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Relationship between stepping and kicking behavior and milking management in dairy cattle herds

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A B S T R A C T

We studied the relationship between behavior during milking with milking parlor management, measuring the occurrence of steps and kicks, and cow-related factors. We also investigated the link between stepping and kicking during milking and udder health. A total of 2,903 direct observations of milking behavior were collected in 44 dairy herds in the north of Portugal. The results showed great variability in the occurrence of stepping and kicking among herds during milking. Mixed linear and logistic regression models for factors associated with stepping and kicking were developed. Cows in tandem milking parlors took fewer steps ($P < 0.003$) than in herringbone ones, although in the tandem milking system, more kicking occurred than in parallel and herringbone systems. Milking room temperatures of more than 27°C led to a higher frequency of kicks among cows ($P < 0.010$). The practice of overmilking also produced a significantly greater frequency of cow stepping ($P < 0.001$). Primiparous cows stepped a third less frequently than did greater parity cows but showed a greater tendency to kick compared with the multiparous ones. Cows with somatic cell counts for more than 200,000 cells/mL at the time of the visit also showed a trend toward higher kicking frequency. The results suggest that animal welfare measures, like kicking and stepping, are suitable for epidemiologic studies. Significant interactions were observed when animals were affected by challenging health and welfare situations.

Introduction

There is clear evidence that the assessment of cattle welfare must include the milking routine (Wenzel, 2003; Rousing et al., 2004) because it is a daily process throughout the lifetime of dairy cows. Milking is a convenient time to assess animal welfare because cows show their sensitivities, which could lead to different stress responses if consistent behavioral routines are interrupted (Hopster et al., 1998; Gygax et al., 2008). Behaviors during milking may be relevant as part of a welfare management tool serving to indicate cow welfare problems relating to udder health, milking techniques, skin lesions, and quality of handling routines in the individual herd (Rousing et al., 2004).

There is an increasing interest in the analysis of animal welfare by scientifically evidenced animal-based measurements following the recommendation of the European Food Safety Authority (EFSA, 2009). From previous observational studies during milking, increases in physiological rhythms (defecation and urinating) are considered as signs of acute stress (Grandin, 1997). Stepping, kicking, and constant movement are also indicative of agitation and stressful situations (Grandin, 1993), which might inhibit milk ejection (Willis, 1983) and decrease milk yield (de Vries et al., 2011). Thus, increased movement could be used to assess cow comfort during milking (Rousing et al., 2004). As these are animal-based measurements, they also allow comparisons to be made of

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animal welfare under different production systems and conditions (Hemsworth et al., 1989).

In commercial farms, various milking environments and management systems are used, but in all cases, the aim is to provide maximum animal welfare conditions for animal comfort. In fact, the appropriateness of milking technical design, equipment, operation, and personnel is well recognized (EFSA, 2009), and many factors with a major effect on the welfare of dairy cows arise from the husbandry system (Whay et al., 2003).

Slippery floors cause cow discomfort in the milking system and in all cow traffic areas. The risk assessment carried out by EFSA (2009) showed that inappropriate flooring in passageways, feeding, and milking areas posed the largest risk for welfare associated with behavioral problems, fear, and pain in cubicle houses. They have been reported as having a negative effect on cow comfort, locomotion, and claw health (Fjeldaas et al., 2011) and will likely result in poor milk production. However, Rousing et al. (2004) found no lameness or other signs of leg disorders with stepping and kicking during milking.

Ambient temperature may be a measure of animal welfare. Cows have a preferred range of 5-25°C, and cows exposed to temperatures above 25°C have lower feed intake, decreased daily milk production, and reduced metabolic and conception rates (McDowell et al., 1976). Heat stress occurs in the most adverse cases (Roenfeldt, 1998; Kadzere et al., 2002). However, temperature is rarely studied in connection with milking.

Overmilking, conditioned by milk management, is associated with a greater harmful effect on teat tissue than milking frequency, increasing the risk of new infection and mastitis (Peterson, 1964). For the assessment of cattle welfare, the type of milking parlor also leads to different observation periods, different body areas of the animal (conditioned by pit, rump, and kick rail bail configurations) from the cupping position, and animals confronted or not with the stockperson: all these factors modify cows' responses.

Stepping is more frequent in nervous and anxious animals (Metz-Stefanowska et al., 1992). The process of handling is also modified by the age of the cow with better adaptation over time, indicating a degree of habituation (Uetake et al., 2004; Gygax et al., 2008).

