How is the association of teat-end severe hyperkeratosis on udder health and dairy cow behavior?

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SUMMARY

The teat-end hyperkeratosis is a pathology which causes pain and discomfort in dairy cows, increasing the risk of infection of the mammary gland. A total of 2,957 dairy cows from 43 farms in Portugal were examined to evaluate the teat-end, degree of hyperkeratosis and callosity on 11,828 teats. Factors related to milking management, characteristics of the individual and udder health status with the most severe level of hyperkeratosis were evaluated. The link between stepping and kicking during milking and the levels of hyperkeratosis was also investigated. Most animals observed (70%) showed signs of hyperkeratosis and 12% showed the teat-end more serious types, thick and extreme. The incidence of severe hyperkeratosis was highly variable between farms (1-35%) and front teats showed twice the risk compared to back teats (P <0.000). A trend towards higher levels of hyperkeratosis in cows with higher milk production was identified (P <0.094). Hyperkeratosis increased with parity (P <0.000) and for the period 61-180 days of lactation (P <0.000). Over-milking influenced hyperkeratosis levels (P <0.002). Higher levels of hyperkeratosis were observed on cows with a higher incidence of mastitis (P <0.004). More steps (P <0.025) but no kicks during milking were also associated with higher levels of hyperkeratosis. The results suggest that hyperkeratosis needs to be monitored in order to prevent and control this pathology, ensuring the udder health and welfare of dairy cows.

Keywords: Holstein-Friesian; callosity; welfare; over-milking; parity; lactation

RÉSUMÉ

Existence d'une relation entre l'hyperkératose sévère du trayon et le comportement des vaches laitières?

L'hyperkératose de l'extrémité du trayon est une pathologie douloureuse qui provoque un inconfort chez les vaches laitières ainsi qu'une augmentation du risque d'infections de la glande mammaire. Dans 43 fermes au Portugal, 11.828 trayons de 2.957 vaches ont été examinés pour évaluer leur extrémité, le degré d'hyperkératose et la callosité. La gestion de la traite, les caractéristiques de l'animal, et la santé du pis ont été évaluées pour le degré d'hyperkératose le plus sévère pendant la traite. Le rapport avec le nombre de pas et de ruades pendant la traite et le niveau d'hyperkératose a aussi été évalué. La plupart des animaux observés (70%) souffraient d'hyperkératose et 12% d'entre eux possédaient un trayon avec une extrémité très endommagée et épaisse. L'incidence de l'hyperkératose sévère s'est révélée très variable selon les fermes (1-35%) et il a été observé que les trayons avant avaient deux fois plus de risque de développer une hyperkératose sévère que les trayons arrière. De même, il a été observé, chez les vaches ayant une grande production laitière, une incidence accrue de cette maladie. Cette incidence augmente aussi avec le nombre de vêlage et entre le 61ème et le 180ème jour de lactation. La sur-traite favorise les taux d'hyperkératose (P<0.002). Il a aussi été observé des taux d'hyperkératose plus importants chez les vaches avec une plus grande incidence de mastite (P<0.004). L'augmentation du nombre de pas sans ruades pendant la traite a été associée avec des taux d'hyperkératose plus hauts. Les résultats suggèrent que l'hyperkératose a besoin d'être monitorée afin d'éviter et de contrôler cette pathologie, ainsi que d'assurer la santé du pis et le bien être des vaches laitières.

Mots-clés : Holstein Friesian; callosité; bien-être; surtraite; parturition; lactation.

Introduction

Hyperkeratosis of the teat canal is the result of a physiological process of adaptation of the mammary gland. The onset of hyperkeratosis is profoundly influenced by the over-riding effects of climate, seasonal and environmental conditions, milking management, herd milk production level and genetics of individual cows. Various agents and mechanisms, causing a number of forms of trauma or lesions, may affect the condition of the teats of the milking dairy cow such as milking duration, intensity of vacuum and over-milking [6, 21, 15]. In fact, hyperkeratosis has been suggested as an earlier indicator of problems associated with milking management and milking equipment. In addition, other studies revealed associations of shape of extremity and length of teat in the animals, milk production and parity with teat-end callosity [3, 7, 30]. In this sense, much more hyperkeratosis may be a measure of the performance and management of the herd [29].

