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Article

Integration of Differential and Total Somatic Cell Counts for Udder Health Classification: Association with Milk Lactose Concentration

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Abstract: Mastitis is one of the most important diseases in dairy cattle, impairing milk yield, composition, and animal welfare. Somatic cell count (SCC) is the traditional indicator of udder health, but it does not provide information on leukocyte composition. Differential somatic cell count (DSCC) complements SCC by quantifying the proportion of neutrophils and lymphocytes, improving mastitis detection and characterization. Lactose, the main milk carbohydrate, is a potential biomarker of mammary epithelial integrity and declines during intramammary inflammation. This study aimed to classify milk samples based on combined SCC and DSCC thresholds and to evaluate the association between udder health status and lactose concentration. A total of 1,083 milk samples were collected from 50 multiparous Romanian Spotted cows housed in a free-stall system with an automated milking robot over a two-year period. Sixteen samples (1.5%) could not be classified due to missing or invalid SCC/DSCC values and were excluded from group comparisons. The remaining 1,067 samples were classified as healthy udder (DSCC < 75%, SCC < 200,000 cells/mL), subclinical mastitis (DSCC ≥ 75%, SCC < 200,000), clinical mastitis (DSCC ≥ 75%, SCC ≥ 200,000), or chronic mastitis (DSCC < 75%, SCC ≥ 200,000). Mean lactose concentration was 4.82 ± 0.37% (range 1.94-5.31%) and decreased progressively from healthy udders to chronic mastitis. The Kruskal–Wallis test confirmed significant differences among groups ($p < 0.001$, $\eta^2[H] = 0.074$), with Dunn's post-hoc tests showing significantly lower lactose in clinical and chronic mastitis compared with healthy udders ($p < 0.001$ and $p < 0.003$, respectively). Lactose was negatively correlated with SCC (r_s=-0.46) and DSCC (r_s = -0.40), whereas SCC and DSCC were strongly positively correlated ($r_s = 0.83$). These findings suggest that lactose concentration may serve as a sensitive indicator of udder health, and its integration with SCC and DSCC provides a robust, non-invasive approach for early detection and monitoring of intramammary inflammation.

Keywords Udder health, Differential Somatic Cell Count, Somatic Cell Count, Milk Lactose, Mastitis Classification

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1. Introduction

Mastitis remains one of the most common and economically significant diseases affecting dairy cows worldwide. It reduces milk yield, impairs milk quality, and increases culling rates, thereby causing substantial financial losses to the dairy industry. Somatic cell count (SCC) has long been considered the gold standard for monitoring udder health at both cow and herd levels, but it does not provide information about the cellular composition of milk leukocytes. The differential somatic cell count (DSCC), which quantifies the proportion of polymorphonuclear neutrophils and lymphocytes, has recently been introduced as a complementary tool to improve the accuracy of mastitis detection [1-3]. DSCC enables differentiation between neutrophil-dominated inflammatory responses, commonly associated with active intra-mammary infections, and lymphocyte-predominant profiles, which may reflect either recovery of immune homeostasis or chronic inflammation [1,2].

Milk lactose concentration has also emerged as a promising biomarker of udder health and mammary epithelial integrity. Lactose is the major carbohydrate in milk and a key regulator of its osmotic pressure, driving water influx and milk secretion. Several studies have consistently reported that lactose concentration decreases during mastitis,

reflecting epithelial cell damage and altered mammary permeability [4,5]. Monitoring lactose, particularly in combination with SCC or DSCC, may therefore enhance the detection of subclinical mastitis and provide additional insight into the physiological effects of intra-mammary infections on milk composition.

Although there is growing interest in the combined use of SCC and DSCC, few studies have investigated their relationship with milk lactose concentration under field conditions. A better understanding of this relationship could improve early detection of udder inflammation, inform herd health management, and support evidence-based treatment and prevention strategies.

The objective of the present study was to classify milk samples into udder health categories using combined SCC and DSCC thresholds and to evaluate the association between udder health status and lactose concentration. We hypothesized that lactose concentration would decrease progressively with worsening udder health and that significant negative correlations would exist between lactose, SCC, and DSCC.

2. Materials and Methods

2.1 Animals and Sampling

A total of 1,083 milk samples were collected from 50 multiparous Romanian Spotted cows (Simmental breed) housed in a free-stall system on a commercial dairy farm in Romania. The farm was equipped with a fully automated milking system (1 milking robot per 50 cows). Sampling took place over a two-year period, from May 2022 to September 2024, covering multiple stages of lactation. Milk samples were obtained during routine milking sessions, following standard hygienic procedures, and were immediately cooled and transported to the laboratory for analysis.

Sixteen samples (1.5%) could not be classified into udder health categories due to missing or invalid DSCC or SCC values and were therefore excluded from group comparisons. The remaining 1,067 samples were included in the statistical analysis.

