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Relationship Between Hyperkeratosis, Teat Conformation Traits, Microbiological Isolation, and Somatic Cell Count in Milk from Dairy Cows

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Abstract

Maintaining teat-end integrity in dairy cows is essential to preventing intramammary infections (IMIs) in dairy cows, yet the relationship between hyperkeratosis, teat conformation, and mammary health remais underexplored. This study evaluated the relationship between teat-end hyperkeratosis, teat conformation traits, microbial colonization, and somatic cell count (SCC) in milk from 170 cows on ten commercial dairy farms in Santa Catarina, Brazil. During two farm visits, milk and teat-end swab samples from paired teats (one with hyperkeratosis, one without) were analyzed for microbial growth and SCC. SCC data were transformed into somatic cell scores (SCS). Results showed no significant association between hyperkeratosis and mastitis microorganisms, although environmental microorganisms tended to be more frequent in hyperkeratotic teats ($p = 0.0778$). Major microorganisms in milk were significantly associated with higher SCC ($p = 0.0132$). No relationship was observed between teat conformation traits and hyperkeratosis. These findings suggest that hyperkeratosis may subtly influence the teat canal to environmental bacterial colonization, underscoring the need for improved milking management practices to minimize hyperkeratosis and associated mastitis risks.

Keywords: contagious microorganisms; environmental microorganisms; mastitis; teat shape; teat length

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1. Introduction

Bovine mastitis remains the most prevalent and economically impactful disorder affecting dairy herds worldwide [1–3]. This inflammatory condition, primarily caused by the invasion and multiplication of pathogens in the mammary gland, leads to significant physical–chemical changes in milk and damage to glandular tissue [4,5]. Mastitis typically occurs when pathogens enter through the teat canal, especially after milking, when the teat sphincter remains open, allowing microbial colonization [5,6]. The transmission of these pathogens is often linked to mechanical milking, contaminated hands, or environmental exposure [7,8]. Consequently, adopting proper milking practices, particularly those focused on maintaining teat-end health, is critical for mastitis prevention and control.

The mammary gland is equipped with multiple defense mechanisms, including anatomical barriers and immune responses, to protect against mastitis-causing pathogens [9,10]. The teat end serves as the first line of defense, with structures such as the sphincter muscle and the keratin plug playing essential roles in preventing bacterial invasion [11–13]. The sphincter muscle ensures teat canal closure between milkings [14], while the keratin plug acts as both a physical barrier and a bacteriostatic agent, inhibiting bacterial migration into the gland cistern [15]. However, conditions like hyperkeratosis—an excessive buildup of keratin around the teat orifice—can compromise these protective mechanisms, increasing the risk of intramammary infections (IMIs) [16,17].

Hyperkeratosis has been widely associated with heightened susceptibility to IMIs in dairy herds [18–22]. This condition disrupts the teat canal's protective barrier, facilitating microbial colonization and the development of IMIs [23–26]. Several factors influence the development of hyperkeratosis, including teat shape, position, milking frequency, overmilking, lactation stage, milking management practices, environmental conditions, and genetic predisposition [27–30]. Although the exact mechanisms by which hyperkeratosis increases IMI risk remain unclear, it is hypothesized that the condition impairs the teat canal's natural defenses, allowing microorganisms to penetrate more easily [26].

Bacterial colonization of teat skin is a major concern for the dairy industry, as it is more prevalent than viral infections and a significant source of new IMIs [31]. Microorganisms such as *Staphylococcus aureus*, *Streptococcus dysgalactiae,* and *Trueperella pyogenes* are commonly found on teat skin and readily colonize lesions caused by trauma or infection [32]. Additionally, hyperkeratosis has been linked to the presence of environmental microorganisms like *Streptococcus uberis* and *Escherichia coli* in the teat canal, further emphasizing its role in mastitis development [33,34]. Although teat-end hyperkeratosis is considered a potential risk factor for teat canal impairment, increased local contamination, and, consequently, mastitis, scientific evidence fully supporting this causal pathway is still lacking. Specifically, a direct and conclusive association between the presence of hyperkeratosis and microbial contamination of the teat canal remains unestablished [26].

