



## BEHAVIOURAL RESPONSES OF PRIMIPAROUS AND MULTIPAROUS DAIRY COWS TO THE MILKING PROCESS OVER AN ENTIRE LACTATION\*

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### Abstract

The objective of this study was to examine associations between milking temperament, parity and milk production traits in primiparous and multiparous dairy cows. Twenty-one primiparous and nineteen multiparous Holstein Friesian cows were involved in the investigation on a Hungarian dairy farm. Cows' stepping behaviour, milk yield and average milking speed were recorded once a month over an entire lactation, during morning milking. Milking temperament was scored by direct human observation on a 5-point-scale (1=very nervous, 5=very quiet) during udder preparation and milking. Multiparous cows showed a little more excitable behaviour at milking than during udder preparation (Mann-Whitney  $U=14165.00$ ,  $P=0.032$ ), and they were calmer at pre-milking preparation than primiparous cows (Mann-Whitney  $U=14046.00$ ,  $P=0.001$ ). Milking temperament of multiparous cows during udder preparation was associated with milking speed: nervous cows let down their milk slower ( $F=9.102$ ,  $df=1$ ,  $P=0.003$ ). Further experiments are needed to repeat the milking temperament test along with sensors measuring heart rate variability of cows in order to better understand the milking behaviour of cows.

**Key words:** dairy cow, lactation, milking temperament, milking speed, milk yield

In intensive dairy farming modern housing and milking technologies mainly determine the welfare of animals. Dairy cows can reach their full performance potential only in an adequate production environment, in which they feel comfortable. Regarding the milking system, discomfort due to human presence as well as the milking process itself induces stress in cows, hereby milk ejection problems can occur (Van Reenen et al., 2002; Bruckmaier, 2005) and this also makes cows difficult

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\* This work was supported by the Research Centre of Excellence under Project 8526-5/2014/TUD-POL.

to handle (Rushen et al., 1999). It is well-known that there is a complex relationship between behaviour during milking, reaction to human, daily milk yield, milking system, physiological indicators and health status of dairy cows (Rousing et al., 2004).

Milking temperament is a main characteristic of dairy cows that determines the level of comfort during the milking process. Stepping behaviour at milking is a behavioural indicator of stress (Metz-Stefanowska et al., 1992; Breuer et al., 2000) mostly shown by nervous cows (Wenzel et al., 2003). Namely, excitable cows are more susceptible to stress caused by routine handlings and have more extensive responses to the milking process than calm ones (Voisinet et al., 1997). Stressed animals require not only more handling during milking (Rushen et al., 1999), but they may kick the hand of the milker or kick off the cluster during milking. Fearfulness in cows can result in slower milk let-down and milk retention (Munksgaard et al., 2001). Better understanding of the milking behaviour of cows helps us to make the milking procedure easier and quicker and to avoid a lot of problems such as injuries of handler, poor milk quality, low milk yield and also profit loss.

Temperament is generally described in terms of the type and volume of behavioural response to handling by human (Burrow, 1997; Voisinet et al., 1997; Buchenauer, 1999) or to a novel environment (Sutherland et al., 2012), and it expresses the sensibility of cattle to stressors. Temperament of dairy cows has been often evaluated subjectively in different scoring systems, such as 1-3, 1-4 or 1-5 scales in a milking parlour or in an automatic milking system to identify their milking behaviour (Visscher and Goddard, 1995; Lewis and Hurnik, 1998; Sewalem et al., 2011).

Temperament has been reported to be influenced by many factors including age/parity (Roy and Nagpaul, 1984; Burrow et al., 1988; Tózsér et al., 2003) and lactation stages (Hagen et al., 2004), too. Numerous publications have reported correlation between milk production and temperament of dairy cows (Gupta and Mishra, 1978; Roy and Nagpaul, 1984; Lawstuen et al., 1988). Cows with unfavourable temperament produced less milk and their milk ejection was worse compared to those having calm temperament, which had higher milk yield and more favourable milking speed. Furthermore, Purcell et al. (1988) revealed that parlour score is related to milk production across herds, whereas flight distance and approach distance are not useful indicators of milk production. At the same time, in some published studies there was no relation between temperament and milk production traits (Khanna and Sharma, 1988; Budzyńska et al., 2005).