The impact of severe hyperkeratosis in the health and well-being of cows is less known. It is well established that poor welfare conditions have direct negative effects on physiology, behaviour, disease susceptibility and productivity of an animal [5]. Severe hyperkeratosis is per se a well-being problem and chronical pathological stages have not potential to be reduced. It is also possible that severe hyperkeratosis is connected to other diseases like mastitis, with negative effects on milk production and economy. This type of injury reduces the ability of immunity of teats due to the appearance of hyperkeratosis causing an increased risk of infection by microorganisms which cause mastitis [16]. Some studies have demonstrated a high correlation between hyperkeratosis and appearance of clinical mastitis, especially in pointed teats [22] and subclinical mastitis [13]. Cows with clinical mastitis presented higher callosity of teats than cows without mastitis in both periods before and after the incidence of mastitis, whenever it manifests after the first and before the sixth month of lactation, indicating a relationship between the biological development of hyperkeratosis and mastitis [22].

Animal disease may be associated with pain, distress or discomfort. Cow behaviour and disease such as mastitis are gauges of cow comfort. Thus, deviation in cow behaviour might be indicative of cow discomfort. There is still a lack of knowledge concerning cow behaviour and aspects impacting on behavioural changes during mastitis and milking, and whether behavioural changes might serve as a tool for its early detection. Together with ethology, epidemiology is well accepted for assessing animal welfare and behaviour to ensure that relevant risk factors are investigated which facilitates giving correct advice to the farmers and success control.

To improve prevention of this animal welfare problem, the main objective of this study was to first determine the percentage of hyperkeratosis in a representative sample of Portuguese dairy farms and to find associations between cows with severe hyperkeratosis and of subclinical mastitis and behavior accounting simultaneously for several factors at farm and cow level.

Materials and methods

ANIMAL MATERIAL

Data collection was carried out between June and December 2010 in 43 dairy farms in Northern Portugal in free stall production systems with cubicles ranged from 47 to 187 average herd size. This region is considered as the region with the greatest importance in the production of milk in Portugal. A total of 2957 dairy cows of Holstein-Friesian breed were evaluated in the production phase, at the end of milking. Data consisted on data analysis from the farm and cow records from the milking recording scheme, and cow data from observation at milking.

MEASUREMENTS

Teats-end were classified at the same time as evaluating hyperkeratosis, after milking and prior to application of disinfectant. In the present study, the proposed system by Klaas *et al.* [2005] was used, where the teat shape is classified as being inverted, smooth, round, or pointed.

The evaluation of the teat callosity appealed to the methodology of Neijenhuis *et al.* [2000], through which it is possible to divide the callus into two groups: smooth and rough, with four categories each one. In this classification system, N means absence of hyperkeratosis and 1A, 1B and 1C ratings correspond to smooth calluses, in an increasing disposition of the thickening of the teat-end. In rough classifications, 2A shows low roughness, 2B and 2C medium to high and 2D extreme roughness.

The somatic cell counts were obtained using data from the milk recording scheme, considering the average value of the observations immediately before and after visiting the farm.

For milking behaviour, the average time of observation per cow at milking (time elapsed between entering the milking stall and re-entering the cowshed) was 13.0±3.1 minutes. The behavior of animals was evaluated following the methodology of Rousing et al. [2006] regarding the definition of stepping and kicks.

All evaluations of the morphology of teats, as well as hyperkeratosis and milking behaviour were performed by the same operator (first author).