Given the significant impact of hyperkeratosis on mastitis risk, we aimed to investigate the influence of teat conformation traits on hyperkeratosis, the relationship between microbial colonization and hyperkeratosis, and the association of hyperkeratosis with somatic cell count (SCC). While previous research has established the link between hyperkeratosis and IMIs, our study provides additional insights by examining how teat conformation traits and microbial colonization of the teat skin interact with hyperkeratosis to influence SCC and mastitis risk. A key methodological distinction of our study is the use of an intra-animal comparison design, in which each cow contributed one teat with hyperkeratosis and one contralateral teat without, allowing us to isolate the effect of hyperkeratosis while minimizing inter-animal variability. This approach differs from studies that compare cows with and without hyperkeratosis at the herd level. By integrating assessments of teat-end condition, bacterial load, and SCC, in relation to the hyperkeratosis status; our findings offer a more comprehensive understanding of the mechanisms underlying IMI susceptibility. Our hypothesis is that teats with hyperkeratosis and bacterial contamination on the teat skin have higher SCC and an increased incidence of intramammary infections.

2. Materials and Methods

2.1. Herd and Data Collection

This study was conducted on ten commercial dairy farms affiliated with the official milk recording service of the Santa Catarina State Breeders Association (ACCB), located in the Meio-Oeste and Planalto Serrano mesoregions of Santa Catarina, Brazil. The study region experiences a humid subtropical climate, classified as Cfa (hot summers) and Cfb

(mild summers) according to the Köppen climate classification [35]. The herds consisted of Holstein (74.7%), Jersey (23.5%), and crossbreed Holstein–Jersey cows (1.7%) at different days in milk and different parities. Cows within the same herd were milked consistently at the same time each day, either two or three times daily, using a piped milking system. A standardized premilking routine was applied to all cows, including forestripping, application of a pre-dipping solution with a 30 s contact time, and drying of the teats with a disposable paper towel prior to milking unit attachment. After milking unit removal, a post-dipping solution was applied to all teats. The dairy farmers participating in the study performed regular maintenance of the milking equipment according to the manufacturer's recommendations. Two technical visits were conducted on each farm, spaced approximately three months apart, to assess teat-end hyperkeratosis, evaluate teat conformation traits, collect milk samples for microbiological analysis and SCC, and obtain teat swabs for further examination. The interval between visits was chosen to allow for detectable progression of teat-end hyperkeratotic lesions, considering the rate of epithelial renewal and the chronic response to mechanical milking. Additionally, this period is appropriate for monitoring changes in intramammary infection dynamics and fluctuations in SCC, providing a balance between diagnostic sensitivity and logistical feasibility. The study design was based on cross-sectional evaluations conducted during two periodic visits to the same herds. The total population screened in this study comprised 870 lactating cows from ten commercial farms, which were visited and evaluated on-site. From this population, 170 cows were selected based on strict inclusion criteria, namely the presence of one teat with hyperkeratosis grade 3–4 and the contralateral teat with grade 1–2. This approach aimed to implement an intra-animal comparison design, whereby each cow served as her own control by contributing one teat with hyperkeratosis and one contralateral teat without. This allowed for the isolation of the effect of hyperkeratosis while reducing inter-animal variability. The final dataset comprised 340 udder quarter-level observations from 170 cows.

2.2. Evaluation of Hyperkeratosis Severity and Teat Morphometric Parameters

The assessment of hyperkeratosis severity was conducted on all lactating cows immediately after milking and before the application of a post-milking disinfectant. Teat-end hyperkeratosis severity was visually scored on a 1-to-4 scale, as described in [17], with representative images shown in [36]. This scoring system categorizes the condition of the teat ends as follows: 1—no ring formation; 2—small ring formation; 3—rough ring formation; and 4—extensive rough ring formation. Cows selected for this study met the inclusion criteria of having at least one teat with a hyperkeratosis score of 3 or 4 and a contralateral teat with a score of 1 or 2. Cows exhibiting signs of clinical mastitis were excluded from the study. After the initial evaluation of all cows and the identification of those meeting the inclusion criteria, milk and swab samples were collected at the next milking. Samples were taken from two contralateral mammary quarters of each study cow, meaning the selected quarters were diagonally opposite within the udder (e.g., front-left and rear-right or front-right and rear-left), rather than adjacent.

