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Short communication: Heart rate variability, step, and rumination behavior of dairy cows milked in a rotary milking system

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ABSTRACT

Step behavior, heart rate (HR), the high-frequency (HF) component of heart rate variability (HRV), the ratio of the low-frequency (LF) and HF components (LF/HF ratio) as well as rumination behavior during milking were investigated in dairy cows milked in a high-capacity rotary milking system (n = 49) to study animals' stress responses to the milking process. Cardiac parameters were analyzed for undisturbed standing (baseline) and for the stages of the morning, afternoon, and evening milking processes (i.e., driving animals from the barn to the pre-milking holding pen, pre-milking holding pen, preparation, milking, and waiting after milking in the milking stall). During driving, HR was greater than during all other stages. After driving, a gradual decrease in HR was observed. The HF decreased during driving, indicating a decrease in vagal tone compared with baseline. When animals were in the holding pen, vagal tone decreased, whereas sympathetic tone increased with lower values than recorded for baseline and driving. During preparation, HF values were still lower than those recorded for baseline. The recovery of the autonomic activity was observed following preparation as indicated by increased HF and decreased LF/HF ratio during milking and waiting stages. During milking, 53.1% of the animals ruminated. The frequency of steps was greater during preparation (3.7 ± 1.8 steps/min) than during milking (0.7 ± 0.4 steps/min) and waiting after milking (1.6 ± 1.0 steps/min). Our results suggest that being in the holding pen is stressful for cows; however, vagal predominance from the onset of milking, the low frequency of steps, and the high prevalence of rumination during milking suggest a possible welfare benefit of the investigated rotary milking system.

Key words: rotary milking parlor, heart rate variability, step behavior, rumination behavior, dairy cow

Short Communication

In recent decades it has been shown that the effects of milking technology on bovine welfare can be assessed using the variability in consecutive interbeat intervals (**IBI**) [i.e., heart rate variability (**HRV**) (Kovács et al., 2014)]. Changes in heart rate (**HR**) and HRV have been used in dairy cows to assess stress in large cow-capacity parlors with stationary milking stalls (Kovács et al., 2013; Kézér et al., 2015) or to compare totally and semi-automatic milking systems in terms of animal welfare (Hagen et al., 2005; Gygax et al., 2008). Introducing automatic milking systems is an option for dairy farmers having herds with 70 to 100 lactating cows; however, in large-scale dairies mostly conventional milking parlors are in operation. Even though recent research has focused on parlor performance in rotary milking systems (**RMS**) by the evaluation of cow throughput (Nitzan et al., 2006; Edwards et al., 2012, 2013), information is lacking on the behavioral and physiological aspects of RMS.

To fill this gap in the literature, we investigated step and rumination behavior, HR, and power spectral components of HRV in dairy cows milked in a conventional RMS. Due to the reduced milking time compared with side-opening design conventional milking systems (Nitzan et al., 2006) and the continuous visual contact between cows during milking, we hypothesized that animals milked in the RMS may show lower stress reactions compared with those found by earlier studies investigating parallel or herringbone milking parlors with stationary milking stalls and side-opening design.

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All procedures involving animals were approved by the Pest County Government Office, Department of Animal Health (permit number: PE/EA/1973–6/2016; Hungary). Measurements were carried out in a large-scale farm in Hungary (45°47′15.9″N, 18°25′56.4″E) with a herd of more than 1,900 lactating cows in October 2016. Forty-nine mid-lactation multiparous Holstein cows were selected for the study from the clinically healthy animals with optimal BCS (means ± SD; 2.6 ± 0.1) for their age, DIM, and milk production (means ± SD; age = 5.2 ± 0.9 yr; parity = 2.8 ± 0.6; DIM = 142.3 ± 12.5; milk yield = 45.8 ± 7.2 kg/d).

Animals were adapted to the 72-stall RMS operated on the farm (BouMatic Excaliber 360, BouMatic, Madison, WI). Milkings took place between 0430 and 0630 h, between 1230 and 1430 h, and between 2030 and 2230 h after the cows being driven by the herdsperson from the barns to the pre-milking holding pen. The milking parlor had one entrance lane and cows left the parlor through one exit lane and used a "face-in" configuration of the rotary platform. When the cow had completed milking, she first had an opportunity to back off the platform of her own accord; if this did not happen, then a "cow motivator" gently encouraged the cow to back off the platform. Four operators worked: one for pre-milking teat cleaning, one for unit attachment, one for application of postmilking teat dip, and one for tending to any problems occurring while cows were traveling around. Automatic teat cup detachment was used.