STATISTICAL ANALYSIS

Statistical analyses were performed using Stata, version 11 (Stata Corporation, College Station, TX, USA). A mixed logistic regression model (model 1) was performed, to study the association between severe hyperkeratosis (≥ 1B) in milk production (model 1). A second mixed logistic regression model (model 2) was used to study the relationships with somatic cell count (model 2). A third model (model 3) was employed to study the association with behaviour at milking. In the three models, we have taken in account two hierarchical levels (the average number of cows per holding and cow) of observations. The average number of cows per holding and cow were considered as random effects responsible by clustering of observations. The major question in this study was to investigate levels in hyperkeratosis and was therefore forced to stay in all models. Other predictors of interest were type of callus of teat, type of milking, vacuum level, over-milking, milking time, parity and stage of lactation of cows. The callus was differed as dichotomous variable, smooth or rough according to the methodology of Neijenhuis *et al.* [2000]. The type of milking was established as a categorical variable with three classes: herringbone, parallel and tandem milking. The vacuum level was obtained using the existing vacuum gauge in the milking machine and was considered a dichotomous variable with two classes: 42kpa ≤ and> 42kpa. The over-milking was classified as a dichotomous non-existent variable (0) when the teats were removed immediately after cessation of milking, or existent (1) when the cluster remained after cessation of milking. The milking time was a dichotomous variable with two classes: ≤ 10 and > 10 minutes. Parity was defined as a categorical variable with three classes: first, second and third or more. Stage of lactation was also a categorical variable with four classes: 1st stage: ≤ 60 days; 2nd stage: 61-120; 3rd stage: 121-180 and

4th stage:> 180 days. In model 2, the distribution of SCC was highly skewed, therefore the variable was transformed to the logarithmic scale with base 10 (lgSCC) prior to statistical analysis.

In model 3 steps worked as a dichotomous variable in two classes: ≤ 6 steps and> 6 steps and kicks as a dichotomous variable as absent (0) or present (1).

The modelling was done manually, either by elimination of non-significant variables such or by the addition of new variables. For each variable eliminated or introduced, confounding factors were evaluated by comparing the coefficient of variables included. Confounding factors were considered present if a coefficient fluctuated more than 25% and eliminated or inserted variable was then kept in the model even if the p-value> 0.05, and the selection process continued. All two-way interactions were evaluated one by one for subsequent addition to the model if significant (p<0.05). Model validation was performed by examining residuals with respect to equal variance and a normal distribution, and all models were found to be valid.

Results

The average number of animals per farm was 87.3 ± 65.0, which animals averaged 2.4 ± 1.5 and 206.2 ± 137.5 lactations and days in lactation respectively. About 64% of the animals were in the first and second lactation. In average, cows daily yielded 28.7 ± 8.6 kg, reflecting a production to 305 days of 10113.0 ± 1830.5 kg and somatic cell count was 268,900 ± 539,500 cells / ml.

About 72% of observed teats were round shaped, while smooth teats made up 17% of the sample and the pointed and inverted ones retracted 9% and 1% of the total displayed teats respectively. The dry teats or teats with injuries were excluded and amounted to 1.4% (Figure 1).

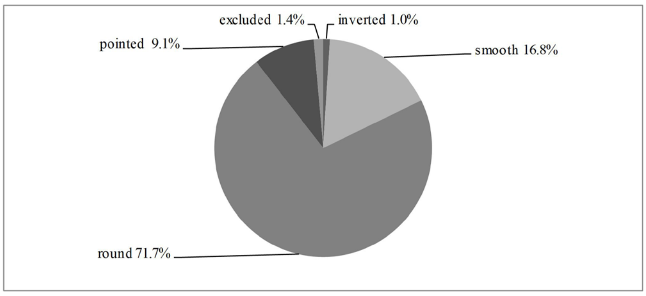

Figure 1: Classification of teat-end shape of the cows.

About 30% of teats didn't present any type of hyperkeratosis and about 29% of teats were located at a level of slight hyperkeratosis with smooth teat-end callous ring. On the other hand 25% of teats showed rough teat-end callous ring and 12% teats exhibited more severe hyperkeratosis (thickened extreme) (Table I).