2.2 Milk Analysis

All samples were analyzed in an ISO-certified laboratory to ensure quality and traceability of results. Somatic Cell Count (SCC), Differential Somatic Cell Count (DSCC), and lactose concentration (g/100g) were measured simultaneously using a CombiFoss™ 7 FT analyser (FOSS, Hillerød, Denmark), which combines flow cytometry (Fossomatic™ module) for SCC/DSCC determination and mid-infrared spectroscopy (MilkoScan™ module) for milk composition. Analyses were performed according to the manufacturer's protocols and international reference standards.

2.3 Statistical Analysis

Data were analyzed using R software (R version 4.5.1). Before analysis, SCC values were multiplied by 1,000 to convert them from thousands of cells/mL to absolute cell counts. Each milk sample was classified into one of four udder health categories based on combined SCC and DSCC thresholds:

(1) Healthy udder: DSCC < 75% and SCC < 200,000 cells/mL,
(2) Subclinical mastitis: DSCC ≥ 75% and SCC < 200,000 cells/mL,
(3) Clinical mastitis: DSCC ≥ 75% and SCC ≥ 200,000 cells/mL,
(4) Chronic mastitis: DSCC < 75% and SCC ≥ 200,000 cells/mL.

Descriptive statistics (mean, standard deviation, median, minimum, and maximum) were calculated for lactose concentration in each group. Normality was tested using the Shapiro-Wilk test, which showed significant deviations from normal distribution for all groups ($p < 0.001$). Therefore, differences in lactose concentration among the four groups were assessed using the Kruskal-Wallis test, a non-parametric alternative to one-way ANOVA.

Where a significant global difference was detected, Dunn's post-hoc tests with Bonferroni correction were applied for pairwise group comparisons. Effect sizes were expressed as $\eta^2[H]$ (eta squared for the Kruskal–Wallis test) and effect sizes for Dunn's pairwise comparisons were calculated as $r = Z / \sqrt{N}$, where r represents the effect size coefficient, Z is the standardized test statistic from the Dunn comparison, N is the total number of observations across the two groups, and $\sqrt{N}$ is the square root of N. Effect sizes were interpreted as small (r = 0.1), medium (r = 0.3), or large (r = 0.5) effects. Associations between DSCC, SCC, and lactose concentration were further explored using Spearman's rank correlation coefficients (r_s).

Statistical significance was set at $p < 0.05$ for all analyses. Because multiple milk samples were collected from the same cows over the two-year period, observations may not be fully independent. Therefore, correlation results should be interpreted with caution, as they reflect sample-level associations rather than fully independent experimental units.

3. Results

3.1. Descriptive Statistics

Of the 1,083 milk samples collected, 1,067 could be classified into udder health groups and were included in the statistical analysis: 774 healthy udders, 137 subclinical mastitis, 116 clinical mastitis, and 40 chronic mastitis. Sixteen samples (1.5%) could not be classified due to missing or invalid DSCC or SCC values and were excluded from group comparisons.

Across the 1,067 milk samples, the mean lactose concentration was 4.82 ± 0.37% (range: 1.94-5.31%). When classified by udder health status, lactose concentration decreased progressively from healthy cows to those with chronic mastitis. Mean lactose was highest in cows with a healthy udder (4.85 ± 0.33%) and decreased in subclinical (4.79 ± 0.43%), clinical (4.71 ± 0.34%), and chronic mastitis (4.48 ± 0.64%) groups.

These distributions are visualized in Figure 1, which presents box plots for each udder health group, with the mean marked by a white diamond and individual data points displayed as jittered dots. The figure clearly illustrates the progressive decline in lactose concentration from healthy to chronically affected udders and the greater variability observed in chronic mastitis, reflecting a more severe and heterogeneous disruption of mammary secretory function.