According to prior studies it can be supposed that the relationship of temperament and milk production is determined by many factors. In order to better understand temperament of cows during milking behavioural observations have been carried out over an entire lactation. This is essential because the true milking behaviour of cows cannot be represented by observing only one stage of lactation. Namely, milking temperament of animals can be influenced by direct environmental effects as well as inner factors related to milk production and health status. The purpose of this study was to detect the direct behavioural reaction reflecting the milking temperament of cows during the udder preparation and milking in order to analyse the effect of parity on temperament and to identify the relationship of milking temperament with milk production traits, such as milk yield and milking speed, through an entire

lactation. We hypothesized that milking behaviour of cows differs in the two milking stages (udder preparation and milking) and also differs in the parities. Furthermore, we hypothesized that there are some associations between milking behaviour and milk production traits.

### **Material and methods**

Twenty-one primiparous (mean±s.d. for age: 2.35±0.17, for days in milk: 54.86±27.02) and nineteen multiparous (mean±s.d. for age: 4.16±0.69, for days in milk: 52.32±31.86) Holstein Friesian cows were randomly chosen from a Hungarian herd for the investigation in January. At the beginning of experiment selected cows were in early phase of their lactation: 8 cows of the primiparous group were below 50 milking days (mean±s.d. for age: 2.22±0.10, for days in milk: 25.00±12.41) and 13 cows were between 50 and 100 days (mean±s.d. for age: 2.42±0.17, for days in milk: 73.23±12.92). As for the multiparous cows, 10 animals were below 50 milking days (mean±s.d. for age: 4.35±0.82, for days in milk: 26.30±14.61) and 9 cows were between 50 and 100 days (mean±s.d. for age: 3.94±0.48, for days in milk: 81.22±16.05). The cows were kept in loose housing cubical systems with a yard. The cows were all fed a total mixed ration (TMR) at a fodder board in a separate feeding area. Milking took place in a double-row herringbone milking parlour twice a day (in the morning between 05:00-08:00, in the afternoon between 16:00-18:00). It had eight milking compartments on each side. Cows were not fed with any concentrate in the parlour. A waiting area was located in front of it. Animals were milked simultaneously by the same two handlers (one milker per side) during morning and evening milking on the observation days in the entire lactation.

Observations in the milking parlour were carried out in each month of a year (lactation), generally one week before official milk recording. Cows' behaviour was recorded once in ten consecutive months from January up to November, excluding July, during morning milking time. This resulted in 7–10 observations of milking temperament for each individual cow with fewer observations for cows which were earlier in their dry period. Milking temperament was evaluated by direct human observation on a 5-point scale on each occasion, during the whole duration of udder preparation and milking procedure (Budzyńska et al., 2005) (Table 1). So, each cow each month received two temperament scores by the same observer (prior to milking and during milking in morning milking time). Milk yield (l) and average milking speed (l/min) were also recorded individually on each observation day per milking by using ALPRO herd management.

All analyses were carried out in SPSS version 22.0. Mann-Whitney U tests were used to determine the effect of parity on temperament assessed before and during milking. Mann-Whitney U tests were also used to assess the difference between temperament before and during milking. Spearman rank coefficients were applied to relate temperament before milking to temperament at milking. To test the association between behaviour during udder preparation, during milking and milk production

traits, multivariate general linear models were used in the case of primiparous (1st model) and multiparous cows (2nd model), separately. The dependent variables as milk yield and milking speed, the fixed factors as temperament score during udder preparation and temperament score at milking and the covariate as milking days were included in the models. Previously, it had been tested but not been confirmed, that the interaction of temperament score during milking and temperament score at milking associates with the milk yield or milking speed. Consequently, the interaction as fixed factor has not been involved into our models. Estimated marginal means were calculated for milk yield and milking speed by factors. Normal distributions of milk yield, milking speed data and the models' residuals were proven graphically using the QQ plots and by Shapiro-Wilk tests. Levene's test was applied to confirm that the error variance of milk production traits is equal across groups in both models.

