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Prevalence and Associated Risk Factors of Teat-End Hyperkeratosis in a Commercial Dairy Herd

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Abstract

Teat-end hyperkeratosis (THK) is a welfare concern in dairy cows, associated with pain, discomfort, and increased mastitis risk. Identifying risk factors associated with THK may support targeted preventive management. This cross-sectional observational study aimed to determine the prevalence of THK and identify factors associated with its occurrence. A total of 492 Holstein-Friesian cows were evaluated at the milking parlor of a dairy farm located in Évora, Portugal. All four teats per cow (n = 1968) were scored on a 4-point scale: no lesion (1), smooth ring (2), rough (3), and very rough skin (4). Parity, days in milk, teat length, teat-end shape, udder health, and milking procedures were assessed as potential factors associated with THK. A mixed-effects ordinal logistic regression model was used to evaluate the associations between THK and the selected factors. Overall THK prevalence was 54.4%, with a median score of 2 (range: 1–4; IQR: 1–2). Score distribution was 45.6% (score 1), 43.0% (score 2), 10.3% (score 3), and 1.1% (score 4). Round teat ends were associated with greater odds of severe THK compared with flat teat ends (OR = 6.4; p = 0.016). Longer milking duration (OR = 2.7 per 100 s; p = 0.002) and a history of mastitis (OR = 80.0; $p < 0.001$) were also positively associated with THK severity. These findings highlight opportunities to mitigate THK through optimized milking management and breeding strategies. Notably, factors such as mastitis and prolonged milking time are also associated with culling, potentially masking the true burden of THK on animal welfare.

Keywords: milking management; teat lesions; tegument; udder health; welfare

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Academic Editor: Erminio Trevisi

Received: 15 May 2026
Revised: 2 July 2026
Accepted: 13 July 2026
Published: 16 July 2026

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1. Introduction

The teat canal is an important first line of defense that protects the mammary gland against invasion by mastitis-causing pathogens [1]. It is closed by a circular sphincter at its distal end and lined with a keratin layer that forms a ring around the teat opening. This keratin has both bactericidal and bacteriostatic properties, helping to prevent bacteria from entering the mammary gland [2]. Teat-end hyperkeratosis (THK) is a physiological response to the forces exerted on the teat canal during manual or mechanical milking, as well as during calf suckling [3]. The degree of hyperplasia of the teat keratin layer is a dynamic condition that can change throughout the lactation [4]. However, an exacerbated response may compromise the barrier function of keratin [2], allowing microorganisms to proliferate in keratin layers, which increases the incidence of clinical mastitis [5]. In this

situation, the keratin layer becomes thickened and excessive, and the sphincter, previously able to close tightly between milkings, loses this ability, increasing the risk of bacterial invasion [6]. Thus, severe cases of THK are a welfare concern associated with pain, and its chronic pathological stages are unlikely to be reversible [7].

THK is a multifactorial condition; therefore, it is important to distinguish among its potential contributing factors, including the effects of different mechanisms that can cause trauma and teat lesions, as well as its possible occurrence as a consequence of other pathologies [7]. Thus, the prevalence of THK on a farm can vary widely depending on several factors, which can be broadly grouped into animal characteristics, milking management practices, and milking equipment [8,9]. Though milking-machine characteristics may vary between farms [10], related issues can generally be prevented through regular monitoring and maintenance. In contrast, those associated with animal characteristics and milking management can be more complex and less easily controlled.

Since THK is one of the most prevalent pathologies in dairy cows [11], significant improvements regarding long-term selection and milking management practices are necessary [12,13]. Therefore, this study aimed to assess the prevalence of THK and its associated factors, with a focus on variables related to animal characteristics and milking management.