Animals were housed in 2 freestall barns with 1,000 cows each. Cows were kept in 4 pens/barn. Group size was 235 animals/pen with a space allowance of 6.2 m^2/cow calculated for the whole barn area including feeding space. Cubicles were bedded with sand. Cows were fed a TMR once a day at 0900 h and water was available ad libitum.

Cow behaviors performed after entering the milking parlor were recorded by the same 4 observers on each experimental day. Step behavior was defined and recorded, whereas stepping frequency was calculated according to Wenzel et al. (2003) for preparation, milking, and waiting stages. Rumination during milking was considered if the cow ruminated a minimum of 70% of the time spent in the milking stall. It was recorded by 4 observers who situated over the rotary parlor.

Heart rate was recorded using a Polar Equine T56H mobile recording system (Polar, Kempele, Finland), as described by Kovács et al. (2016) over one day from each animal, for 6 main stages of the morning, afternoon, and evening milkings (Table 1). Four portable video cameras (Legria HF M36, Canon, Tokyo, Japan) were installed in the milking parlor allowing subsequent matching of the stages of the milking process and the IBI recordings. Start and end points of pre-milking stages (i.e., driving and holding pen) were recorded based on direct human observations. If the holding pen stage exceeded 15 min, it was subdivided into three 5-min substages, where the first sample covered the first and the third the last 5 min of the time spent in the holding pen.

The Kubios HRV software (version 2.2, Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland) was used to analyze IBI. Baseline data (2 IBI samples) for a given milking were collected within 1 h before the milking process was started, based on direct human observation. Artifacts were corrected as described by Kovács et al. (2016). In each time domain, HR was quantified. For computing frequency-domain HRV, IBI data were subjected to fast Fourier transformation of power spectrum analysis (Akselrod et al., 1981). Equal length of 5-min IBI samples were used to quantify the normalized power of the high-frequency (**HF**) band and the quotient of

Table 1. The investigated main stages of the measurement

Stage number	Stage description	Definition	No. of samples/substages
1	Baseline[1]	The cow is standing at any site of the barn without any disturbance from her herdmates; the cow finished feeding or walking by 5 min before the start of recording.	2
2	Driving	Time lag between letting out of the stable's gate and entering the pre-milking holding pen	1
3	Pre-milking holding pen	Time lag between entering the holding pen and stepping into the milking parlor	3
4	Preparation	Admission + udder preparation Admission: time lag between entering the milking stall and beginning of udder preparation Udder preparation: first contact between animal and milker until attachment of all teat cups	1
5	Milking	Time lag between the attachment and the removal of the last teat cup	1
6	Waiting after milking	Time lag between the removal of the last teat cup and stepping out of the milking stall with all 4 legs	1

[1]Baseline data were collected within 1 h before the start of the actual (morning, afternoon, or evening) milking process based on direct human observation.

the low-frequency (**LF**) and HF components (**LF/HF ratio**). The HF component reflects the vagal modulation of the heart, whereas the LF/HF ratio provides information on the sympathovagal balance in dairy cattle (Kovács et al., 2014). Recommendations of von Borell et al. (2007) were considered by setting the limits of spectral components (LF: 0.05–0.20 Hz, HF: 0.20–0.58 Hz).

All analyses were carried out by R 3.4.1. statistical software (R Development Core Team, 2017). A general linear mixed model (package nlme in R) was fit to the data (Pinheiro and Bates, 2000) with random effects for each cow. The fixed effects were the stages of the measurement, time of milking (morning, afternoon, evening), the duration of driving, the time spent in the pre-milking holding pen, parity, BCS, milk yield of the actual milking, frequency of steps during the stages of preparation, milking and waiting after milking, and the presence or absence of rumination. Heart rate, HF, and LF/HF ratio were inserted into the models as response variables. Log-transformation of HR, HF, and LF/HF ratio was applied to satisfy the normality and variance homogeneity assumptions of the models. For multiple comparisons, the Tukey-Kramer correction was used at the significance level of $P < 0.05$.

Differences in stepping frequencies performed during preparation, milking, and waiting phases were calculated by a general linear model. The fixed effects were the time of milking, the time spent in the pre-milking holding pen, parity, BCS, milk yield of the actual milking and the stages of milking (preparation, milking, waiting after milking). Cows served as random factors and square-root transformation of the stepping rate was the outcome variable. The significance level was set at $P < 0.05$.