Table 1. Description of the 5-point scale of dairy temperament test

Point	Definition
1	very nervous, continual and vigorous stepping and kicking
2	continual and vigorous stepping but there is no kicking
3	occasionally vigorous leg movements
4	quiet standing with few slight leg movements
5	very quiet, no leg movements

Using the multivariate general linear models primiparous and multiparous cows were divided into  $2 \times 2$  groups by milking behaviours individually scored on every observation day through the lactation. First group involved nervous cows having 1, 2 and 3 temperament scores (cows with vigorous leg movement). Primiparous cows and multiparous cows were excitable before milking in 13 and 7 cases, respectively, whereas during milking in 10 and 16 cases, respectively, considering all observations during the lactation. Second group included calm cows having 4 and 5 temperament scores (quiet standing cows). Primiparous cows and multiparous cows were calm before milking in 176 and 171 cases, respectively, whereas during milking in 173 and 168 cases, respectively, considering all observations during the lactation.

## Results

Concerning all observations, during udder preparation the cows did not get score 1, they received score 2 in 3 cases (0.82%), score 3 in 17 cases (4.63%), score 4 in 104 cases (28.34%) and score 5 in 243 cases (66.21%). While during milking the cows did not receive score 1, they got score 2 in 1 case (0.27%), score 3 in 25 cases (6.81%), score 4 in 115 cases (31.34%) and score 5 in 226 cases (61.58%), considering the entire lactation. In total, the prevalence of nervous behaviour was 5.45% (in 20 cases) during udder preparation and 7.08% (in 26 cases) during milking.

There was no difference in temperament scores recorded during udder preparation and milking either in the case of all cows (Mann-Whitney  $U=64052.00$ ,  $P=0.174$ ) or primiparous cows (Mann-Whitney  $U=17809.50$ ,  $P=0.956$ ). However,

temperament score was higher before milking than during milking in multiparous group (Mann-Whitney  $U=14165.00$ ,  $P=0.032$ ) (Table 2). Score prior to milking was positively correlated with score during milking in the case of all cows ( $r=0.21$ ;  $P<0.001$ ), also in the case of primiparous ( $r=0.16$ ;  $P<0.05$ ) and multiparous ( $r=0.24$ ;  $P<0.01$ ) animals.

Table 2. Descriptive statistics data of milking temperament by milking stage (udder preparation=UP, milking=M) and by cow parity

Observations during the entire lactation by cow group	All cows (N of cases= 367)		Primiparous cows (N of cases= 189)		Multiparous cows (N of cases= 178)	
	Milking stages					
	UP	M	UP	M	UP	M
Temperament scores						
mean±S.D.	4.59±0.61	4.54±0.63	4.51±0.62 a	4.50±0.66	4.69±0.60 b	4.58±0.59 a
median	5.0	5.0	5.0	5.0	5.0	5.0

Values with different superscripts (a, b) significantly differ ( $P<0.05$ ) by Mann-Whitney U tests.

Significant difference was shown between primiparous and multiparous cows in behaviour during udder preparation (Mann-Whitney  $U=14046.00$ ,  $P=0.001$ ). The multiparous group had higher temperament score than primiparous one. However, behaviour of the 2 groups did not differ during milking (Mann-Whitney  $U=15884.00$ ,  $P=0.282$ ) (Table 2).