Stress in cows during milking not only impairs welfare but also causes challenges to metabolic and immune status (somatic cell count [SCC]) (Moberg, 2000). Cows with compromised immune systems are predisposed to mastitis and other types of infectious and metabolic diseases (Goff et al., 2002). Animal behavior may be one of the first things that changes when an animal is affected by health or welfare challenges. Changes in behavior can precede clinical signs of stress or disease (Kyriazakis and Tolkamp, 2010). Concurrent milking behavior variations and teat lesions have been reported (Rousing et al., 2004). Such knowledge can be useful for the development of welfare assessment protocols during milking because the potential for mastitis to induce behaviors associated with sickness is not reported. The welfare and comfort of dairy cows are vital to promote a high level of productivity. Early detection using animal welfare indicators of discomfort is critical to optimize treatment and prevention plans, minimize adverse effects on animal welfare, and therefore reduce economic loss (Welfare Quality, 2009; Grandin, 2010).

Few studies involving direct observations of milking using animal-based measures, environment, and handling have been performed. The purpose of this study is to investigate, first, the effect of management and cow factors on stepping and kicking and, second, the effect of stepping and kicking behavior and udder health on milk yield.

Materials and methods

Description of dairy herds

This observational study was carried out on 44 commercial dairy herds located in the northern part of Portugal. This region represents 38% of the national milk production (FENALAC, 2011).

From June to December 2010, the selected herds were all visited once. The average herd size in this study was 107.8 with Holstein Friesian as the only breed (range, 31-380) and an average annual milk yield per cow of 10,147 kg on 305-day lactation (range, 3212-17,292). The average daily milk yield of the herds was 26.9 kg (range, 4.2-65.1) per day. Cows were housed in loose housing cubicle systems with concrete floors and were all fed a total mixed ration at a fodder board in a separate feeding area. Milking took place in a milking parlor twice a day. The distribution of observations according to the different factors studied in the analysis is shown in Table 1.

Observations of behavior during milking

The behavior of animals was evaluated following the methodology of Rousing et al. (2006) regarding the definition of stepping and kicks. Stepping was defined as taking low and vertical leg lifts, and kicking was a high powerful leg lift often directed backward (Breuer et al., 2000; Rousing et al., 2006). The number of steps, kicks, defecations, and urinations observed during milking (morning or afternoon turn) was written down on notation sheets by a single observer (first author). The observer attended to on-farm training applying the methodology of Rousing et al. (2006).

Table 1
Distribution of observations (n = 2,903) according to variables included in the models of the study and number of stepping and kicking per cow in 44 Portuguese dairy herds

Factors	Categories	Cow level observations	Herd level observations	Stepping/cow		Kicking/cow	
				Median	Range	Median	Range
Type of milking parlor	Herringbone	2,195	32	5	0-58	1	0-7
	Parallel	419	4	6	0-46	2	0-10
	Tandem	453	8	4	0-27	1	0-7
Temperature (°C)	≤20	766	10	4	0-46	1	0-10
	21-27	1,563	20	6	0-58	1	0-7
	≥27	791	14	5	0-51	2	0-7
Overmilking	Yes	1,630	24	6	0-58	1	0-7
	No	1,490	20	4	0-46	1	0-10
Parity	First	1,050	44	5	0-54	1	0-10
	Second	844	42	5	0-34	1	0-5
	Third or greater	1,060	40	6	0-58	1	0-7
SCC[a]	Below cutoff	2,596	40	5	0-58	1	0-10
	Above cutoff	358	38	5	0-41	1	0-7

SCC, somatic cell count.
[a] SCC: cutoff = 200.000 cells/mL.

The number of animals monitored was set by the observer during training sessions in pilot farms. A maximum of 6 animals were observed at any one time between teat cup attachment and teat cup removal in the milking parlor. The position of the observer was 3 m from the exit point where the animals could be seen well. 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 percentage of animals observed per farm was 38%-100% in milking courses, which ran no longer than 150 minutes. A total of 2,903 observations were collected.