2. Materials and Methods

2.1. Farm and Animals

This cross-sectional observational study was conducted on a commercial intensive dairy farm with 1025 animals, located in Évora, Portugal. A total of 492 lactating Holstein-Friesian cows were evaluated, including 140 (28.5%) in the first, 85 (17.3%) in the second, 64 (13.0%) in the third, and 203 (41.2%) in the fourth or higher lactation. Average daily milk yield was 33.1 kg/cow, with milk protein and fat contents of 3.56% and 5.03%, respectively, as recorded by the National Dairy Development Program (NDDP). Replacement heifers were reared on the farm, with calves initially housed individually and subsequently transferred to group housing systems. Heifers were first inseminated at approximately 13 months of age. Cows were milked twice daily (02:30–07:00 h and 14:30–19:00 h) in a 2×20 parallel milking parlor operating at a fixed vacuum level of 41 kPa. Automatic teat cup detachment was set at a milk-flow threshold of 0.45 kg/min. Each milking session lasted approximately 4.5 h, including animal handling and equipment cleaning. Milking equipment was maintained according to the manufacturer's recommendations and inspected semi-annually by a certified technician.

The milking protocol followed a standardized hygiene procedure. Operators wore clean gloves and maintained hand hygiene throughout each session. Fore-stripping was performed on all quarters prior to unit attachment, with milk visually assessed for abnormalities. Pre-dipping was applied to all teats with a 30 s contact time, followed by drying with individual disposable paper towels. Teat cups were attached approximately 90 s after initial stimulation. Post-dipping was applied immediately after unit removal. Cows producing abnormal milk or undergoing antimicrobial treatment were identified and milked separately. Equipment was washed and disinfected at the end of each milking session.

2.2. Teat Evaluation

During the afternoon milking THK was scored, after cluster removal and before post-dipping, using a 1–4 scale (Figure 1) according to Mein et al. [4] and Cardozo et al. [12]. Each teat was first visually inspected without contact, followed by gentle handling to examine the teat end. A headlamp was used to ensure adequate lighting. Teat morphology was evaluated by measuring the teats' length and the shape of the teat-end. Teat length was measured according to Haverkamp et al. [14] and classified as: long > 6.5 cm; normal

4.5–6.5 cm; or short < 4.5 cm. Teat-end shape was classified, by visual inspection, according to the methodology adapted from Chrystal et al. [15]: round teat ends exhibit a smooth, uniform curvature; flat teat ends present a planar surface; and pointed teat ends show a more tapered and protruding morphology.

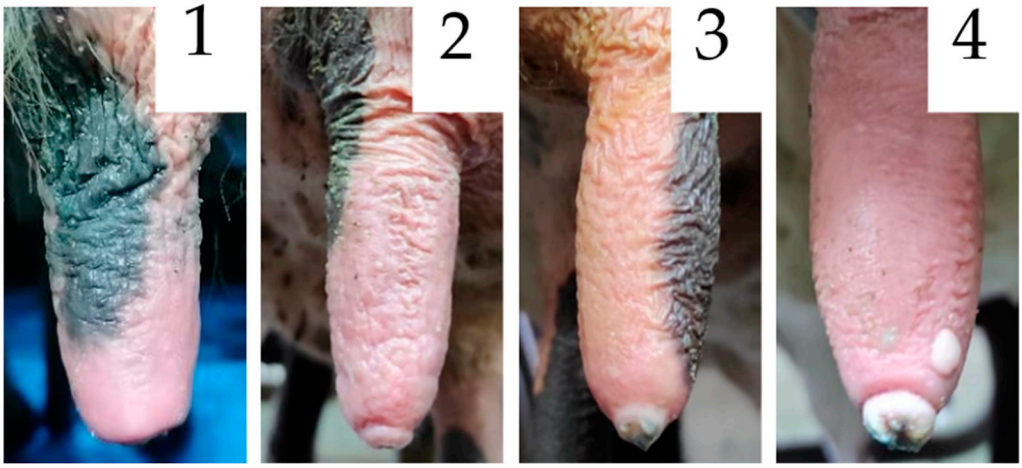


Figure 1. Teat-end hyperkeratosis classification system: No hyperkeratosis (**1**)—teat end smooth, without a visible ring, with a small and uniform orifice; slight to moderate ring (**2**)—teat end with a slight to moderate ring, where the ring surface may be smooth or slightly firm, but there is no evident keratin deposition; rough ring (**3**)—teat end with a rough ring and keratin thickening of 1–3 mm; very rough ring (**4**)—teat end with an extremely rough ring, with keratin thickening $\geq$ 4 mm. The ring is coarse and cracked, giving the teat end a flower-like appearance.