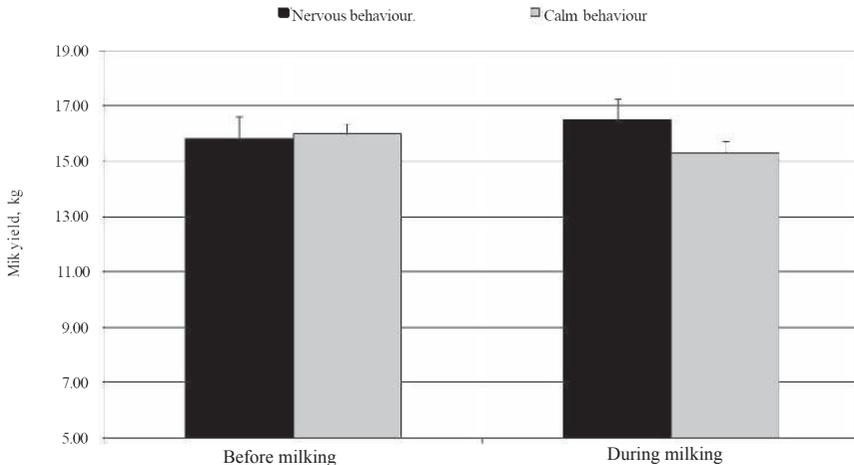


Figure 1. Estimated marginal means and S.E. of milk yield by behaviour type recorded before and during milking for primiparous cows (N of cases = 189)

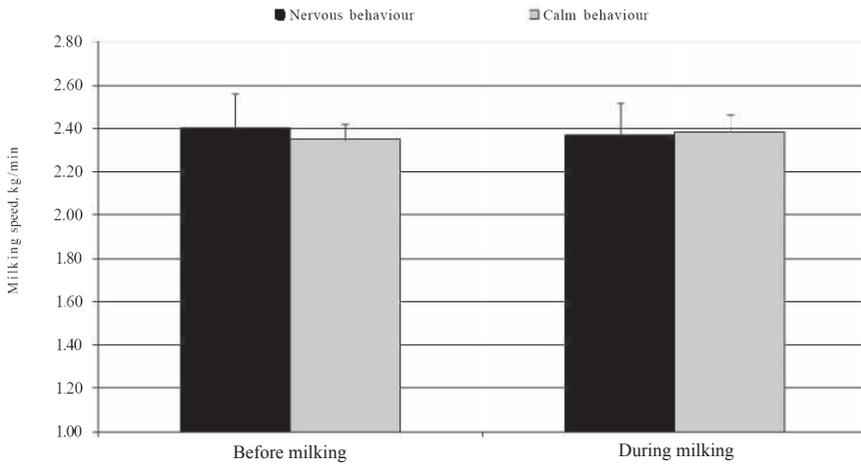


Figure 2. Estimated marginal means and S.E. of milking speed by behaviour type recorded before and during milking for primiparous cows (N of cases = 189)

