

The effect of different levels of teat-end hyperkeratosis on mammary infrared thermograph and mastitis in dairy cows

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Summary: The object of this study was to assay different levels of teat hyperkeratosis and to determine the connections between teat thermographic characteristics, somatic cells count (SCC) and mastitis in dairy cows. A total of 920 teats of 230 Lithuanian Black and White cows were evaluated to assess teat-end conditions and the thermographic characteristics were determined before evening milking. Teats of the animals were grouped into four different classes of hyperkeratosis. Additionally, quarters of udder were divided into three different classes based on the California mastitis test (CMT) and clinical signs: group 1 (healthy), group 2 (subclinical mastitis), and group 3 (clinical mastitis). The 44.3% of the teats were given a score of N (No ring), 41.1% of the teats were scored S (smooth ring), 11.8% of teats were given a score R (rough ring) and 2.9% of teats were given a score VR (very rough skin). The N and S groups had more healthy udder quarters than R and VR groups ($P<0.001$). Analysis of thermographic images at the teat sinuses showed that group 1 had lower teat temperature (0.93-1.32 °C) than group 2 and group 3 ($P<0.01$). There was a significant positive correlation between milk SCC and temperature of the teats evaluated by hyperkeratosis scores N, S, and R. The results of the present study clearly showed that there was a significant connection between different levels of hyperkeratosis and teat temperature in all groups, indicating a greater risk to mastitis.

Keywords: Dairy cows, infrared thermography, mastitis, somatic cell count, teat-end hyperkeratosis

Süt sığırlarında farklı seviyelerdeki meme başı hiperkeratozun meme infrared termografi ve mastitis üzerine etkisi

Özet: Bu çalışmanın amacı süt sığırlarında meme başı hiperkeratozun farklı seviyeleri ile mastitis, somatik hücre sayısı ve meme başı infrared termografi özellikleri arasındaki bağlantıları belirlemektir. 230 Litvanya Siyah Beyaz sığırında toplam 920 meme başı değerlendirildi ve akşam sağımından önce termografik özellikleri belirlendi. Hayvanların meme başı özelliklerine göre hiperkeratozları dört seviyede gruplandırıldı. Ek olarak meme lobları Kaliforniya Mastitis Testi (CMT) ve klinik belirtilerine göre Grup 1 (sağlıklı), Grup 2 (subklinik mastitis) ve Grup 3 (klinik mastitis) olarak üç farklı sınıfa ayrıldı. Araştırmada, değerlendirilen meme başlarının %44.3'üne N (halka olmayan), %41.1'ine S (pürüzsüz halka), %11.8'ine R (keratinleşmiş halka) ve %2.9'una VR (çok keratinleşmiş halka) puanı verildi. Araştırmada, N ve S grupların R ve VR gruplarına oranla daha sağlıklı meme loblarına sahip olduğu gözlenmiştir ($P<0.01$). Meme başı sinüs infrared termografi analizlerinde 1. Grubun, 2. ve 3. gruplara oranla daha düşük meme başı sıcaklığı (0.93-1.32) sahip olduğunu göstermiştir ($P<0.001$). Hiperkeratoz N, S ve R seviyelerindeki gruplarda süt somatik hücre sayısı ve meme başı sıcaklıkları arasında önemli pozitif korelasyon saptanmıştır ($P<0.001$). Bu çalışmanın sonuçları bütün gruplarda hiperkeratozun farklı seviyeleri ile meme başı sıcaklığı arasında önemli bir bağlantı olduğunu açık bir şekilde göstermektedir.

Anahtar sözcükler: Infrared termografi, mastitis, meme başı hiperkeratoz, somatik hücre sayısı, süt sığırcılığı.

Introduction

Bovine mastitis is one of the most frequent diseases in dairy farms and has an important economic effect on the dairy farming sector (9). Particularly, because of reduction in milk production, subclinical mastitis leads to more significant economic losses compared to other mastitis types (9,19). In Italy, the cost of mastitis was reported

around 318 euros/head and it was an essential criterion in cow culling (30).