Teat conformation traits, including teat length, shape, placement, and inter-teat distance, were evaluated during the first milking prior to machine attachment. Teat length (cm) was measured vertically along the teat wall from base to tip using a measuring tape. Inter-teat distance was determined by measuring the space between adjacent quarters. Teat shape was classified into four distinct categories: cylindrical, pointed, round, and small (Figure 1). For teat placement assessment, a standardized 1–9-point linear classification system was employed, where scores of 1, 5, and 9 represented teats positioned at the quarter periphery, quarter center, and near the median suspensory ligament, respectively.

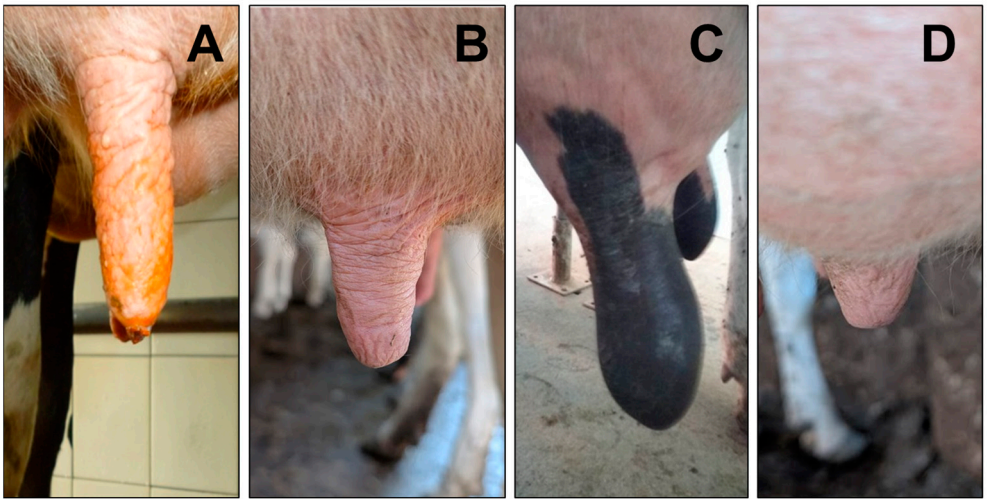


Figure 1. Classification of Teat Shapes into Four Distinct Categories Pointed (**A**), Cylindrical (**B**), Round (**C**), and Small (**D**).

2.3. Sample Collection

Sample collection was performed by the same researcher during two technical visits to each farm, ensuring consistency. A total of 170 cows and 340 mammary quarters were evaluated.

Swab samples from the teat ends were collected before the attachment of the milking unit and after performing pre-dipping. For this purpose, individual sterile flexible swabs (Stuart-Copan-PC® Swab 111C) were used, applying gentle friction only to the teat ends. After sampling, each swab was immediately transferred to a tube containing sterile semi-solid Stuart transport medium [37]. The samples were then frozen and transported to the Animal Microbiological Diagnostic Center (CEDIMA) at CAV/UDESC in Lages, SC, Brazil.

Milk samples from the selected mammary quarters were collected aseptically for SCC analysis and microbiological culture, following the standards established by the National Mastitis Council [38]. Initially, teats were sanitized with 70% alcohol, and the first three squirts of milk were discarded. Sterile 15 mL conical-bottomed (Falcon) vials with caps were used for storing milk samples. These vials were properly labeled, placed in thermal boxes containing recyclable ice, and subsequently frozen for later transport to CEDIMA (CAV/UDESC) for microbiological culture.

For SCC analysis, milk samples were collected from the same mammary quarters and stored in standard collection bottles containing the preservative Bronopol® (2-bromo-2-nitropropane-1,3-diol). The samples were then sent to the Milk Quality Laboratory at UNC/CIDASC, located in Concórdia, SC, Brazil.

2.4. Microbiological Analysis

The isolation and identification of microorganisms from milk and teat-end swab samples were performed according to the guidelines of the National Mastitis Council [38], Quinn [37], and Oliveira [39]. The collected samples were thawed at room temperature, homogenized, and a 10 µL aliquot of each milk and swab sample was inoculated onto 5% sheep blood agar plates using a disposable calibrated loop. The inoculated plates were incubated at 37 °C for 24 to 72 h, with periodic observations made every 24 h to assess colony development. The samples that exhibited bacterial growth were analyzed based on their macroscopic characteristics and subsequently stained using the Gram technique. Following this, the microorganisms were identified through biochemical tests specific to each bacterial group, according to the methods proposed by Quinn [37] and Oliveira [39].