2.3. Data Collection

Additional individual-cow-level data were retrieved from the farm management software (DairyPlan C21, GEA, version 5.2.), including parity, days in milk (DIM), somatic cell count (SCC), milking duration (i.e., machine-on time), mastitis history, and occurrences of manual milking cluster removal. Manual cluster removal was performed in cows with typically slower milking, often due to one or more quarters, leading operators to override automatic detachment to ensure complete milk removal. This practice may result in prolonged milking and increased exposure of the other teats to overmilking. The individual SCC (expressed as number of cells $\times$ 10^3/mL) was analyzed using an automatic cell counter within the NDPP regular sampling protocol.

2.4. Statistical Analysis

All statistical analyses were performed using R software, version 4.5.2. [16].

Descriptive statistics were calculated to summarize the distribution of THK scores across explanatory variables. Continuous variables were assessed for appropriate scaling. Milking duration was expressed per 100 s increase to facilitate interpretation, and SCC was log-transformed to approximate normality. The primary outcome variable was the THK score, analyzed as an ordinal variable (scores 1–4).

Univariable associations between each explanatory variable and THK scores were evaluated using mixed-effects ordinal logistic regression models, including cow as a random effect to account for clustering of teats within cows, thus considering the individual teat as the unit of observation. This analysis was performed solely as a preliminary assessment to avoid potential confounding in unadjusted results. A multivariable mixed-effects ordinal logistic regression model was then constructed to identify factors independently associated with THK severity. The model included biologically relevant variables and those identified as significant in univariable analyses. Fixed effects considered included teat

length, teat-end shape, parity, DIM, SCC, milking duration, mastitis history, and cluster removal (automatic vs. manual), with cow included as a random effect. Post hoc pairwise comparisons for categorical variables were performed using estimated marginal means with Tukey adjustment.

Model stability was assessed by examining convergence diagnostics and the condition number of the Hessian matrix. Multicollinearity among explanatory variables was evaluated using variance inflation factors, calculated from an equivalent linear model, with values < 3 considered acceptable. Model results are presented as odds ratios (OR) with 95% confidence intervals (CI).

An additional analysis, considering SCC as a secondary outcome, was performed, using a linear regression model. Since SCC represents a cow-level variable, analyses were conducted at the cow level to avoid pseudo-replication arising from multiple teats per cow. Explanatory variables included parity, DIM, milking duration, mastitis history, cluster removal, and THK. For this model, THK severity at the cow level was summarized as the mean THK score across the four teats, providing an overall indicator of teat-end condition per cow. Sensitivity analyses were conducted to assess the robustness of the association between THK and SCC, using alternative cow-level summaries of THK severity (mean, median, and maximum score per cow). In addition, the relationship between milking duration and DIM was also explored using linear regression. Results are presented as exponentiated coefficients, representing multiplicative effects on SCC. Statistical significance was set at $p < 0.05$ for all models.

3. Results

3.1. Descriptive Characteristics

In this study, cows had a mean of 3.3 ± 2.2 lactations (median: 3; IQR: 1–5) and 154.5 ± 90.8 DIM (median: 147.5; IQR: 84–219 days). The SCC averaged 127.1 ± 348.6 × 10^3 cells/mL and 79 cows were identified with a history of mastitis. The mean milking duration was 384 ± 123 s (median: 354.5 s; IQR: 301.0–439.0 s) and 84 cows were milked with manual cluster removal.