Assuming the cow as an epidemiological unit, we calculated the frequency of severe hyperkeratosis and teats with rough teat-end callous ring (2B, 2C and 2D). The frequency of severe hyperkeratosis ranged between 1% and 34.8% of animals per farm, where 67.4% and 25.6% of the farms showed a higher proportion than the 10% and 20% of moderate and extreme hyperkeratosis respectively, with roughened teat-end callous ring (Figure 2).

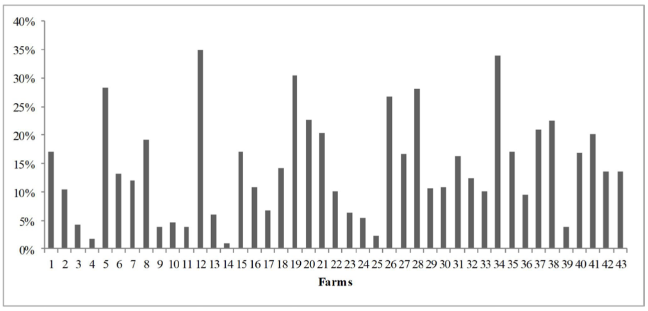

Figure 2: Frequency of severe hyperkeratosis on the farms under study.

The front teats are about 10% more affected by hyperkeratosis than back ones (Table II) (P <0.05). Regardless of the degree of hyperkeratosis its frequency is always higher in the front teats (75%) than in back ones (65%). Moderate hyperkeratosis to extreme reached 35% of the front teats and the back ones 26% (Figure 3).

Type of hyperkeratosis	None	Thin	Moderate	Thick	Extreme
Smooth callous ring	30.1%	28.8%	12.8%	3.0%	-
Thick callous ring		10.4%	5.9%	3.4%	5.6%

Table I: Distribution of type of hyperkeratosis and teat callosity

Type of hyperkeratosis	Teats	Inverted	Smooth	Round	Pointed
Thick callous ring	front	3.7%	16.5%	36.0%	50.3%
	rear	3.2%	12.2%	27.5%	41.2%

Table II: Frequency of hyperkeratosis according to location and type of teats

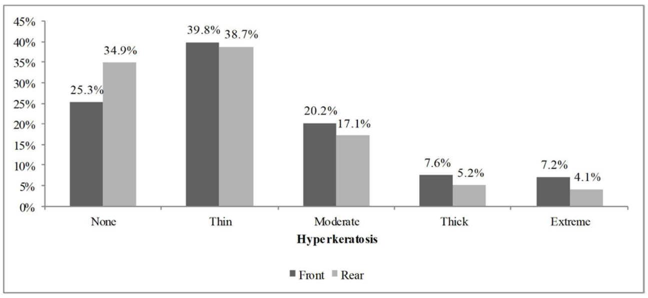

FIGURE 3: Frequency hyperkeratosis of the front and rear quarters of the cows.

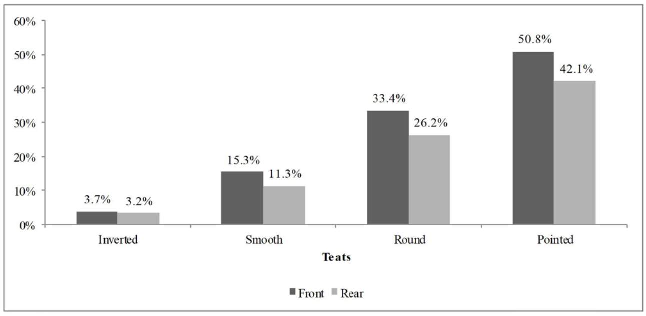

FIGURE 4: Hyperkeratosis ratio ≥ 1B according to the type of the teat-end.