Milking management, udder health, and parity

Related information was recorded at herd level with respect to resources (milking parlor type, milking turn of observation [morning and afternoon], floor type [cement, beaded cement, tile, and rubber mat], ambient temperature in the milking parlor, number of milking machines, type of teat cup attachment [manual and automatic], vacuum level, music at milking [yes and no], time for milking preparation, milking side of the cow [left and right], the presence of flies [yes and no], concentrate at milking [yes and no], time of milking, overmilking [yes and no], and teat disinfection after milking [yes and no]) together with cow factors at cow level (cow identification and hyperkeratosis assessed with the methodology of Neijenhuis et al. (2000)). Animal-level variables except milking behavior were also recorded between entering the milking stall and teat cup attachment to the cow's side. Milk yield per observed cow was registered after milking from the milking computer program of the farm. All farm and animal-level variables were recorded by the same person.

The assessment was completed with individual cow data records such as milk yield from the milking computer program of the farm. Milk SCC, calving dates, number of calving, and days in milk (DIM) were retrieved from the Associação para o Apoio à Bovinicultura Leiteira do Norte. The SCC data retrieved were from the 2 (before and after) closest monthly test-milking occasions (monthly corresponding to the sampling date of each farm visit in this study).

Statistical analysis

All statistical analyses were performed using the STATA software, version 11 program (Stata Corporation, College Station, TX). As part of data management, variables were checked for normal distribution. The distribution of SCC was highly skewed; therefore, the variable was transformed to the logarithmic scale with base 10 (lgSCC) before statistical analysis.

A causal model was created to identify potential intervening factors (Dohoo et al., 2010). Variables considered as intervening were not used for further modeling, whereas those with $P \leq 0.2$ in univariable logistic regression were considered for further analysis. In addition, collinearity of the $P \leq 0.2$ variables was investigated using Spearman rank correlations test. If correlations reached 60%, one of the correlated variables was chosen to be included in the multivariable analysis using the criteria of biological plausibility, fewer missing observations, and ease and reliability of measurement (Dohoo et al., 2010). Some categorical variables were regrouped to reduce number of levels and increase degrees of freedom. Regrouping of categories was done based on prior biological knowledge or to produce an approximately equal number of animals in each level. Modeling was done manually, both by backward elimination of nonsignificant variables and by forward selection. For each eliminated or entered variable, confounding was assessed by comparing the coefficient change of included variables. Confounding was considered as present if a coefficient changed >25%, and the eliminated or entered variable was then retained in the model even in case of $P > 0.05$, and the selection process continued. Two-way interactions were investigated once a main-effects model had been developed. In all the models, 2 (herd and cow) hierarchical levels accounted for clustering of observations.

All variables of behavior were examined for occurrence in individuals and in the herd. The defecation and urination indicators rarely occurred (2.1% and 1.8%) and were, therefore, not included in the statistical analysis. Hyperkeratosis was not analyzed further because this variable was not properly tailored to the practical experimental design scope.

The associations between stepping and kicking occurrence and environmental factors and cow parity during milking were determined with mixed linear (model 1) and logistic regression (model 2). The predictors of interest were type of milking parlor, overmilking, temperature of the milking parlor, and parity. Type of milking parlor was a categorical variable with 3 classes: herringbone, parallel, and tandem. Overmilking was classified as a dichotomous variable as nonexistent (0), when the teat cups were taken off immediately after the flow of milk had stopped; or present (1) when the teat cups remained on the udder after milk flow had stopped (modified from Natzke et al., 1982). Temperature was a categorical variable with 3 classes: ≤20, 21-27, and ≥27°C (according to Kadzere et al., 2002). Parity was a categorical variable with 3 classes: first, second, and third or greater. The SCC variable was also dichotomized according to a threshold of 200,000 cells/mL used as an udder health indicator (Fall and Emanuelson, 2009). Mixed linear regression models were designed to evaluate the relationship between milk yield and stepping and milk yield and kicking (models 3 and 4). The predictors of main interest were stepping and kicking, and both variables were therefore forced to stay in these models. Stepping was dichotomized, and a value (stepping events at the observation period) of 6 was used as the cutoff value according to our data distribution for an orientation of theoretical reference values to the closest situation of the study context. Kicking was dichotomized between nonexistent (0) or present (1). To model the fixed part of the lactation curve, the model included a function of DIM, established by the curve of Ali and Schaeffer (1987). In short, a new variable was created $g = (DIM/305)$ and another new variable $w = \log(305/DIM)$ and then g, g^2, w, and w^2. This mathematical function was included in the models as a covariate to account for factors whose effect can change over time, that is, over several test-milking occasions within lactation so the lactation curve has to be fit. Two separate analyses were carried out; 1 for primiparous cows (model 3) and 1 for cows of third and greater parity (model 4). The reason for studying primiparous and cows from third parity separately was the known difference in the shape of the lactation curves of milk yield. In addition, because first and third or greater parity have the most differentiated behavior (Hemsworth et al., 1989), only models for these 2 groups were displayed. The likelihood ratio test for the herd random effect was computed by comparing the model with both herd and cow random effects to the one with cow only, which was significant in a chi-square distribution. Model validation was carried out by examining residuals with respect to equal variance and normal distribution, and all models were found to be valid.