3.2. Teat-End Hyperkeratosis Assessment

A total of 1968 teats from 492 cows were evaluated, with a mean THK score of 1.8 ± 0.7. Overall, 45.6% of teats showed no THK (score 1), 43.0% presented a slight and smooth callosity ring (score 2), and 11.4% showed more severe THK (10.3% with score 3 and 1.1% with score 4). The prevalence of THK at the teat level was 54.4%. At the cow level, 58.7% of cows presented at least one affected teat. Among affected cows, the majority showed lesions in all four teats (88.2%), while a smaller proportion exhibited involvement restricted to the forequarters (7.3%), hindquarters (2.4%), or both (2.1%).

3.3. Teat Morphology

Teats were classified across all length categories, with the highest THK prevalence in the normal and long categories. Overall, 47.6% of teats had normal length, 43.5% were long, and 8.9% were short. Normal-length teats were the most frequent in THK scores 1 and 2 (49.1% and 52.0%, respectively), while long teats predominated in higher THK categories, accounting for 73.3% of teats with THK score 3 and 90.9% of those with THK score 4 (Figure 2).

Overall, 55.5% of teat ends were classified as round, 42.5% as flat, and 2.0% as pointed. For THK score 1, round teat ends were more frequent than flat teat ends (53.6% vs. 45.5%), and similarly for THK scores 2 (47.5% and 48.7%, respectively). Pointed teat ends were rare

across all categories and were not observed in THK scores 3 and 4. Most teats with THK score 3 (92.6%) had round ends, and all teats with score 4 were round (Figure 3).

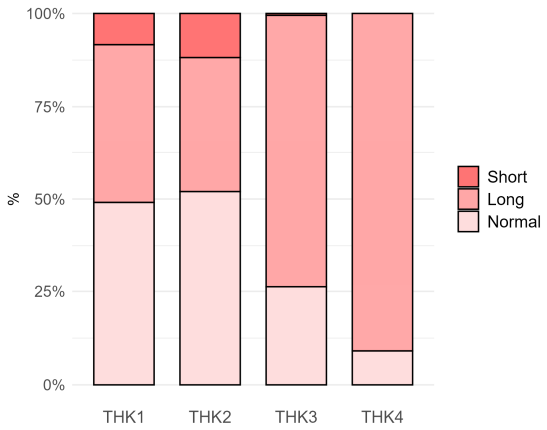


Figure 2. Frequency of teat-length classification (short, normal, and long) across teat-end hyperkeratosis (THK) scores (1, 2, 3, and 4) in 1968 teats.

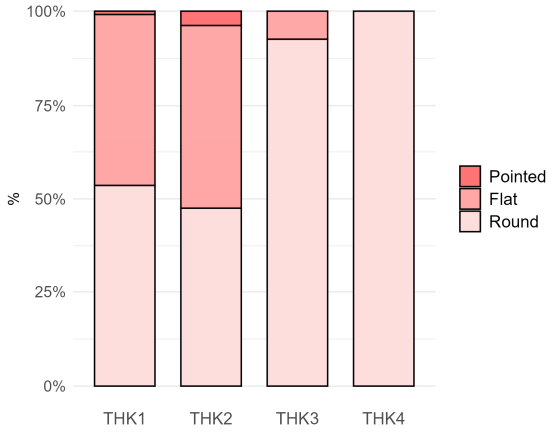


Figure 3. Frequency of teat-end shape classification (pointed, flat, and round) across teat-end hyperkeratosis (THK) scores (1, 2, 3, and 4) in 1968 teats.

3.4. Multivariable Analysis for THK

All variables included in the multivariable mixed-effects ordinal logistic regression model were associated with THK in the univariate models ($p \leq 0.006$). In the final model, only teat-end shape, milking duration, and mastitis were significantly associated with THK severity ($p < 0.001$; Table 1).