The same trend was found when classification 1B was established as a base limit of hyperkeratosis, according to Neijenhuis *et al.* (2000). When we analyze the most serious classes of hyperkeratosis we observe a similar trend regarding only to the appraisal of teat-end callosity, that is to say, higher levels of this pathology in the front teats where pointed and round teats showed a higher frequency of hyperkeratosis (Figure 4). In general it was found that the highest yields of milk per cow corresponded to higher levels of hyperkeratosis (Figure 5). Furthermore, high levels of hyperkeratosis showed high cell counts (P<0.01) (Table IV). For the remaining predictors results are identical to those presented above.

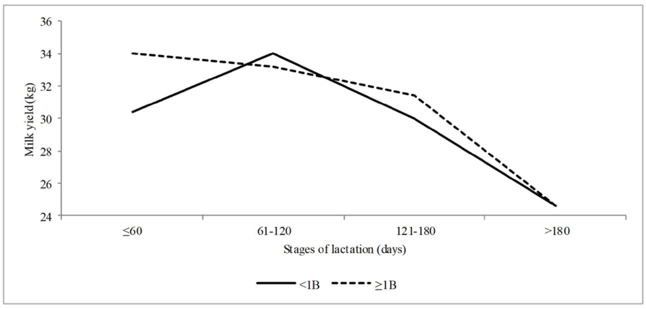

FIGURE 5: Distribution of daily milk yield per lactation according to the level of hyperkeratosis.

Model 1 (n= 2737)		"Odds ratio"	P value	95% CI
Daily milk yield		1.01	0.094	[0.99-1.02]
Front callosity	Smooth	Base		
	Thick	2.84	0.000	[1.68-4.80]
Rear callosity	Smooth	Base		
	Thick	1.34	0.237	[0.83-2.16]
Parlour type	Herringbone	Base		
	Paralel	1.51	0.158	[0.85-2.66]
	Tandem	1.69	0.170	[0.80-3.57]
Vacuum level	≤42 kpa	Base		
	>42 kpa	1.40	0.160	[0.87-2.25]
Tandem x vacuum>42 kpa		0.20	0.001	[0.07-0.51]
Over-milking	Yes	Base		
	No	0.51	0.002	[0.33-0.79]
Milking time	≤10 minutes	Base		
	>10 minutes	0.88	0.627	[0.54-1.45]
Parity	First	Base		
	Second	1.65	0.000	[1.32-2.05]
	Third or more	1.10	0.000	[1.62-2.46]
Stage of lactation	≤60 days	Base		
	61-120 days	2.59	0.000	[1.86-3.60]
	121-180 days	3.37	0.000	[2.41-4.70]
	>180 days	2.77	0.000	[2.08-3.70]

TABLE III: "Odds ratio" of severe hyperkeratosis using the milk yield (Kg/day) of cows observed during milking process in 43 farms

A trend to increase the degree of hyperkeratosis with increasing milk production was observed. In the front teats the rough callus showed higher levels of hyperkeratosis (P <0.001) than the smooth callus. And in front teats observed twice the risk of hyperkeratosis (2.84) relative to the back ones (1.34). There were no differences (P>0.05) between different types of milking for the degree of hyperkeratosis, as well as the level of vacuum greater or less than 42 kPa. However, we found an interaction between tandem milking and vacuum level (P = 0.001). The absence of over-milking resulted in a lower degree of hyperkeratosis (P <0.01) compared to its practice, for instance, milking time showed no differences (P>0.05). The parity and stage of lactation influenced (P<0.001) the level of hyperkeratosis, being more severe with increasing lactation order and the days of lactation, regressing in the last stage of lactation (Table III). Regarding behaviour, the animals that expressed more than six steps during milking had a higher degree of hyperkeratosis (P<0.05), whereas less kicks were observed (Table V).