Gram staining, coagulase test, and biochemical tests were then conducted to characterize the isolates according to the methods proposed by [39].

Microorganisms were categorized as either major microorganisms (including *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus* spp., *Streptococcus uberis*, species from the *Streptococcus equinus* complex (SBSEC), *Klebsiella* spp., *Escherichia coli*, and *Serratia* spp.) or minor microorganisms (*Trueperella pyogenes*, *Bacillus* spp., *Corynebacterium* spp., *Enterobacter* spp., and coagulase-negative *Staphylococci* (CNS)) [40]. In addition, microorganism analysis considered the modes of transmission, distinguishing between environmental and contagious sources. The "mixed infections" category was used exclusively when both environmental and contagious microorganisms were identified in the same sample. For mixed infections classification, we considered only samples with pure and profuse growth (>30 colonies/10 µL of milk) of up to two distinct colony morphotypes. This approach allowed us to account for cases of mixed etiology without arbitrarily assigning a single transmission source. The same classification criteria were applied to microorganisms isolated from the teat ends of the same cows, allowing for a direct comparison between milk and teat-skin results. Milk samples were classified as contaminated when more than two distinct bacterial morphologies were observed on the same culture plate, suggesting likely contamination during sample collection [41]. These samples were excluded from the microorganism-specific analysis. Environmental microorganisms typically associated with sampling contamination tended to grow in low numbers (<10 colonies/10 µL of milk) and were not considered as causative agents unless in pure or dominant growth. The SCC was analyzed using a flow cytometry absorption method with a Bentley 2000™ instrument (Bentley Instruments Inc., Chasca, MN, USA).

2.5. Statistical Analysis

SCC data were transformed into somatic cell score (SCS) using the equation: SCS = ((log2 (SCC/100,000) + 3), as described by [42]. The relationship between SCS and other indicators of mammary gland health (including microbial isolation, microorganism characterization, and hyperkeratosis score) was analyzed using analysis of variance, by the MIXED procedure of the SAS statistical package (version 9.3, SAS Institute Inc., Cary, NC, USA). Residual normality was tested using the Kolmogorov–Smirnov test, and mean differences were compared using the Tukey test at a 5% significance level. The following statistical model was used to assess the effects of microbial colonization and hyperkeratosis.:

$$Y_{ijkl} = \mu + herd_i + quarter_j + group_k + microorganism_l + microorganism * group + e_{ijkl}$$

where Y_{ijkl} = somatic cell score per mammary quarter belonging to the i-th herd, in the j-th mammary quarter, in the k-th group, in the l-th microorganism class (growth, classification of microorganisms in milk and swab); µ = overall mean; $herd_i$ = effect of the i-th herd; $quarter_j$ = effect of the j-th mammary quarter (j = front and back); $grupo_k$ = effect of the k-th group (k = with hyperkeratosis and without hyperkeratosis); $microorganism_l$ = effect of the l-th treatment (l = growth (negative and positive) or classification (major and minor)); microorganism * group = interaction effect between microorganism and group; and e_{jjkl} = experimental error.

The association between mastitis microorganism (in milk and teat-end swabs) and hyperkeratosis was evaluated using the Chi-square test (χ^2) at a 5% significance level (FREQ procedure in SAS). Differences with *p*-values between 0.05 and 0.10 were interpreted as indicative of a trend toward significance.

For the evaluation of the effect of teat conformation traits on the hyperkeratosis score, the data were subjected to analysis of variance according to the following statistical model:

$$Y_{ijk} = \mu + herd_i + quarter_j + conformation\ (quarter)_k + e_{ijk}$$

where Yijk = individual hyperkeratosis score per mammary quarter, belonging to the i-th herd, the j-th mammary quarter, and the k-th udder conformation class (shape or placement); μ = overall mean; herd_i = effect of the i-th herd; quarter_j = effect of the j-th mammary quarter (l = front and rear); teat conformation trait (quarter)_k = effect of the k-th teat conformation (k = teat shape [cylindrical, round, small, and pointed] or teat placement [1, 5, and 9] or continuous variables such as average teat length or average teat distance); and eijk = experimental error.