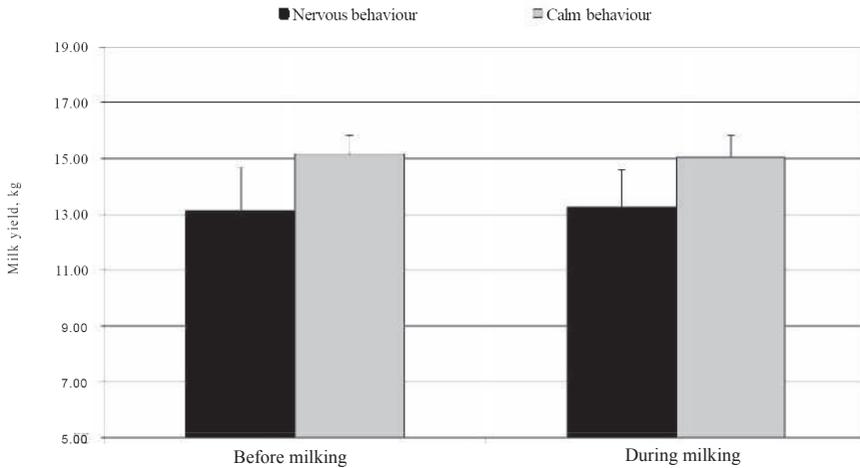
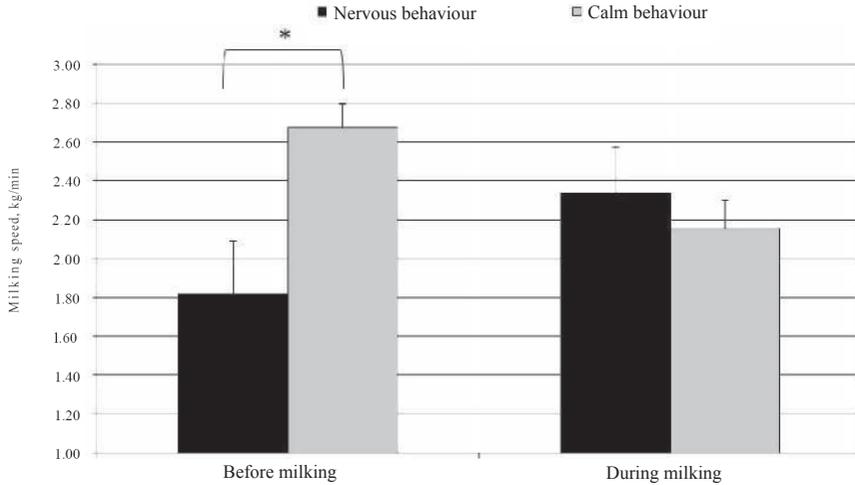


Figure 3. Estimated marginal means and S.E. of milk yield by behaviour type recorded before and during milking for multiparous cows (N of cases = 178)

In the case of primiparous cows, milk yield was not associated with the behaviour either during udder preparation ( $F=0.05$ ,  $df=1$ ;  $P=0.812$ ) or during milking ( $F=2.67$ ,  $df=1$ ;  $P=0.104$ ) (Figure 1). Milking speed was associated with the behaviour neither during udder preparation ( $F=0.12$ ,  $df=1$ ;  $P=0.728$ ) nor at milking ( $F=0.01$ ,  $df=1$ ;  $P=0.899$ ) (Figure 2). In the case of multiparous cows, milk yield was not associated with the behaviour either during udder preparation ( $F=1.56$ ,  $df=1$ ;  $P=0.212$ ) or during milking ( $F=1.78$ ,  $df=1$ ;  $P=0.183$ ) (Figure 3). However, the behaviour

during udder preparation was associated with milking speed ( $F=9.10$ ,  $df=1$ ;  $P=0.003$ ). Milking speed was significantly higher if cows during udder preparation were calm (mean $\pm$ S.E.= $2.67\pm 0.12$  kg/min) compared to nervous cows (mean $\pm$ S.E.= $1.81\pm 0.27$  kg/min). At the same time, the behaviour during milking was not associated with milking speed ( $F=0.58$ ,  $df=1$ ;  $P=0.447$ ) (Figure 4).



Means accompanied by an \* differ at  $P<0.01$

Figure 4. Estimated marginal means and S.E. of milking speed by behaviour type recorded before and during milking for multiparous cows (N of cases = 178)

## Discussion

In the present study, temperament of primiparous and multiparous dairy cows was determined by their milking behaviour in the parlour. The dairy temperament test used (Burrow, 1997) is based on a 5-point linear scale similar to Visscher and Goddard (1995), Lewis and Hurnik (1998), Budzyńska et al. (2005) and Sewalem et al. (2010, 2011). This test is based on steps, kicks and leg movements of cows as the direct signs of discomfort or restlessness during milking routines (Wenzel et al., 2003; Hagen et al., 2004).