The anatomical characteristics of teats for a healthy milking process are a crucial issue and especially teat canal is the main barrier against the invasion of mastitis pathogens into udder (14,24). The integrity of teat orifice is needed to protect against bacterial colonization of the

quarter (4). Guarín et al (12) indicated that there were significant relationships between teat anatomical characteristics, teat dimensions, hyperkeratosis, and subclinical mastitis at quarter level. The hyperkeratosis is associated with clinical mastitis (13), reduced milk production (8), increased somatic cell count (8,11) and new intramammary infections (29). Additionally, the infrared thermography is a novel and non-invasive tool to measure the temperature of cow's udder in veterinary studies. As known, there is a heat circulation over bloodstream in mammary gland and other active organs (1). Hovinen et al. (15) and Çolak et al. (6) found that infrared thermography has an important capacity in the detection of temperature increases ($>1^{\circ}\text{C}$) in cow's udder with the clinical mastitis.

The purpose of this study was to assay different levels of teat hyperkeratosis and to determine the relationships between teat thermographic characteristics, SCC and mastitis in Lithuanian Black and White cows.

Materials and Methods

This study was carried out in the herd of Black and White cattle breeders' association in Southern Lithuania. The experiment was completed in February 2017. A total of 920 teats of 230 Lithuanian Black and White cows (73 cows of first parity, 61 of the second parity and 96 cows of the third parity) were used in this study. Before the study, the cows were examined according to a general clinical examination plan (an average rectal temperature of 38.8°C , without signs of lameness, metritis, digestive and respiratory disease). The cows were kept in a loose housing system and fed with a balanced feed ration according to their physiological needs throughout the year. Feeding scheduled to 06:00 AM and 18:00 PM every day. The teat-end conditions and thermographic traits were evaluated on each animal and teat-end conditions were measured based on Teat Club International guideline (18). The four different levels (score) of hyperkeratosis were named: no ring, where the teat- end is smooth with a small even orifice (N); smooth ring, where a raised ring encircles the orifice (S), rough ring, where a raised roughened ring with isolated fronds of old keratin extending 1-3 mm from the teat canal orifice (R), and very rough, where a raised ring with rough fronds of old keratin (VR). The quarters of udders were divided into three groups according to the CMT test and clinical signs: group 1 (healthy), group 2 (subclinical mastitis), and group 3 (clinical mastitis). All the measurements were performed at the same time for all analyzed animals. Thermographic measurements of udder quarters of cows, milk SCC, and CMT tests were carried out before morning milking and, clinical signs of udder quarters were evaluated during the morning milking. Thermal images were taken with an infrared thermographic camera (FLIR Systems E4)

positioned 1 m from the lateral side of the udder before milking. Assessment of the teats was carried out at a 90° angle. All cows were acclimated to environmental temperature for 10 minutes before thermal testing. Moreover, the temperatures of mammary in cows were measured at teat sinuses. After colorimetric thermal mapping with software, the elements (pixels) were converted to give the surface temperatures ($^{\circ}\text{C}$). Thermographic images were analyzed using the FLIR software (FLIR Tools version 2.1). The temperature in teat sinuses was rated on four different points: point 1 (left front), point 2 (right front), point 3 (left rear) and point 4 (right rear) (Figure 1).

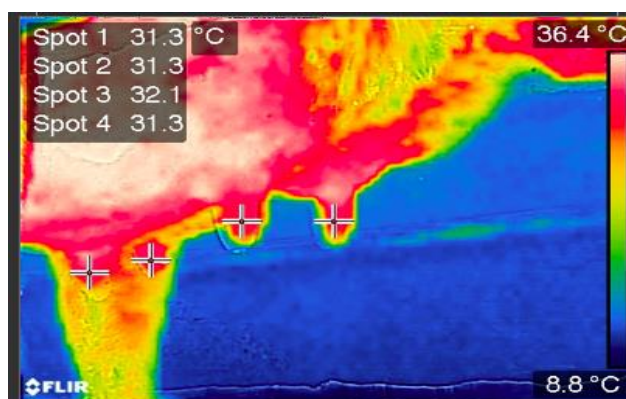


Figure 1. Multispectral (MSX) image
Şekil 1. Multispectral (MSX) görüntü

The SCC in milk was determined using "Somascop" device (CA-3A4, 2004; Delta Instruments, the Netherlands), which uses on flow cytometry technology in State enterprise "Pieno tyrimai". Besides, CMT was used in addition to physical examination of cows.

Data were analyzed using SPSS (Statistical Package for the Social Sciences, 13.0). Pearson's Chi-square test was used to identify statistical differences among groups. Value of $P < 0.05$ was considered significant in statistical evaluations.