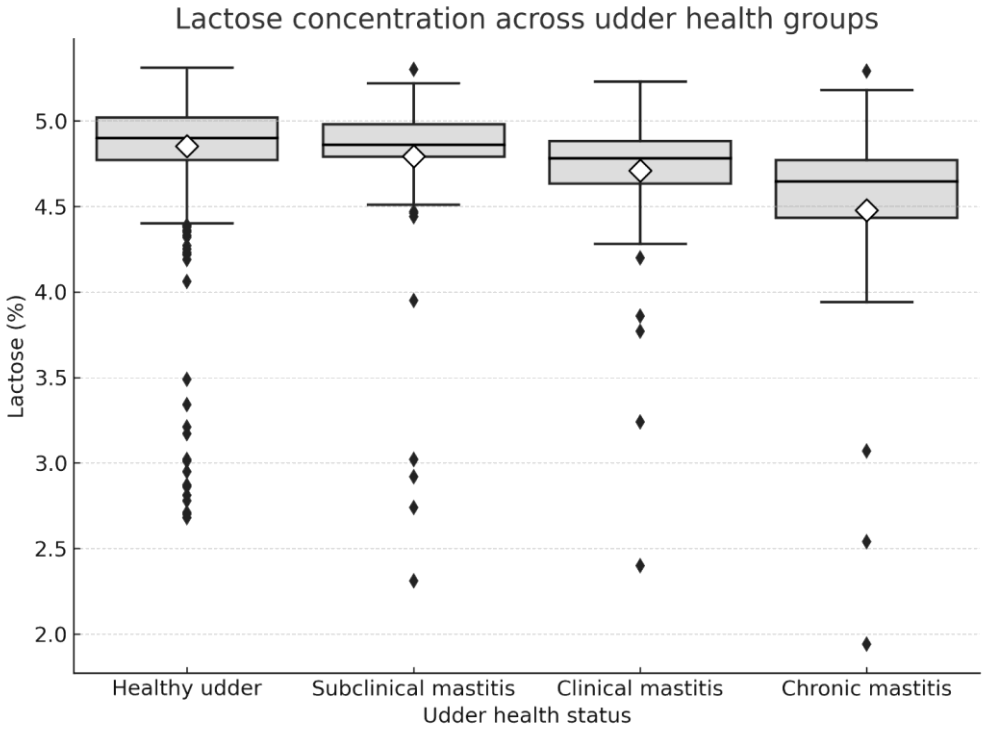

Figure 1. Boxplots of lactose concentration (%) across udder health groups (Healthy, Subclinical, Clinical, Chronic mastitis).

3.2. Group Comparisons

Shapiro-Wilk tests indicated that lactose was not normally distributed in any of the four groups ($p < 0.001$), justifying the use of non-parametric tests. The Kruskal-Wallis test confirmed a statistically significant difference in lactose concentration among the four groups ($p < 0.001$, $\eta^2[H] = 0.074$, indicating a moderate effect size), demonstrating that udder health status has a meaningful impact on milk lactose levels.

Dunn's post-hoc pairwise comparisons revealed that lactose was significantly lower in cows with clinical mastitis compared with healthy udders ($p < 0.001$, $r = -0.206$) and in chronic mastitis compared with

healthy udders ($p < 0.001$, $r = -0.199$). Significant differences were also found between subclinical and clinical mastitis ($p = 0.001$, $r = -0.123$) and between subclinical and chronic mastitis ($p < 0.001$, $r = -0.152$). No significant difference was observed between healthy and subclinical mastitis ($p = 0.44$) or between clinical and chronic mastitis ($p = 0.21$).

These pairwise comparisons are summarized visually in Figure 2, which displays a heatmap of Bonferroni-adjusted p-values. Darker blue shades indicate lower p-values, highlighting the strongest statistical differences between groups.

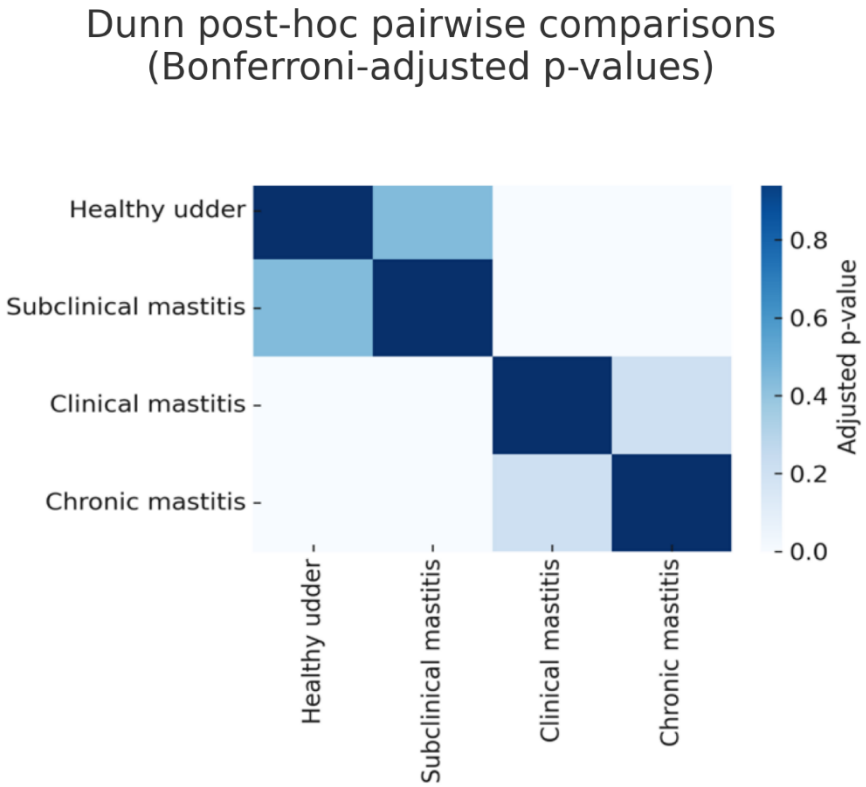

Figure 2. Heatmap of Bonferroni-adjusted p-values from Dunn's post-hoc comparisons of lactose concentration between udder health groups.