3. Results

Table 1 presents the association between hyperkeratosis and microbiological variables derived from milk and swab samples, including microbial growth status (negative or positive), microorganism classification (major or minor), and transmission type (environmental, contagious, or mixed infections).

Table 1. Frequency distribution of microorganisms isolated from milk and teat-end swab samples, grouped by microbial growth status, microorganism classification (major or minor), and transmission mode (environmental, contagious, or mixed infections), for teats with hyperkeratosis (number = 170) and teats without hyperkeratosis (n = 170).

Sample Origin	Variable	Category	Teat-End Condition				$p > \chi^2$ *
			With Hyperkeratosis		Without Hyperkeratosis		
			N	%	N	%	
Milk	Growth	Negative	114	50	114	50	=1.0000
		Positive	56	50	56	50	
	Classification	Major Microorganisms	27	55.1	22	44.9	=0.3835
		Minor Microorganisms	29	46.8	33	53.2	
	Transmission	Environmental	39	59.1	27	40.9	=0.0778
		Contagious	9	34.6	17	65.4	
		Coinfection	8	42.1	11	57.9	
Swab	Growth	Negative	66	49.6	67	50.4	=0.9542
		Positive	30	49.2	31	50.9	
	Classification	Major Microorganisms	14	53.8	12	46.1	=0.5221
		Minor Microorganisms	15	45.4	18	54.5	
	Transmission	Environmental	4	33.3	8	66.7	=0.4625
		Contagious	15	53.6	13	46.4	
		Mixed infections	10	52.6	9	47.4	

* Differences were considered statistically significant at $p < 0.05$ and trends at $0.05 \leq p \leq 0.10$.

In milk samples, 50% of teat ends with hyperkeratosis and 50% without hyperkeratosis showed no microbial growth, indicating an equal distribution of negative growth across both conditions. Similarly, growth was equally distributed, with 50% for each condition. Major microorganisms were slightly more frequent in teat ends with hyperkeratosis (55.1%), although this difference was not statistically significant ($p = 0.3835$). In contrast, minor microorganisms were more associated with teat ends without hyperkeratosis. A trend was observed for environmental microorganisms to be more frequently associated with hyperkeratotic teat ends ($p = 0.0778$).

The average SCC was 483,932 cells/mL. Microorganism type significantly influenced SCC levels, with mammary quarters infected by major microorganisms showing higher

counts ($p = 0.0132$). Additionally, a strong relationship ($p < 0.0001$) was observed between SCS and bacterial growth in milk samples, as summarized in Table 2.

Table 2. Means ± standard errors of the mean (SEM) of somatic cell score (SCS) based on the growth and classification of microorganisms isolated from microbiological cultures of milk and teat-end swabs.

Sample Origin	Explanatory Variable	Class	Number of Samples	X ± SEM
Milk *	Growth	Negative	228	2.27 ± 0.191 [b]
		Positive	112	4.24 ± 0.273 [a]
	Classification	Major Microorganisms	49	5.36 ± 0.441 [a]
		Minor Microorganisms	62	3.86 ± 0.385 [b]
Swab **	Growth	Negative	133	3.06 ± 0.254 [b]
		Positive	61	3.98 ± 0.435 [a]
	Classification	Major Microorganisms	26	3.91 ± 0.571 [a]
		Minor Microorganisms	33	3.70 ± 0.516 [a]

Means followed by different letters indicate statistical differences (* $p < 0.05$ or ** $p < 0.10$).

Udder quarters with microorganisms detected in the teat-end swab showed a tendency ($p = 0.0713$) for higher SCS compared to those with negative swab cultures. However, no significant association ($p = 0.7887$) was found between microorganism classification and SCS in the swabs (Table 2).

Similarly, no significant relationship ($p = 0.9883$) was observed between SCS and hyperkeratosis score per mammary quarter. The mean ± standard error for cows with hyperkeratotic teats was 3.26 ± 0.227, while cows with non-hyperkeratotic teats had a mean score of 3.26 ± 0.228.

Bacteriological growth was absent in 61.4% of the milk samples collected. Contagious microorganisms were identified in 15.3% of the samples, and environmental microorganisms were found in 8.7%. Among the major microorganisms, *Staphylococcus aureus* was the most prevalent, detected in 4.9% of the samples. Among minor microorganisms, coagulase-negative *Staphylococcus* (SCN) had the highest prevalence, detected in 9.8% of the total analyzed quarters (Table 3).