Discussion

Veterinarians and others require a simple and reliable method for evaluating teat health in dairy herds. Protocols

Model 2 (n= 2750)		**ratio**	**P value**	**95% CI**
interaction		-3.09		
LSCCS*		0.38	0.004	[0.12-0.64]
Rear callosity	Smooth	Base		
	Thick	1.05	0.000	[0.52-1.57]
Front callosity	Smooth	Base		
	Thick	0.30	0.220	[-0.18-0.78]
Parlour type	Herringbone	Base		
	Paralel	0.41	0.155	[-0.15-0.96]
	Tandem	0.54	0.149	[-0.19-1.28]
Vacuum level	≤42 kpa	Base		
	>42 kpa	0.37	0.121	[-0.97-0.83]
Tandem x vacuum>42kpa		-1.63	0.001	[-2.58- -0.68]
Over-milking	Present	Base		
	Absent	-0.63	0.003	[-1.05- -0.22]
Milking time	≤10 minutes	Base		
	>10 minutes	-0.08	0.722	[-0.58-0.40]
Parity	First	Base		
	Second	0.50	0.000	[0.28-0.72]
	Third or more	0.67	0.000	[0.46-0.88]
Stage of lactation	0-60 days	Base		
	61-120 days	0.96	0.000	[0.63-1.29]
	121-180 days	1.19	0.000	[0.86-1.52]
	>180 days	0.94	0.000	[0.67-1.22]

*LSSCC: linear score of somatic cells count.

Table IV: Ratios mixed effects and P value for severe hyperkeratosis (≥ 1B) in the logistic regression model using linear score of somatic cells of cows observed during milking in 43 farms

Model 3 (n= 2957)		**coefficient**	**P value**	**95% CI**
Intersection		-0.72		
Steps in milking	≤6	Base		
	>6	0.21	0.025	[0.03-0.40]
Kicks in milking	Missing	Base		
	Present	-0.50	0.000	[-0.77- -0.23]

Table V: Coefficients of mixed effects and P value for severe hyperkeratosis (≥1B) the regression model using logistic behaviour in milking cows observed in 43 farms for assessment are normally based on a visual classification system that may help to identify or resolve problems related to milking management, environment or the milking machine. However, hyperkeratosis in dairy cows is a multi-factorial problem. Results of our study suggest that hyperkeratosis needs to be monitored throughout lactation, in order to prevent and control risk factors, ensuring the well-being and health of the udder of dairy cows.

Some milking machine characteristics and some milking management practices are involved in the onset of hyperkeratosis. The interaction found among level of vacuum and tandem milking has some connection with the fact that it is an older system, sometimes associated with the lowest frequency of maintenance of milking equipment and the milking operators identified with being less qualified. Mein *et al.* [2003] and Mein *et al.* [2004] state that the influence of the vacuum level in hyperkeratosis is because if there is higher vacuum, the difference of pressure is greater between the different phases of pulsing, and consequently, the force of pressure exerted by liners on the teats will be greater stimulating the appearance of hyperkeratosis. However, other researchers also reported that increases of flow rate of milk with high milking vacuum, results in greater removal of keratin which will lead to a stimulation of its production into the teat canal [32, 10]. The over-milking is the most important cause of damage to teats and causes loss of keratin of the teat canal [8]. The presence of over-milking showed significant differences in the degree of hyperkeratosis of the animals, which contradicts that reported by others [23, 25, 26]. Another factor analyzed but which revealed no effect on the level of hyperkeratosis was milking time per animal. According to Mein *et al.* [2001] milking time with milk flow lower than 1 kg/minute has a great importance on the condition of the teats. This time is mainly influenced by milking udder preparation for milking and the automatic tuning of the teats and the setting when ACRs exist and are used. The fact that the milking time has not influenced the degree of hyperkeratosis may be associated with the accounting, since the milking time was registered by milking cycle times and average milking cow contrasted, since otherwise it would be difficult to collect field data.