The dairy temperament test is based on a subjective scoring system; however, the results of an extra test done on 29 Holstein Friesian cows during pre-milking udder preparation confirmed a close correlation between temperament scores of three different observers ( $r=0.735$ ;  $P<0.01$ ). A previous study (Lewis and Hurnik, 1998) reported a slightly lower association ( $r=0.661$ ;  $P<0.05$ ) between Holstein dairy cows' milking temperament scores recorded by two attendants. These results suggest that the dairy temperament point scale can be easy to learn for every observer; however, the lower value also refers that nervous behaviour in the parlour might be less consistent, and therefore more difficult to detect on a one-day observation (Lewis and Hurnik, 1998).

Based on the temperament scoring scale in our investigation, the number of cases, in which Holstein Friesian cows had poor temperament during udder preparation and milking was low. This finding has been supported by most of the previous studies. Using a 4-point scale (1: docile, 4: aggressive) 85–90% of cows were ranked by Khanna and Sharma (1988) in calm classes, while the prevalence of excitable behaviour was only 2 to 4%. Budzyńska et al. (2005) found that 91.6% of cows were classified as quiet animals (1–3 temperament scores) and excitable behaviour (4–5 temperament scores) was shown only by 8.4% of cows. 89.15% of Holstein cows had desired temperament in the investigation of Sewalem et al. (2010). It appears that Holstein Friesian cows are generally calm, probably due to their long selection in milk production.

The stepping behaviour of primiparous cows did not differ before and during milking. However, multiparous cows were a little more nervous at milking than during udder preparation. They probably felt uncomfortable in this milking system owing to the distended udder resulting from the high level of daily milk yield. Kovács et al. (2013) found no difference in heart rate of Holstein cows between the different phases of milking routines in a milking parlour (udder preparation, milking and waiting in the milking stall after milking). Corresponding to Szentléleki et al. (2008), in the present study the behaviour before milking was weakly correlated with the milking behaviour by Spearman rank correlation coefficient, which suggests that these are not the same traits, but there is some positive association between them. It can be supposed that cows with their milking temperament respond with different intensity to different kind of stimulus during udder preparation and milking. The milking stages are most likely not to stimulate the animals to the same extent. Cows' behaviour at udder preparation might express their reaction to handling by human or udder sensitivity from mastitis. On the other hand, behaviour of cows at milking might reflect their direct reactions towards the machine milking process. More studies (Rushen et al., 1999; Hagen et al., 2005) stated that generally, there is no considerable stress on the basis of heart rate in conventional milking parlours during milking, however handling and technological processes around milking can generate stress for cows (Kovács et al., 2013). Hopster et al. (2002) ascertained that cows could be stressed by milking due to human-animal interaction. Cows handled aversively during milking had more leg movements during udder preparation, so they were harder to handle. Hagen et al. (2004) found that kicking was the most frequent between the beginning of udder cleaning and the cluster attachment in a herringbone milking parlour. Kicking behaviour during milking reduces milking efficiency, increases the total milking duration, and it is a source of injuries for milker (Rushen et al., 1999; Sewalem et al., 2011).

Parity can impact the milking temperament of cattle as well. In the present study, multiparous cows were calmer while preparing udder by milker than primiparous ones. However, during milking the cows of different parities behaved similarly, which means that multiparous cows were as excited as the primiparous ones. Regarding the entire milking routine, Kovács et al. (2013) showed by data of heart rate variability that multiparous cows had lower levels of stress than primiparous ones. These findings may refer that primiparous cows have not got enough time to get suf-

ficient experience in milking routine. Consequently, they react more susceptible to any stimulus of milking stages than multiparous animals. As for multiparous cows, their behavioural reaction during milking might be also influenced by the level of milk production. Some studies reported that cows in older age become calmer during milking process. Roy and Nagpaul (1984) found that dairy cows in the sixth parity had the best temperament. This result is supported by Khanna and Sharma (1988), who showed that temperament of indigenous cows tended to become milder beyond fourth lactation. It can be supposed that cows with higher parity have habituated to milking routines and technology, in addition to having a slightly reduced daily milk yield, so they are not stressed in the familiar milking technology.