Microbiological analyses of teat-end swabs revealed a higher prevalence of contagious microorganisms. Of the 17.6% of microorganisms isolated from the teat-end swab samples, 7.4% were identified as *Corynebacterium* spp., classified as a minor microorganism. Among the environmental microorganisms, *Trueperella* spp. and *Bacillus* spp. were the most prevalent, each accounting for 3.7% of the identified samples (Table 3).

For the teat conformation traits, we found that the mean ± standard deviation of teat length was 6.05 ± 1.23 cm in the front quarters and 4.87 ± 1.06 cm in the rear quarters. The distances between teats were 12.73 ± 4.02 cm (front) and 5.67 ± 3.36 cm (rear). The distribution of teat conformation (shape and placement) is presented in Table 4.

Table 3. Microorganisms isolated from milk culture samples and teat-end swab samples.

Microorganisms Isolated in Milk	Number of Samples (%)	Microorganisms Isolated in Swab	Number of Samples (%)
Contagious microorganisms:	28 (15.3)	Contagious microorganisms:	19 (17.6)
SCP	13 (7.1)	SCP	8 (7.4)
Staphylococcus aureus	9 (4.9)	*Corynebacterium* spp.	8 (7.4)
Corynebacterium sp.	6 (3.3)	*Staphylococcus aureus*	3 (2.8)

Table 3. *Cont.*

Microorganisms Isolated in Milk	Number of Samples (%)	Microorganisms Isolated in Swab	Number of Samples (%)
Environmental microorganisms:	16 (8.7)	Environmental microorganisms:	16 (14.8)
Bacillus spp.	4 (2.2)	*Trueperella pyogenes*	4 (3.7)
Nocardia spp.	3 (1.6)	*Bacillus* spp.	4 (3.7)
Streptococcus equi	3 (1.6)	*Serratia* spp.	2 (1.9)
Streptococcus spp.	2 (1.1)	*Yersinia* spp.	2 (1.9)
Streptococcus dysgalactiae	1 (0.5)	*Nocardia* spp.	1 (0.9)
SBSEC	1 (0.5)	*Streptococcus uberis*	1 (0.9)
Streptococcus uberis	1 (0.5)	*Streptococcus equi*	1 (0.9)
Yersinia spp.	1 (0.5)	*Enterobacter* spp.	1 (0.9)
SCN	18 (9.8)	SCN	2 (1.9)
Others	9 (4.9)	Others	5 (4.6)
No growth	113 (61.4)	No growth	66 (61.1)

SCP—Staphylococcus coagulase positive. SCN—Staphylococcus coagulase negative. SBSEC—Streptococcus bovis/Streptococcus equinus complex.

Table 4. Distribution of dairy cows' teat conformation traits.

Teat Conformation	Front Quarter	Rear Quarter
	N (%)	N (%)
Shape		
Pointed	43 (25.4)	44 (26.0)
Round	10 (5.9)	17 (10.0)
Cylindrical	113 (66.9)	94 (55.7)
Small	10 (1.8)	14 (8.3)
Placement		
1	56 (33.1)	8 (4.8)
5	89 (52.7)	45 (26.9)
9	24 (14.2)	114 (68.3)

1: Represents teats positioned at the periphery of the udder quarter; 5: Indicates teats located at the center of the udder quarter; 9: Corresponds to teats positioned at the median suspensory ligament.

No significant association was found between teat length ($p = 0.3176$) or distance between teats ($p = 0.3798$) and teat-end hyperkeratosis. Similarly, no relationship was observed between teat placement ($p = 0.1468$) or teat shape ($p = 0.5671$) and hyperkeratosis scores (Table 5).

Table 5. Means ± standard error of the mean (SEM) for teat-end hyperkeratosis scores according to teat conformation traits.

Variable	Number of Samples	Mean ± SEM
Shape		
Pointed	87	2.41 ± 0.081
Round	27	2.62 ± 0.151
Cylindrical	207	2.43 ± 0.052
Small	17	2.62 ± 0.244
Total	338	-
Placement		
1	64	2.46 ± 0.141
5	134	2.55 ± 0.068
9	138	2.34 ± 0.084
Total	336	-

Means did not differ significantly ($p > 0.05$); no letters assigned.