Results

The prevalence of stepping and kicking of cows at least once during milking per herd in our study is presented in the Figure and ranged from 9.7% to 90.6% and from 0% to 38.7%, respectively. The number of steps and kicks (mean ± standard deviation) per milking cow was 6.75 ± 5.96 and 0.12 ± 0.32, respectively.

Results from the analysis of stepping (model 1) are presented in Table 2. Tandem milking was significantly associated with fewer steps than herringbone milking. The occurrence of overmilking led to

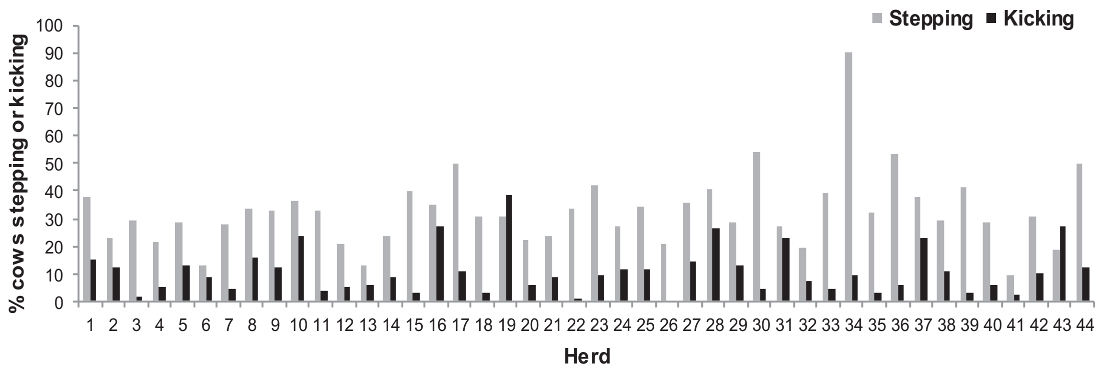

Figure. Prevalence of cows that have shown stepping and kicking behavior at least once during milking in 44 dairy herds.

significantly more steps. The number of steps differed between the first and third or greater parity, with the latter class revealing more steps.

Odds ratios of kicks influenced by milking management (model 2) are presented in Table 3. The risk of kicks was significantly higher in parallel than herringbone type of milking parlor. Temperature of the milking parlor above 27°C also had significantly higher odds of kicks than ≤20°C, and multiparous cows showed fewer kicks than primiparous cows. Finally, there was a trend of higher risk of kicks in cows with SCC above 200,000 cells/mL.

Fixed effects of the full model for primiparous (model 3) and for cows with 3 or more parities (model 4) are presented in Table 4. An interaction was found in the model for primiparous cows with high SCC and more than 6 steps with a slightly higher milk production. In the model for elder cows, more than 6 steps were associated with a slightly lower milk production. Furthermore, the interaction of high SCC and the occurrence of kicks were associated with lower milk production.

Discussion

Considering that milking is a daily task, carried out in conventional milking parlors usually twice a day, it is the ideal place to detect situations of a decline in well-being. Therefore, it is important to study the behaviors of steps and kicks and their relationship with the parity of animals, productivity, and udder health. Furthermore, these behaviors can vary depending on the environmental conditions of management, such as type of milking, ambient temperature, and the practice of overmilking.

In the context of this observational study, based on the use of animal-based measurements for assessing animal welfare, a relationship between management, SCC, behavior, and milk production was found. Our results indicated that nonoptimal conditions lead to cow discomfort that can affect milk production and udder health. The effect was evident despite the limitation that some factors (e.g., human-animal relationship) to complete a multicriteria evaluation were not recorded in the present survey. So, the extent to which milking management affects dairy cows must still vary because of other factors, but we have focused on what could be observed during the real time of the milking routine. It may lie in a greater understanding to have measured biological indicators such as cortisol in milk or heart rates in primiparous versus older cows and evaluate if they are harmonized with the animal-based measures we observed.