Compared with flat teat ends, round teat ends had higher odds of severe THK (OR = 6.4; 95% CI: 1.4–29.0; $p = 0.016$), while pointed teat ends also showed increased odds, although with wide confidence intervals (OR = 64.0; 95% CI: 1.4–2952.2; $p = 0.033$). Milking duration was positively associated with THK severity, with each additional 100 s of milking increasing the odds of more severe THK by approximately 2.7-fold (OR = 2.71; 95% CI: 1.5–5.1; $p = 0.002$). Cows with a history of mastitis showed a strong positive association with THK (OR = 80.0; 95% CI: 16.4–389.5; $p < 0.001$). Teat length, parity, and cluster removal were not significantly associated with THK after adjustment ($p > 0.05$).

Post hoc pairwise comparisons confirmed that no significant differences were observed across teat-length categories ($p > 0.05$). For teat-end shape, round teat ends showed significantly higher odds of THK compared with flat teat ends ($p = 0.043$), whereas comparisons involving pointed teat ends were not statistically significant.

Table 1. Multivariable mixed-effects ordinal logistic regression model results with odds ratio (OR) and 95% confidence intervals (CI) for teat-end hyperkeratosis (THK; n = 1968 teats).

Variable	Category	OR	95% CI	p-Value
Teat shape	Round vs. Flat	6.4	1.4–29.0	0.016
	Pointed vs. Flat	64.0	1.4–2952.2	0.033
Teat length	Long vs. Normal	1.2	0.3–5.4	0.810
	Short vs. Normal	4.3	0.4–42.8	0.210
Parity	per lactation	1.2	0.9–1.7	0.138
Milking time	per 100 s	2.7	1.5–5.1	<0.001
Mastitis	Yes vs. No	80.0	16.4–389.5	<0.001
Cluster removal	Manual vs. Automatic	2.8	0.3–23.8	0.254

3.5. Multivariable Analysis for SCC

The SCC was analyzed at the cow level using log-transformed values. In the multivariable model, history of mastitis was strongly associated with increased SCC, with affected cows showing approximately a 2.1-fold increase in SCC compared to cows without a history of mastitis ($\beta = 0.726$; SE = 0.149; $p < 0.001$). DIM was also positively associated with SCC, with each additional 10 days associated with an approximate 2.1% increase in SCC ($\beta = 0.021$; SE = 0.006; $p < 0.001$). Milking duration was negatively associated with SCC, with each additional 100 s of milking associated with approximately 12% decrease in SCC ($\beta = -0.125$; SE = 0.062; $p = 0.045$). The association between milking duration and SCC showed some variability across model specifications, with the effect ranging from borderline ($p = 0.054$) to statistically significant ($p = 0.042$). THK severity, expressed as the mean score per cow, was not significantly associated with SCC ($p = 0.577$). Similar results were obtained when THK severity was summarized using the median and maximum values per cow, with no significant association observed between THK and SCC across all model specifications. Parity and cluster removal were also not significantly associated with SCC ($p = 0.581$ and $p = 0.804$, respectively).

4. Discussion

This study evaluated the prevalence and severity of THK in a large commercial dairy farm, identifying animal- and management-related factors associated with THK occurrence. Most teats were either free of lesions or presented mild THK, whereas severe lesions were less frequent. Morphological traits such as teat-end shape showed significant associations with THK severity. Management practices, particularly milking duration, were associated with increased lesion severity, whereas SCC remained below clinical and subclinical mastitis thresholds, indicating overall good udder health at the herd level.

The THK evaluation is not usually included in dairy cattle welfare-assessment protocols, and therefore, maximum recommended prevalence levels are lacking. The present findings suggest a lower prevalence of THK compared with that reported in other studies [7,14,17]. In the studies by Breen et al. [17] and Cerqueira et al. [7], prevalences of 93% and 70% were reported, respectively. Nevertheless, both studies consistently reported a lower prevalence of severe THK (scores 3 and 4) and a predominance of mild hyperkeratosis (score 2).