Results

Teat hyperkeratosis: According to results, only 11.8% of the teats were found as rough (R) and 2.9% as very rough for teat hyperkeratosis (Figure 2), while 44.3% of the teats were classified as no ring (N), 41.1% were classified as a smooth ring (S).

The percentages of hyperkeratosis for front and rear quarters are presented in Figure 2. The prevalence of the hyperkeratosis score was significantly ($\chi^2=40.377$; $DF=3$, $P < 0.001$) higher for rear quarters than for front quarters. The percentage of teats classified in the worst classes of hyperkeratosis (R and VR) for front and rear quarters were 12.8% and 18.7%, respectively (Figure 2).

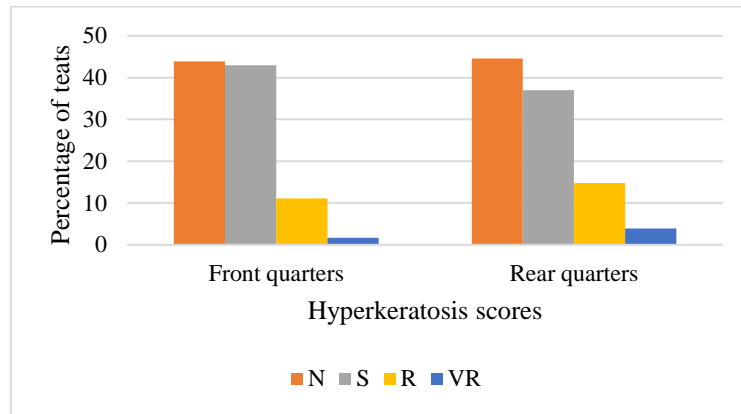


Figure 2. The incidence of teat hyperkeratosis for front and rear quarters
Şekil 2. Ön ve arka meme loblarında hiperkeratoz yoğunluğu

Table 1. Teat hyperkeratosis in dairy cows with different lactation levels (%)
Tablo 1. Farklı laktasyon seviyelerindeki süt sığırlarında meme başı hiperkeratozu

Teat end score	Lactation 1	Lactation 2	Lactation 3>
N	52.25 ^a	37.40 ^b	42.60 ^c
S	42.56 ^a	48.37 ^b	32.73 ^c
R	3.81 ^a	12.60 ^b	20.00 ^c
VR	1.38 ^a	1.63 ^b	4.68 ^c

^{a, b, c}: The differences among values in the same line were significant (χ^2 , $P < 0.001$)

The effect of lactation number on teat hyperkeratosis is presented in Table 1. Multiparous cows had increased degrees of hyperkeratosis when compared with the younger ones ($P < 0.001$). Regarding the teat-end condition of primiparous cows, only 5.19% of the teats were classified as rough (R) and as very rough (VR). The percentage of teats assigned to R and VR classes in the second parity and multiparous cows had 2.74-4.75 times than primiparous R and VR classes (Table 1).

There were significant differences between front and rear teats for the prevalence of hyperkeratosis in first, second and third (≥ 3 lactations) lactation cows ($P < 0.001$). The prevalence of the hyperkeratosis score was significantly ($\chi^2 = 8.423$; $DF = 3$, $P < 0.05$) higher for rear quarters than for front quarters. The percentage of teats classified in the worst classes of hyperkeratosis (R and VR) equaled 12.8% in front quarters and 18.7% in rear quarters. Rear teats of first lactation cows showed more prevalence of hyperkeratosis compared to front quarters ($\chi^2 = 9.692$, $DF = 3$, $P < 0.05$); in second lactation cows we did not establish a statistically significant difference between hyperkeratosis scores of rear and front teats ($\chi^2 = 1.752$, $DF = 3$, $P > 0.05$); rear quarters of the multiparous cows were more likely to hyperkeratosis ($\chi^2 = 40.029$, $DF = 3$, $P < 0.0001$) than front quarters.

Mastitis and teat hyperkeratosis: Regarding quarters of the udder, which was divided into three groups according to the CMT test and clinical signs, the percent of udder quarters with healthy, subclinical, and clinical mastitis were 74.1% (682 of quarters), 8.7% (80 of

quarters) and 17.0% (158 of quarters), respectively. There were significant ($P < 0.05$) differences between front and rear udder quarters. Rear quarters had more subclinical and clinical mastitis compared to front quarters (1.28 and 1.40 times). These results clearly showed that there were significant relationships between teat condition and udder quarter cases ($\chi^2 = 258.072$, $DF = 6$, $P < 0.001$). Teat condition was significantly ($\chi^2 = 84.239$, $DF = 3$, $P < 0.001$) better on healthy udder quarters of group 1 when compared to group 3.