The time of milking, the time spent in the pre-milking holding pen, parity, BCS, and the milk yield of the actual milking did not affect stepping frequency. Stepping occurred more often during the stage of preparation (3.7 ± 1.8 steps/min) compared with milking and waiting (0.7 ± 0.4 and 1.6 ± 1.0 steps/min, $P < 0.001$ for both comparisons), whereas stepping frequency did not differ between milking and waiting ($P = 0.235$). Previous studies observed lower frequency of steps during the stage of preparation (Wenzel et al., 2003; Gygax et al., 2008); however, the slightly increased stepping rate found in this study was probably associated with the adjustment of the animals to the rotary platform, not with stress. Hagen et al. (2005) observed a greater stepping frequency, whereas others reported similar stepping frequencies during milking compared with our findings in small capacity (4–12 cows) parallel and tandem parlors (Wenzel et al., 2003; Gygax et al., 2008).

Cardiac parameters recorded for preparation, milking, and waiting stages were not influenced by the time of milking (morning/afternoon/evening), parity, BCS, the frequency of steps or the presence or absence of rumination, which was 53.1% in the study population. It was more surprising that the duration of driving and the time spent in the holding pen also had no effect on HRV recorded for the milking stages.

Heart rate, HF, and LF/HF ratio did not differ between the 2 baseline recordings (undisturbed standing). During the stage of driving, HR increased from baseline (Figure 1a), reaching greater values compared with all other stages ($P < 0.001$ for all comparisons). After driving, a gradual decrease in HR was observed but it remained greater compared with baseline while cows were in the pre-milking holding pen ($P < 0.01$). Heart rate increased again during preparation compared with baseline ($P < 0.001$) and was greater than recorded for the last 5 min of the holding pen ($P = 0.025$). Increased HR in the pre-milking holding pen, and elevated values even during preparation, milking, and waiting after milking are supposed to reflect the prolonged effect of increased physical activity of animals during driving. It is difficult to differentiate whether HR increase correlates with stress or rather with anticipation since positive and reverse emotions may lead to similar changes in HR (von Borell et al., 2007). Heart rate showed a gradual decrease from milking ($P = 0.030$ compared with preparation) being still greater than baseline during the stage of waiting in the milking stall ($P = 0.005$).

Cows showed decreased HF and increased LF/HF ratio (Figure 1b and 1c) compared with baseline with the onset of driving ($P < 0.001$ for both parameters). When animals were in the holding pen, HF decreased onwards with lower values than measured for baseline ($P < 0.001$ for all substages) and driving ($P = 0.020$, $P = 0.017$, and $P = 0.021$ for holding pen 1, 2, and 3), whereas LF/HF ratio increased for the 3 stages of the holding pen ($P = 0.032$, $P = 0.030$, and $P = 0.034$, compared with driving). Cardiac autonomic activity did not change during the substages of the holding pen as reflected by HF and LF/HF ratio. The low vagal and high sympathetic tone recorded for the 3 substages of the holding pen support that pre-milking parlors can be stressful locations due to the reduced social space and crowding (Silva and Passini, 2017). During preparation, HF was still lower than baseline ($P < 0.001$) and LF/HF ratio reached its peak ($P < 0.001$ compared with the last 5 min of the holding pen). As no change in HF was found during preparation and neither HR nor LF/HF ratio were influenced by the frequency of steps, increased sympathetic tone during preparation was probably associated with the anticipation for milking