4. Discussion

In our study, no statistically significant relationship was found between the presence of mastitis microorganisms and hyperkeratosis; however, a trend toward an association between hyperkeratosis and environmental microorganisms was detected (Table 1). Refs. [1,43] reported no significant difference in the types of microorganisms based on hyperkeratosis status, and [44] observed no association between hyperkeratosis score and teat skin bacterial count. However [45], found a positive association between the teat microbiota and hyperkeratosis. Similarly [46], provided evidence of some association between infections by major microorganisms and coagulase-negative *Staphylococcus* and teat lesions, although this was not observed in our study (Table 1). These contrasting results highlight the complexity of the relationship between teat-end condition and mastitis microorganisms, which may vary depending on herd management, environmental factors, and pathogen dynamics.

The primary goal of mammary gland health monitoring and control strategies is to reduce new infections, eliminate existing ones, and shorten infection duration through recommended practices. In this context, several studies have reported a positive relationship between the occurrence of teat-end hyperkeratosis and the incidence of intramammary infections (IMI) in dairy cows [18,19,21,43,47,48]. Cows with hyperkeratosis exhibit a reduced ability to prevent the entry of pathogenic microorganisms into the mammary gland, increasing the risk of IMIs [15,49].

The teat end serves as the first line of defense against bacterial invasion, and rougher teat surfaces are more susceptible to IMIs caused by environmental microorganisms. Some bacterial strains on udder skin can utilize keratin from the teat canal for proliferation. In our study, a trend toward higher contamination by environmental microorganisms was observed in teats with hyperkeratosis (Table 1), possibly due to the rough surface facilitating bacterial adhesion and reducing the effectiveness of post-milking teat disinfection [16]. Wieland, M., et al. [50] reported that teats with skin lesions have higher risks of IMI, and this can compromise the effectiveness of the pre-milking hygienic procedures. This is consistent with findings from [51], who reported that quarters with moderate to severe hyperkeratosis had a significantly higher risk of clinical mastitis caused by *E. coli*. Similarly [33], found that hyperkeratosis scores were associated with *E. coli* counts in teat canal swab samples.

Other studies have reported a higher likelihood of new IMIs caused by *Staphylococcus aureus* in teats with severe hyperkeratosis (score 4) [12,46]. In the present study, no significant relationship was found between microorganism classification and teat-end swab samples, with associations observed only in milk samples (Table 2). Environmental microorganisms accounted for 14.8% of isolates in teat-end swab samples, with *Trueperella* spp. and *Bacillus* spp. each showing a prevalence of 3.7%. These findings underscore the importance of proper teat cleaning and disinfection before milking to prevent mastitis caused by environmental microorganisms.

Asadpour, R., et al. [1] reported that 12.8% of isolated microorganisms were environmental agents, with *Streptococcus dysgalactiae* being the most prevalent. In Brazil, approximately 95% of IMIs are caused by *Streptococcus agalactiae*, *Staphylococcus aureus*, *Streptococcus uberis*, and coliforms [52]. In our study, *Staphylococcus aureus* was the most prevalent microorganism in milk samples (4.9%), while *Corynebacterium* spp. was the most common in teat-end swab samples (Table 3). Infections caused by major microorganisms at the start of lactation in primiparous cows are associated with elevated SCC [53].

Higher SCS were observed in mammary quarters with microorganism growth in both milk ($p < 0.0001$) and swab samples ($p = 0.0713$) compared to microorganism-negative quarters. Additionally, the presence of major microorganisms in milk was associated with higher SCS values compared to minor microorganisms (Table 3). Infections caused by

minor microorganisms typically induce less intense SCC responses than those caused by major microorganisms [54,55]. Minor microorganisms are also associated with a higher number of false negatives in IMI diagnosis [5]. Minor or environmental microorganisms may cause false-negative results due to low bacterial load and intermittent shedding, while their high environmental presence increases the risk of contamination and false positives if sampling is not aseptic [56–58]. These challenges highlight the importance of strict sampling protocols and complementary diagnostic methods.