The number of clinical mastitis cases was higher for the udder quarters in which hyperkeratosis scores were very high. The hyperkeratosis scores of N and S teats had 2.17 times more healthy udder quarters ($P < 0.001$) than the hyperkeratosis scores of R and VR teats (Figure 3).

Mastitis and thermographic characteristics: According to the analysis of thermographic images at the teat sinuses, the average temperature of teats was $30.22 \pm 0.123^\circ\text{C}$ (temperature of rear quarters was higher than the front quarters of the udder, 0.08°C). The average temperature of teats in group 1 was $29.72 \pm 0.118^\circ\text{C}$, while it was 0.93°C and 1.32°C higher in group 2 and group 3, respectively, when compared with group 1 ($P < 0.01$).

Figure 4 shows different levels of hyperkeratosis that lead to an increase in teat temperature in each group, which was divided based on CMT and clinical signs (from 1.34°C in group 3 to 1.72°C in group 1; $P < 0.01$). The average milk SCC for group 1, group 2, and group 3 was 137.76 ± 4.40 , 298.53 ± 12.718 and 526.50 ± 44.78 thousand/ml, respectively.

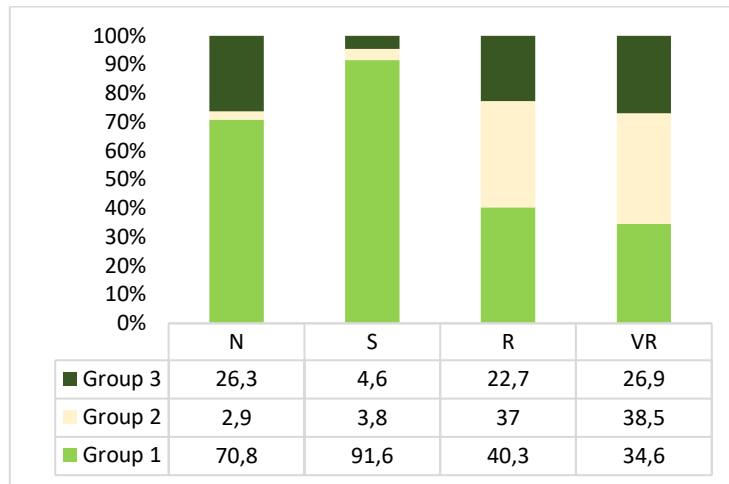


Figure 3. Relationships between teat hyperkeratosis and prevalence of mastitis
Şekil 3. Mastitis yaygınlığı ile hiperkeratoz arasındaki ilişki

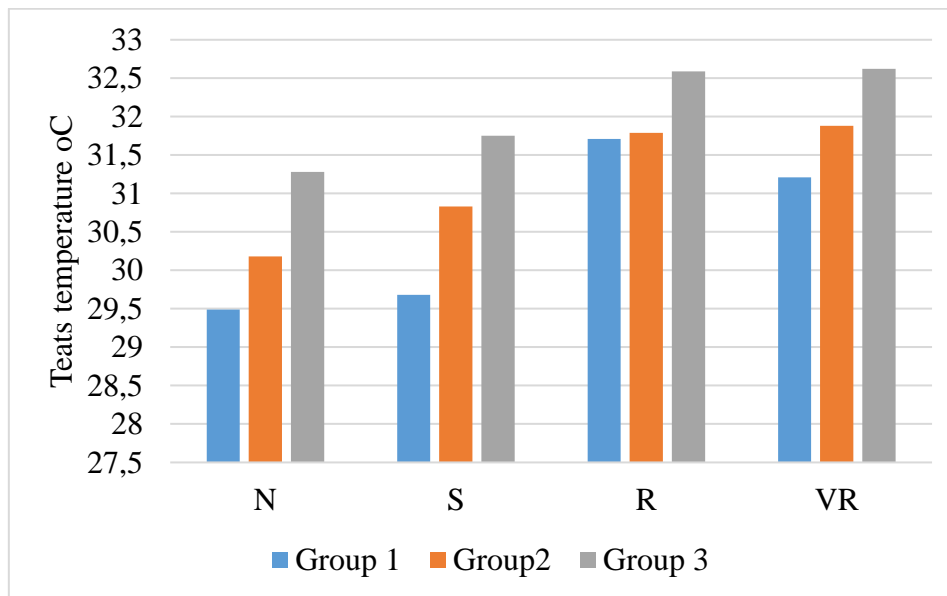


Figure 4. Teat temperature based on the groups of mastitis
Şekil 4. Mastitis gruplarına göre meme başı sıcaklığı

Additionally, the correlation between teat temperature and milk SCC were analyzed. Hyperkeratosis of score VR had no effect on milk SCC depending on thermographic characteristics of teats, while the correlation in this group was the highest. However, a significant correlation ($P < 0.05$) was seen between milk SCC and temperature of teats evaluated by hyperkeratosis scores N, S, and R.