From the several characteristics of the animal which influence the appearance of hyperkeratosis, results of this study for the teat-end shape are similar to those found by Sousa [2008] on 17 dairy farms where most of them (54%) were round followed by 25% pointed, and less frequent were teat smooth (14.5%) and inverted (4%) types. Just as the results we found, other authors [21, 15, 31], reported that pointed teats develop hyperkeratosis earlier and, together with round teats, both grouped high frequencies of this pathology compared to the other teats types. The teat extremity is the region subjected to higher pressure by the collapse of liners during the milking process, so, the channel of inverted and smooth teats are more secure than round or pointed teats [19]. In our study, the front teats position has significantly a higher degree of hyperkeratosis than back ones. Other authors [30, 21] related this finding to the fact that front quarters produce less amount of milk, ending the milking more quickly and thus being exposed to longer periods of over-milking, while the back teats are not completely milked. In this sense, more attention has to be put to the front teats.

The observed large variability for severe hyperkeratosis between farms and where 25% of the farms studied have more than 20% of affected teats reinforce that hyperkeratosis at the dairy farm itself is very variable, and is becoming more common in dairy farms with high yields [9, 29]. In this study, milk production showed this trend to higher levels of hyperkeratosis like referred but not significant ($P = 0.094$).

Cows in first lactation had lower levels of hyperkeratosis than animals with two or more lactations, as observed in other studies [31, 2]. Although there are some concerning the influence of the number of deliveries over hyperkeratosis [29], references of the results of various studies are not completely consistent, since Neijenhuis *et al.* [2000] observed no significant differences between parities for callus and roughness of teat canal.

Levels of hyperkeratosis are influenced by hyperkeratosis days of lactation and are lower to 60 days (1st lactation) and higher in the 2nd and 3rd stage of lactation, which contradict results of previous studies [Neijenhuis *et al.* [2000] and [29], reporting that their hyperkeratosis levels increase up to approximately four months of lactation and thereafter begin to decline. And when mentioned by Gleeson *et al.* [2007] found that increased hyperkeratosis during the first five months of lactation and tended to decrease at the end of this stage.

We have found higher somatic cell counts in animals with higher levels of hyperkeratosis. These results allow deducing that teats with hyperkeratosis are more likely to reveal the mastitis than teats with a slight hyperkeratosis, although in both cases the risk of infection was being increased when compared to animals without hyperkeratosis. Faced with contradictory results of studies on the hyperkeratosis and mastitis [30, 18, 29, 1, 2], the evaluation system used in this study to account for the level of callousness teat canal allows a better interpretation of the changes that have greater relevance in the onset of mastitis and that are related to an increased vulnerability of the teat canal to pathogens. Our results are in line with others that demonstrated a high correlation between hyperkeratosis and the appearance of clinical and subclinical mastitis [13, 21, 22, 31, 4, 14]. According to these authors, it can be used as an important parameter in monitoring and control of mastitis. Pankey and Murdough [1998] had already observed that some bacterial strains present in the skin of the udder have the ability to utilize keratin of teat to proliferate. Thus, the channels of the teats with hyperkeratosis may provide the substrate for some of these bacteria to multiply, increasing the susceptibility of the quarter to new infections.

The association between high levels of hyperkeratosis and behavioral steps during milking agreed with those of Rousing *et al.* [2004], as regards the cows with skin lesions reveal more steps during milking. Not causing severe pain triggers a moderate behavior (steps) in animals. For instance, the kicking behavior might be associated with pain and more acute discomfort as Rousing et al. [2004] have also referred. Similarly to previous studies [20, 11] that report that the practice of over-milking may have harmful effects on the teat and tissue condition with consequences on cow behavior during milking. Thus, behavior in the milking of cows can be an important tool to give more attention to the problem of hyperkeratosis, as well as to mastitis problems.

Conclusions

Severe hyperkeratosis has to be considered a welfare concern, and factors that influenced the appearance such as high yield cows, over-milking, in which affected animals are more prone to mastitis demand higher control. The assessment of hyperkeratosis accompanied by changes in cow behaviour at milking, need to be considered to enable farmers to prevent discomfort and resolve future problems related to udder health.

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