In the current context of productivity with quality, it is essential to identify and eliminate factors associated with poor mammary gland health, including environmental and management conditions as well as animal-specific traits, such as teat conformation traits. According to [59], teat conformation traits are used in animal breeding programs to improve productive lifespan and are associated with lower culling risks as scores approach the ideal. Unfavorable teat conformation traits, such as teats that are too large or positioned too close together, can increase the risk of mastitis in cows [60].

We did not find any relationship between teat shape and the occurrence of teat-end hyperkeratosis (Table 3). Consistent with other studies evaluating teat morphology, the cylindrical shape was the most prevalent (Table 4) [61]. These findings contrast with those of [21], who reported a highly significant correlation between teat-end shape and hyperkeratosis. The same study also noted a relationship between teat length and hyperkeratosis, with longer teats showing a higher prevalence of hyperkeratosis.

Longer teats are more prone to lesions due to incompatibility with mechanical milking equipment [62] and may be a risk factor for mastitis [63]. Based on the classification systems of [59,63], the average teat lengths in this study classify the front udder quarters as medium-length and the rear quarters as short.

However, the literature lacks consensus on the relationship between teat-end lesions and teat conformation traits. The association between teat-end hyperkeratosis and mastitis is well-documented [18]. While several studies have identified risk factors associated with the development of teat-end hyperkeratosis, it remains essential to further investigate all potential contributing factors and to minimize confounding variables in analytical approaches. The development of teat-end hyperkeratosis is a multifactorial process influenced by both animal and management-related variables.

Recent cohort and observational studies have shown that factors such as parity, stage of lactation, milk yield, and milking frequency significantly affect the occurrence and severity of hyperkeratosis [64]. For instance, increasing days in milk and higher parity have been associated with elevated teat-end hyperkeratosis scores, particularly when cows are subjected to overmilking in late lactation [65]. In addition, overmilking and irregular milk flow patterns, such as bimodal letdown, have been linked to a higher prevalence of hyperkeratosis and prolonged exposure of the teat to mechanical stress [64]. In the broader context of udder health, these variables remain key elements for ongoing research aiming to improve milking outcomes and teat-end condition.

Another critical consideration is the evolution of milking equipment over time. Dairy farms continuously improve milking practices to maintain udder health and enhance efficiency, thereby increasing profitability. This progress is supported by advances in milking technology, which may now better accommodate variations in teat anatomy. Considering the association between teat-end hyperkeratosis and increased susceptibility to intramammary infections, the findings of this study reinforce the importance of integrating milking management into programs aimed at improving udder health. Practical strategies to minimize hyperkeratosis should include adjustments to milking machine settings, such as reducing overmilking time and optimizing automatic detachment systems [66], as well as actions that reduce the occurrence of bimodal milk flow by ensuring effective milk ejection

from the start of milking [67]. These measures can significantly decrease milking time and reduce prolonged vacuum exposure to the teat. Additionally, the implementation of double pre-dipping protocols may be recommended as part of routine milking procedures [31]. This multifactorial aspect may explain the absence of a significant association between hyperkeratosis and teat conformation traits in our study, as it likely results from a complex combination of anatomical, physiological, equipment-related, and microbiological factors.

5. Conclusions

This study provides valuable insights on the influence of teat conformation traits on hyperkeratosis, the relationship between microbial colonization and hyperkeratosis, and the association of hyperkeratosis with somatic cell count (SCC) in dairy cows. Although no significant association was found between hyperkeratosis and the presence of mastitis microorganisms, a trend toward a higher prevalence of environmental microorganisms was observed in hyperkeratotic teats. This suggests that hyperkeratosis may compromise the teat's natural defense mechanisms, facilitating bacterial adhesion and increasing the risk of intramammary infections (IMIs) caused by environmental agents. Additionally, major microorganisms in milk were associated with elevated SCC, reinforcing their role in udder health deterioration.

No significant relationship was found between teat conformation traits and hyperkeratosis, indicating that other factors—such as milking practices, equipment settings, and environmental conditions—may play a more critical role in teat-end integrity. These findings highlight the importance of integrating teat-end condition monitoring into mastitis control programs. Practical strategies should include proper teat cleaning and disinfection, regular evaluation of teat-end condition, and optimized milking machine settings to minimize hyperkeratosis development. By addressing both contagious and environmental sources of infection, dairy farmers can improve udder health, reduce SCC, and enhance milk quality.

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