Our assessment of steps and kicks in the course of milking in our study showed a huge variation in the frequency of steps and kicks between herds as previously described by Rousing et al. (2004). Cows in the present study displayed a similar or lower number of step and kick responses than those of previous studies (Hemsworth et al., 1989; Uetake et al., 2004), in which the most nervous cows stepped approximately 7 times and kicked a stockperson approximately 5 times at cup attachment. However, the differences in the study design and the country context make it difficult to compare results. By introducing multivariate analysis, we have confirmed the importance of milking parlor on behavior as a clear factor, as previously described (Willis, 1983). The fewer steps in the tandem milking parlor may be associated with the allowance of more space for the individual animal reflecting improved comfort and consequently a more static position during the milking process. The rationale behind this conclusion is that

Table 2
Fixed-effect coefficients and *P* values for the occurrence of steps from a linear regression model using data of cows observed during milking from 44 dairy herds (Wald $\chi^2 = 54.74$; probability > $\chi^2 = 0.000$)

Variable	Model 1 (n = 2903)	
	Coefficient	*P*
Intercept	7.88	
Milking type		
Herringbone	Baseline	
Parallel	1.12	0.263
Tandem	−2.36	0.003
Overmilking		
Yes	Baseline	
No	−2.73	0.000
Parity		
First	Baseline	
Second	−0.06	0.824
Third or greater	1.09	0.000

Table 3
Odds ratio for occurrence of at least 1 kick using data of cows observed during milking from 44 dairy herds (Wald $\chi^2 = 69.22$; probability > $\chi^2 = 0.000$)

Variable	Model 2 (n= 2903)		
	Odds ratio	*P*	95% CI
Milking type			
Herringbone	Baseline		
Parallel	2.70	0.015	1.21-6.05
Tandem	1.35	0.361	0.71-2.55
Milking temperature (°C)			
≤20	Baseline		
21-27	1.33	0.431	0.65-2.74
≥27	2.76	0.010	1.27-5.97
Parity			
First	Baseline		
Second	0.42	0.000	0.27-0.66
Third or greater	0.30	0.000	0.19-0.47
SCC[a]			
Below cutoff	Baseline		
Above cutoff	1.60	0.068	0.97-2.64

CI, confidence interval; SCC, somatic cell count.
[a] SCC: cutoff = 200.000 cells/mL.

Table 4
Fixed-effect coefficients and *P* values for daily milk yield from 2 linear regression models using data on primiparous cows (model 3; Wald $\chi^2 = 296.34$; probability $> \chi^2 = 0.000$) and cows with 3 or more parities (model 4; Wald $\chi^2 = 715.56$; probability $> \chi^2 = 0.000$)

Variable	Model 3 (n = 949)		Model 4 (n = 944)	
	Coefficient	*P*	Coefficient	*P*
Intercept	46.29		57.65	
SCC[a]				
Below cutoff	Baseline		Baseline	
Above cutoff	−4.256	0.000	−0.707	0.450
Behavior				
Steps[b]	−0.0126	0.721	−0.104	0.004
Kicks[c]	0.7669	0.127	1.083	0.291
Above cutoff SCC × steps[b]	0.648	0.000	—	—
Above cutoff SCC × kicks[c]	—	—	−5.35	0.025
Stage of lactation				
DIM/305	−24.991	0.000	−46.83	0.000
log(305/DIM)	−17.616	0.000	−16.85	0.013
$(DIM/305)^2$	4.466	0.000	16.85	0.000
$(\log(305/DIM))^2$	4.763	0.000	3.227	0.144

SCC, somatic cell count; DIM, days in milk.
[a] SCC: cutoff = 200.000 cells/mL.
[b] Steps: >6 steps during milking.
[c] Kicks: occurrence of kicks during milking.

the behavior of dairy cows is dependent on the interaction between the cows and their physical environment. Physical factors of the facility impose baseline limitations on how the cows interact with the housing conditions used (Krawczel and Grant, 2009). Within these limitations, the ability of cows to engage in natural behavior is further dictated by management routines such as stocking density (Krawczel and Grant, 2009). To our knowledge, these studies of overcrowding and social stress did not contemplate individual social stress at the milking parlor. We suggest that the physical contact between animals in a herringbone parlor may contribute to the increased stepping we observed.

