship between serum zinc concentration, teat canal keratin layer thickness, and somatic cell count (SCC) in dairy cows. Higher serum zinc concentrations were associated with increased keratin layer thickness and lower SCC values, indicating improved udder health. Teat canals with an intermediate keratin layer thickness ( $201\text{--}300 \mu\text{m}$ ) exhibited the lowest SCC, while both insufficient and excessive keratinization were linked to higher SCC. These findings suggest that maintaining adequate zinc status and optimal keratin layer development may contribute to the prevention of subclinical mastitis in dairy cows.

## Veza između koncentracije cinka u serumu, debljine keratinskog sloja sisnog kanala i broja somatskih ćelija kod mlečnih krava

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## Rezime

Cilj ovog istraživanja je bio da se ispita odnos između koncentracije cinka u serumu, debljine keratinskog sloja kanala sise i broja somatskih ćelija (SCC) kod

mlečnih krava. Istraživanje je obuhvatilo 26 mlečnih krava isključenih iz proizvodnje (iz različitih razloga) i upućenih na klanje, pri čemu su analizirane ukupno 104 četvrti vimena. Pre klanja uzimani su uzorci krvi radi određivanja koncentracije cinka u serumu, dok su uzorci mleka iz svake četvrti vimena korišćeni za analizu SCC. Nakon klanja, sise su prikupljene za patohistološko ispitivanje, a merena je debljina keratinskog sloja kanala sise. Utvrđena je značajna negativna korelacija između koncentracije cinka u serumu i SCC ( $r = -0,62$ ;  $p < 0,01$ ), što ukazuje na to da su više vrednosti cinka u serumu povezane sa nižim vrednostima SCC. Koncentracija cinka u serumu bila je u pozitivnoj korelaciji sa debljinom keratinskog sloja kanala sise ( $r = 0,58$ ;  $p < 0,01$ ), dok je debljina keratinskog sloja pokazala značajnu negativnu povezanost sa SCC ( $r = -0,54$ ;  $p < 0,05$ ). Najniže vrednosti SCC zabeležene su u kanalima sisa sa debljinom keratinskog sloja od 201–300  $\mu\text{m}$ , dok su više vrednosti SCC utvrđene u kanalima sa veoma tankim (0–100  $\mu\text{m}$ ) i prekomerno debelim (301–400  $\mu\text{m}$ ) keratinskim slojem. Ovi rezultati ukazuju na to da su adekvatna koncentracija cinka u serumu i optimalna debljina keratinskog sloja kanala sise povezane sa boljim zdravljem vimena i nižim vrednostima SCC, što ukazuje na potencijalnu ulogu statusa cinka i integriteta kanala sise u smanjenju rizika od subkliničkog mastitisa kod mlečnih krava.

**Ključne reči:** mlečne krave, cink, sisni kanal, keratinski sloj, somatske ćelije

### Conflict of interest

The authors declare that they have no conflict of interest.

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