In this study, long teats were more frequently observed among teats with higher THK scores, whereas short teats were rare across all THK categories and not observed in score 4. Although teat length was not retained in the final multivariable model, this pattern is consistent with Sousa [8], who reported that long teats had a 1.91-fold higher risk of developing severe THK compared with short teats. This could be attributed to the overpressure generated by liner collapse, which increases proportionally with teat penetration depth [3], whereas short teats that remain above the collapse zone are less

affected. In contrast, Neijenhuis et al. [5] found no association between teat length and THK severity; nevertheless, a lower threshold for maximum teat length was used compared with the present study. Round-ended teats were more frequently associated with severe THK (scores 3 and 4), followed by flat and pointed teat ends. These findings contrast with Neijenhuis et al. [9], who reported that pointed ends developed THK earlier and more severely, as well with other studies reporting that both pointed and round teat ends were more susceptible than flat ends [5,9,14]. This discrepancy may be explained by the greater protection of the teat canal in smooth-ended teats from collapse pressure during milking [18]. However, the small number of pointed-ended teats in the present study limits definitive conclusions.

Older cows are more likely to present chronic teat-end changes due to prolonged exposure to milking forces and cumulative tissue stress over successive lactations [8]. However, this effect was not retained in the multivariable model, suggesting that the association between parity and THK may be explained by other associated factors, such as history of mastitis and milking-related variables. This indicates that parity itself may not be a direct determinant of THK, but rather reflects cumulative exposure to mechanical stress, particularly in combination with teat morphology and prolonged milking time. Unlike our findings, a longitudinal study has reported an increase in THK during the first 6 to 8 weeks of lactation, going parallel with the increase in milk yield and milking duration [9]. Indeed, Cerqueira et al. [7] reported that THK severity was higher at 61–180 DIM and was also positively associated with higher SCC. Elevated SCC is associated with mastitis, which is more likely to occur in teats with THK [5,11]. In the herd evaluated, cows showed overall good udder health, with average SCC below the thresholds for clinical and sub-clinical mastitis [19], which may explain the absence of a statistical causal association between SCC and THK, as reported in previous studies [1,20–22]. Cows with a history of clinical mastitis or subjected to milking with manual cluster removal have shown to be associated with higher THK scores [5,8,17,23]. Indeed, teat-end lesions have been shown to increase the odds of clinical mastitis [24,25], highlighting the practical importance of controlling THK severity as part of udder health management. The observed association between THK and a history of mastitis, but not SCC, may reflect differences in the temporal nature of these variables, as mastitis history captures cumulative udder health status, whereas SCC, in this case, represents a more transient indicator of current inflammation. The association between milking duration and SCC should be interpreted with caution, as milking duration was significantly associated with DIM. Since SCC tends to increase with advancing lactation [26], whereas cows earlier in lactation typically exhibit higher milk yield accompanied by longer milking times, this relationship may partly reflect underlying differences in DIM rather than a direct effect of milking duration.

Several studies have demonstrated that overmilking significantly increases THK severity [27–29]. Automatic cluster removal helps prevent overmilking by ensuring prompt detachment following milk-flow reduction [7,30], whereas manual cluster removal may prolong milking time, repeatedly exposing animals to excessive mechanical stress [31]. For example, Zucali et al. [32] reported increased risk of severe THK when milking exceeded 5.3 min (318 s). Nevertheless, after adjustment in the multivariate model, cluster removal was not a significant predictor of THK, most likely because milking duration is the main cause of overmilking and not directly whether the cluster is removed automatically or manually.