3.3. Correlation Analysis

Spearman correlations further confirmed these findings, revealing a strong positive association between DSCC and SCC ($r_s = 0.83$), which is expected since both parameters reflect the intensity of mammary inflammation. Lactose was moderately negatively correlated with both SCC ($r_s = -0.46$) and DSCC ($r_s = -0.40$), indicating that lactose declines with increasing severity of intra-mammary inflammation.

4. Discussion

Our results clearly demonstrated a progressive decline in lactose concentration with worsening udder health status, from healthy udders to chronic mastitis. The Kruskal-Wallis test confirmed that these differences were statistically significant ($p < 0.001$), with a moderate effect size, and Dunn's post-hoc analysis highlighted that the most pronounced reductions occurred in clinical and chronic mastitis compared with healthy udders. The increased variability observed in chronic mastitis cases suggests a heterogeneous pattern of mammary damage, consistent with the chronic nature of these infections. Furthermore, Spearman correlations revealed a strong positive association between SCC and DSCC and moderate negative correlations between lactose and both SCC and DSCC, confirming that lactose declines in parallel with inflammatory cell infiltration. The strong correlation between SCC and DSCC was expected, as both reflect inflammatory cell dynamics.

The combined use of SCC and DSCC to classify udder health in our study is consistent with current literature emphasizing the complementary value of DSCC in mastitis diagnostics [1-3]. DSCC provides

information on the cellular composition of milk leukocytes, distinguishing neutrophil-dominated responses—indicative of acute intramammary infection from lymphocyte-predominant profiles that may signal recovery or chronic inflammation [1]. Studies have shown that DSCC enhances the sensitivity and specificity of mastitis detection when combined with SCC, particularly in early or subclinical stages [2,3]. This is in line with our finding that DSCC ≥ 75% was associated with lower lactose even when SCC remained below 200,000 cells/mL, underscoring its role as an early warning parameter.

From a physiological standpoint, the observed decline in lactose concentration can be explained by the effect of intra-mammary infection on mammary epithelial cell activity. Lactose is synthesized in the Golgi apparatus of secretory cells by the lactose synthase complex, and its production is closely linked to osmotic balance and milk volume [4]. Inflammation reduces synthetic activity, increases tight-junction permeability, and allows lactose to leak into the bloodstream, leading to decreased milk lactose content [4,5]. Our results are consistent with previous findings that milk lactose is one of the most sensitive indicators of mammary gland integrity and declines during both subclinical and clinical mastitis [13–15].

Regular monitoring of DSCC, SCC, and lactose offers practical advantages for herd management. DSCC has been validated as a rapid, cost-effective parameter for routine milk recording programs [6–9], with particular value in identifying cows with early or subclinical mastitis. Several studies have highlighted its utility for improving mastitis control strategies, especially when integrated with SCC and other milk recording data [10–12]. Our findings reinforce this approach, suggesting that including lactose concentration in decision-support systems could further improve the early detection of intra-mammary inflammation and reduce production losses.

Finally, from a herd-level perspective, lactose measurement is highly feasible because it can be automatically obtained via mid-infrared spectroscopy during routine milk recording [10,16]. Integrating lactose with SCC and DSCC provides a more comprehensive view of udder health and may allow more targeted interventions, reducing unnecessary treatments and culling, thereby improving both productivity and animal welfare. This supports the concept that milk composition and particularly lactose, serves as a mirror of udder health and a powerful tool for precision livestock management.

A limitation of the present study is that multiple samples were obtained from the same cows over time, which may introduce within-animal correlation and potentially influence the precision of statistical estimates.

5. Conclusions

This study demonstrates that combining SCC and DSCC provides a robust classification of udder health and reveals a clear, progressive decline in lactose concentration with increasing severity of intra-mammary inflammation. Lactose remained unchanged between healthy and subclinical mastitis but decreased significantly in clinical and chronic mastitis, highlighting its sensitivity as an indicator of secretory dysfunction. Notably, DSCC ≥ 75% was associated with reduced lactose even when SCC remained below 200,000 cells/mL, confirming its role as an early marker of inflammation. Incorporating lactose measurement alongside SCC and DSCC in routine herd monitoring can enhance early mastitis detection, support targeted interventions, and improve both productivity and animal welfare.

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