From a practical view, it could have been better to register temperature together with humidity for temperature-humidity index calculations. However, previous findings showed that when the temperature in the milking parlor exceeds 27°C, animals are subjected to a greater environmental challenge, shown through the behavior of kicks, as found in this study. Negative effects of high temperatures have been described before, especially in animals of high genetic merit in relation to milk production (Kadzere et al., 2002; Martello et al., 2009).

Fly avoidance behavior that reflects the increased fly counts also received attention in the present survey for their implications for animal welfare. Cattle attempt to escape this annoyance by taking flight, stomping, kicking their trunk, tail swishing, skin twitching, and head or ear movements, and their presence has been associated with reduced milk production, weight gain, and increased stress (Campbell and Berry, 1989). In the present study, the interaction term of flies and temperature was accounted for in the causal model. However, it did not comply with the criterion on model-building strategy.

Regarding overmilking, this could well exert a detrimental effect on teat and udder tissue conditions with consequences in the cow's behavior (Natzke et al., 1982; Hillerton et al., 2002). In our study, cows that were subjected to overmilking showed more steps, which could be explained by the longer time and higher milking vacuum pressure on the udder that might cause discomfort and consequently more steps. However, this factor was not significantly associated with the other results of this study. We also found that cows with 3 or more lactations had more steps than younger cows, which is in line with previous results (Rousing et al., 2004; Uetake et al., 2004).

Cows in higher parities and with higher levels of SCC were seemingly more prone to being unsettled by the milking situation judged by their higher stepping rates (Gygax et al., 2008). This is opposite to the findings of Rousing et al. (2004). These contradictory results suggest that to some extent stepping behavior may also be related to the human-animal relationship (Munksgaard et al., 2001; Waiblinger et al., 2002). It should be mentioned that the levels of hyperkeratosis are also animal welfare measures, but this requires a specific assessment system that was not developed in this study. We did not find any association between stepping and kicking during milking. This is in agreement with other studies (Rousing et al., 2004; Ishiwata et al., 2005). The kicking prevalence was higher in primiparous than older cows. Kicking demonstrates a defensive behavior to a new situation (Waiblinger et al., 2004; van Reenen et al., 2013). This apparently means, therefore, that primiparous were more agitated and exhibited more kicks and trying to escape from humans earlier (Ishiwata et al., 2005); however, handling after first calving in a special gentle manner led to a reduction in kicking (Hemsworth et al., 1989). In the farms of our study, farmers likely did not handle calving.

As part of the range of factors influencing mastitis, stress has been identified as a risk factor for high SCC (Wegner et al., 1976). In fact, higher frequency of kicking and stepping during milking during the first 3 days after mastitis detection was associated with the presence of mastitis (Medrano-Galarza et al., 2012), which supports the interaction terms in our models of milking behavior and SCC. The fact that more than 6 steps were associated with higher milk production in younger cows is more complex to explain. In general, stepping during milking is positively correlated with daily milk yield. On the other hand, one might expect that greater SCC may impair milk yield (Sharma et al., 2011). The fact that data range of SCC in younger cows of our study was not far from the cutoff (200.000 cells/mL) could explain why milk production was not extensively compromised. Some clinical courses of mastitis do manifest a substantial increase in SCC. A multiparous cow is more likely to yield more milk and thus have more to lose with a challenge, but its natural immunity will also be more compromised (van Knegsel et al., 2007). Finally, kicking is also a reflection of pain and stress as SCC rise. Ivemeyer et al. (2011) reported that herds with a higher incidence of new infections of mastitis showed more kicking during milking. Consequently, kicking was also associated with reduced milk yield.

Conclusions

Milking routine does not always conform to the needs of the cows. Related factors were found in an analysis combining milking behavior, recorded cow-side, management, udder health, and milk yield. This study may be a starting point to further evaluate the use of milking behavior indicators. Kicking and stepping were significantly associated with some factors of milking management. In our study, the occurrence of kicking was related to thermal stress and high SCC and the occurrence of stepping to milking system and overmilking. These animal welfare measures are suitable for epidemiologic studies because significant interactions were observed when animals were affected by challenging health and welfare challenge situations. Further research is needed to identify how milking behavior can precede clinical signs of stress or udder health by focusing on kicking behavior.

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Conflict of interest

The authors declare no conflict of interest.

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