These present findings highlight the importance of monitoring teat morphology and implementing milking practices that minimize mechanical stress on the teat end. Regular assessment of THK scores can serve as a preventive tool, allowing early identification of changes that may predispose to mastitis and enabling timely adjustments in milking

equipment settings or procedures. Nevertheless, it has been suggested that the development of mild THK is a natural response to milking that can help to protect the teat against infection [11]. Overall, these results support the interpretation that THK should not be viewed solely as a pathological condition, but rather as a dynamic indicator of the balance between milking efficiency, equipment settings, and tissue recovery. In this context, breeding programs may also play a complementary role, as teat-end score has been shown to have a genetic background and an unfavorable genetic correlation with milk yield, suggesting that balanced selection objectives are important to prevent deterioration of the teat condition [13]. Future studies integrating longitudinal monitoring of THK with physiological and microbiological markers could provide a deeper understanding of its adaptive and pathological dimensions, contributing to improved udder health management strategies.

From a practical perspective, these findings highlight several management strategies that can be directly applied in commercial dairy farms. Minimizing overmilking by optimizing milking duration is essential, as prolonged machine-on time was consistently associated with increased THK severity. The use of automatic cluster removal systems should therefore be encouraged to ensure timely detachment and reduce unnecessary mechanical stress at the teat-end. In addition, incorporating routine THK scoring into herd management protocols may facilitate early detection and allow timely adjustments in milking practices. Finally, breeding strategies that avoid extreme teat conformations, particularly long and round-ended teats, may contribute to improved teat health over time.

Some limitations should be acknowledged. The cross-sectional design of this study does not allow for establishing causal relationships between the evaluated factors and THK severity. Mastitis history was recorded at the cow level rather than at the individual teat level; therefore, observed associations reflect cow-level effects and may not accurately capture teat-specific relationships. This approach may have led to an overestimation of effect size, although it may also reflect the broader udder health status of affected cows, beyond any direct link between infection and THK at the teat level. Moreover, the study was conducted in a single farm under specific management and environmental conditions, which may limit the generalization of the findings to other farms. Future research involving multiple farms and longitudinal monitoring would help clarify the temporal dynamics of THK development and its impact on udder health.

5. Conclusions

This study demonstrates that teat-end hyperkeratosis severity is multifactorial, influenced by teat morphology, milking practices, and a history of mastitis. These findings reinforce the need for routine teat-end condition scoring as a practical monitoring tool in commercial dairy farms. Targeted interventions, including optimized milking protocols, equipment calibration, and selection against predisposing teat conformations through breeding programs, may substantially reduce hyperkeratosis prevalence and associated welfare compromise. Furthermore, given that key risk factors such as udder health and prolonged milking time are also leading causes of culling, the true impact of teat-end hyperkeratosis on herd welfare is likely underestimated in cross-sectional studies. Longitudinal research is warranted to better characterize the cumulative burden of teat-end hyperkeratosis across lactations and to evaluate the efficacy of targeted management interventions.

Author Contributions: Conceptualization, C.A., C.C. and A.P.; methodology, C.A., C.C., A.G., F.G.S. and A.P.; validation, C.C., A.G., F.G.S. and A.P.; investigation, C.A., A.G. and F.G.S.; resources, C.C. and A.P.; writing—original draft preparation, C.A., A.G. and F.G.S.; writing—review and editing, C.C., A.G., F.G.S. and A.P.; supervision, C.C. and A.P. All authors have read and agreed to the published version of the manuscript.

Funding: This work is funded by National Funds through FCT—Foundation for Science and Technology under Project UID/05183/2025.

Institutional Review Board Statement: Ethical review and approval were waived for this study as it was purely observational in nature, involving no experimental interventions, additional procedures, or handling beyond routine dairy farm management and standard milking practices. All data were collected under normal farm conditions without any modification to animal care or husbandry protocols.

Data Availability Statement: The data presented in this study will be made available on reasonable request from the corresponding author.

Acknowledgments: The authors acknowledge the R&D unit MED—Mediterranean Institute for Agriculture, Environment and Development (https://doi.org/10.54499/UID/05183/2025) and the Associate Laboratory CHANGE—Global Change and Sustainability Institute (https://doi.org/10.54499/LA/P/0121/2020).

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

THK	Teat-end hyperkeratosis
DIM	Days in milk
SCC	Somatic cell count

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