Discussion and Conclusion

The results of the present study demonstrate that increased risks of mastitis in dairy herds are associated with different levels of teat hyperkeratosis. A significant connection in many studies was reported between hyperkeratosis and SCC. On the contrary, such a

connection was not observed in some studies. It is generally thought that a severe degree of hyperkeratosis is most likely associated with a mastitis infection rather than mild hyperkeratosis (5,8,11,17). Neijenhuis (20) reported significant associations between severe degree of teat hyperkeratosis and increased risk of clinical mastitis with a different scale they used. The same author et al (21) has also shown that cows with clinical mastitis had worst teat condition (rough rings) when compared with non-infected animals, especially for animals with clinical mastitis between the second and fifth months of lactation. Breen et al (2) found that only 7% of TEC (greater degree of teat-end callosity) scores were classified as N (no ring). In that study, it was also reported that the majority of TEC scores were score 1A and 1B (thin and moderate smooth callosity

ring); score 2D (extreme thickening, severe HK) was present in 1% of all teats scored during the study period.

The development of hyperkeratosis varies based on a number of lactation. It was found that multiparous cows had increased degrees of hyperkeratosis compared to younger ones ($P < 0.01$). Some studies have indicated that a number of lactations have no effect on hyperkeratosis development (5,8). Emre and Alaçam (8) reported that the development of hyperkeratosis was more prevalent in the fifth lactation and above.

In our study, we detected significantly ($P < 0.05$) higher teat hyperkeratosis in cows for rear quarters than for front quarters of the udder. Trajcev and Nakov (27) reported 60.61% and 39.39% subclinical mastitis for the rear and front quarters, respectively. It was reported that the rear udder quarters had a higher risk of CM incidence when compared to the front udder quarters (19). Our results showed that rear udder quarters had 1.28-1.40 times higher ($P < 0.01$) frequency of subclinical and clinical mastitis cases.

Additionally, we detected new data about thermographic parameters in cases of mentioned pathologies. In fact, several diagnostic tests exist for detection and prediction of mastitis such as milk color, pH test, electrical conductivity (10,22), CMT (16), SCC (28), culture test, biomarkers, proteomic technique, and immunoassay method (3,7,26). Infrared thermography (IRT) is an informative method to determine the temperature changes in the udder and in teats within a distance. In the present study, however, there was not any significant difference between the healthy (evaluated as N) teats and teats with a weak degree of hyperkeratosis (evaluated as S) for the temperature of teats. On the other hand, the temperature of udder quarters was significantly lower in healthy quarters ($P < 0.01$) and was related with increased milk SCC ($P < 0.05$), and with the prevalence of subclinical and clinical mastitis in dairy cows ($P < 0.01$). The IRT is a simple, effective, on-site, and noninvasive method that detects surface heat which is emitted as infrared radiation and generates pictorial images without causing radiation exposure (1,23). Our study shows that the level of hyperkeratosis is associated with increased teat temperature and greater risk of mastitis. This can be useful to extend the knowledge on the risk factors, and on the prevention of this disease (25). According to Porcionato et al. (23), the minimum and maximum temperature in the upper area of the udder was 31.17°C and 35.83°C, respectively. The minimum temperature at the end of the teat in the same study was reported as 29.00°C, while the maximum temperature was 34.80°C.

We investigated the relationships between anatomical characteristics of teats and the prevalence of subclinical mastitis at the quarter level. The results highlight that hyperkeratosis should be considered as an

important problem in the dairy farm we evaluated. Anatomical characteristics of teats in quarter level can be easy and quick criterion in mastitis prevention programs of dairy herds. Additionally, it was concluded that the level of hyperkeratosis is associated with an increase in teat temperature and greater risk of mastitis. This may help to extend our knowledge on the risk factors and on added prevention tools for this disease.

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