One of the milk production traits was associated with milking temperament of cows, which could be shown in the case of multiparous cows in our investigation. Milking temperament during premilking udder preparation was related to milking speed, which indicates that if cows are nervous at premilking, they let down milk slower. When cows are aversively handled during milking, they let down milk more slowly and the residual milk might also increase as a result of stress caused by fear of milker, especially in animals being more susceptible to stress (Rushen et al., 1999; Munksgaard et al., 2001). These are disadvantageous for both dairy farmers by increasing the milking time and dairy cows by suffering from poor welfare. Sutherland et al. (2012) found that milk yield differed by multiparous cows' temperament categorised based on their exit times from the crush, but only when cows were exposed to a novel milking environment. Low responding cows with exit time of more than 4 s had higher milk yield than high responder cows with exit time of less than 2 s. It has to be added that there were no differences in flinch, step and kick behaviour recorded during attachment of the milking cluster between low and high responders in both familiar and novel milking environment. This suggests that the exhibition of the innate temperament of animals is greatly influenced by their milking environment. In a well-known environment they had learned the milking routine, so they are not stressed by this. However, when animals face novel environment, they react with different intensity to the novelty by their innate temperament. Some previous studies had definitely proven the association of temperament with milk production traits in different breeds. Gupta and Mishra (1978) reported that cows with nervous temperament had less milk yield and lower milking speed compared with cows having calm temperament. In another investigation, one of the calmest breeds (Karan Fries) was determined to have the highest milking speed and daily milk yield in contrast to an anxious breed (Murrah buffalo), which had the lowest values of the observed traits (Roy and Nagpaul, 1984). Correlations of milking temperament with milk production traits are also known, calculated by Lawstuen et al. (1988) and Sewalem et al. (2011). Loose associations (0.24–0.36) were found between temperament and milking speed.

Nevertheless, in the case of primiparous cows the association between milking temperament and milk production traits was not observed in the present study, similarly to Van Reenen et al. (2002), who revealed that young cows' behaviour recorded during milking was unrelated to ease of milk removal. Primiparous cows are strongly affected by a lot of environmental and psychological factors in the first lactation.

During this period they have to habituate to the entire milking routine, which probably implicates a change in milking temperament, while the milk production makes a normal lactation curve. In contrast to this, multiparous cows had known the procedure and technology, so they do not have a habituation period in their lactation. Consequently, the difference in cows' milking temperament indicating their individual direct response to environmental stimulus during milking could appear in milk let-down. On the other hand, these results can be caused by the very skewed distribution of temperament score in our study.

In conclusion, this study showed that milking temperament is related to milking speed. This was particularly true of multiparous cows where calm cows during udder preparation let down milk faster. Primiparous and multiparous animals can react to different stimulus in the different milking phases. Primiparous cows are more stressed than multiparous ones during pre-milking process, because it takes time for dairy cows to habituate to the milking routines and technology. All of these emphasized that dairy cows should be milked in a quiet and capable way in order to avoid enhanced stress, extended milking duration and difficult handling through improved milk let-down. The low prevalence of nervous cows in our study indicates that applying a temperament point scale with greater sensibility (e.g. on scale 1–10) might result in better distinction of animals' milking temperament. Further experiments are needed to repeat the temperament test along with sensor measuring physiology parameters of cow (e.g. heart rate, heart rate variability). Better understanding of milking temperament of cows allows us to decrease the effect of stressors, to increase the efficiency of milking as well as the level of animal welfare, and finally to provide better criteria for genetic selection.

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Received: 28 I 2014

Accepted: 18 VII 2014