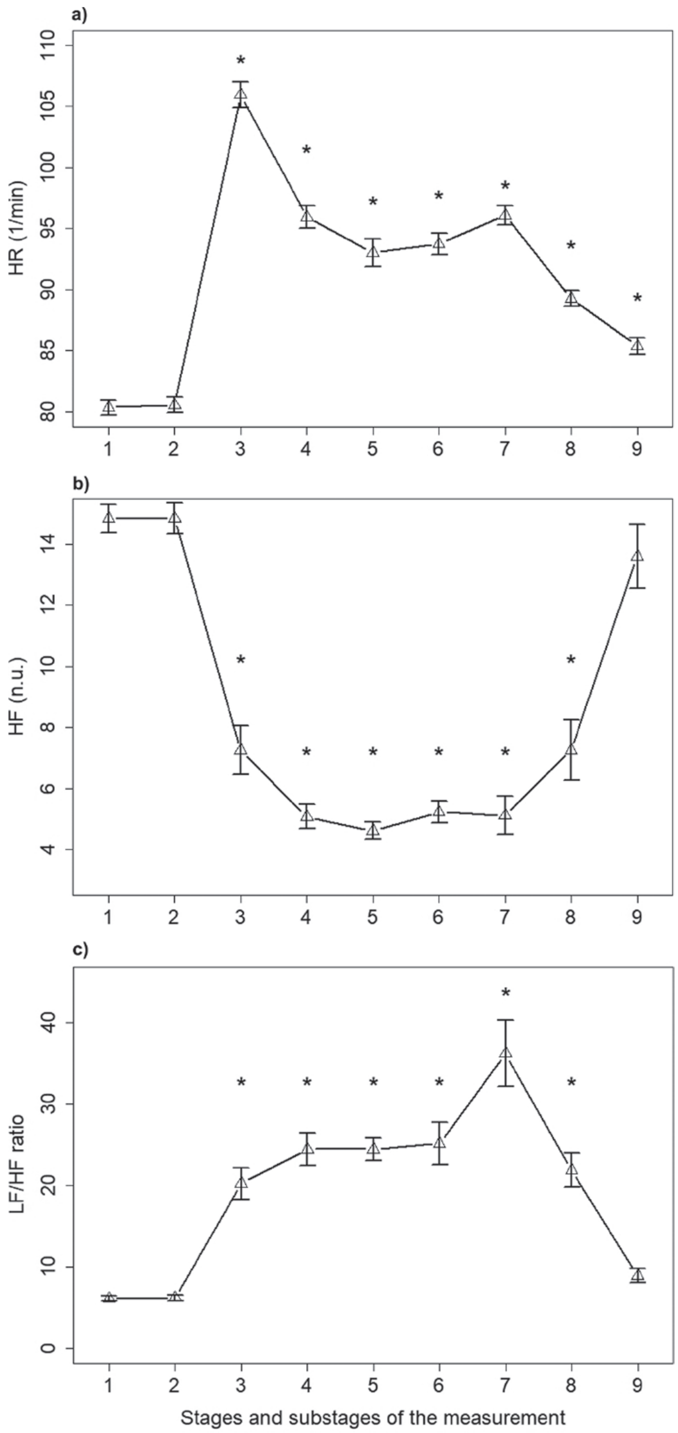

Figure 1. Changes in (a) heart rate (HR), (b) the high-frequency component (HF) of heart rate variability, and (c) the ratio between the low-frequency (LF) and the HF component of heart rate variability (LF/HF ratio) of dairy cows during the milking process in the rotary milking system. Stages and substages of the measurement: 1 and 2 = baseline 1 and 2; 3 = driving; 4 = holding pen/1; 5 = holding pen/2; 6 = holding pen/3 (where /1, /2, and /3 are the first, second, and third selected 5-min periods of the time spent in the holding pen, respectively); 7 = preparation; 8 = milking; 9 = waiting after milking in the milking stall. n. u. = normalized unit. Differences from baseline: $^*P < 0.05$. Error bars indicate SEM.

rather than physical activity or stress. The recovery of the autonomic nervous system activity was indicated by an increase in HF and a decrease in LF/HF ratio during milking from preparation ($P < 0.001$ for both parameters) then both returned to baseline during the stage of waiting. This phenomenon was possibly associated with oxytocin release inducing an increase in vagal nerve activity (Uvnäs-Moberg and Petersson, 2005).

Contrary to the present findings, earlier studies observed reduced vagal tone after milking in parallel (Kovács et al., 2013) and in herringbone milking parlors (Kézér et al., 2015) suggesting that waiting before being released from stationary milking stalls is stressful for the cows in parlors with a side-open design. The low frequency of steps and the high prevalence of rumination in the milking stall in our study may also suggest a high cow comfort during waiting that could be explained by cow exit. Although the exit from the rotary platform was also nonvoluntary in the RMS, the time of the exit was predictable for the cows milked since animals leaved the platform at the end of the "round" if milking was completed. Stall arrangement of the investigated RMS allowed continuous visual contact between cows that might also have lowered the level of stress during the milking and waiting stages. Cattle are motivated to maintain visual contact with each other, especially in restraint situations such as milking (Ewbank, 1968). Moreover, some cows did not leave the platform when milking was completed (i.e., "go-around" cows), which also supports cow comfort.

In summary, vagal predominance, the low frequency of steps during milking and waiting stages, and the high prevalence of rumination in the milking stall suggest that the studied RMS might be less stressful during the stages of milking and waiting than the previously investigated milking systems with side-opening design and stationary milking stalls (Kovács et al., 2013; Kézér et al., 2015). Although autonomic activity did not change with increasing time spent in the pre-milking holding pen, being in the holding pen seemed to be a sensitive period for the animals. Therefore, stress experienced by cows in the holding pen should be decreased by maximizing cow comfort (e.g., use crowd gates and move animals with care).

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