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Short communication: Changes in heart rate variability of dairy cows during conventional milking with nonvoluntary exit

L. Kovács,*[1] J. Tőzsér,* M. Bakony,† and V. Jurkovich‡
*Institute of Animal Husbandry, Faculty of Agricultural and Environmental Science, Szent István University, Páter K. utca 1, Gödöllő 2103, Hungary
†Rumino-Vet Bt, Csillés utca 2, Érd 2030, Hungary
‡Department of Animal Hygiene, Herd Health and Veterinary Ethology, Faculty of Veterinary Science, Szent István University, István utca 2, Budapest 1078, Hungary

ABSTRACT

Heart rate variability (HRV), as a physiological measure of animal welfare, was investigated in 36 cows milked in a parallel milking parlor with nonvoluntary exit. Heart rate variability parameters measured during the morning resting (baseline period) were compared with those measured during different stages of the entire milking process. No differences were found in HRV parameters between the baseline period, preparation, and main milking. A considerable reduction in vagal activity was detected during the movement of the cows to the milking parlor (driving) and while cows were in the holding area. The parasympathetic measures of HRV decreased whereas the sympatho-vagal balance increased compared with baseline. The same pattern was observed regarding the stage between removing the teat cups and leaving the milking parlor (waiting). No differences in any sympathetic measures were observed between the baseline period and any of the milking stages. These findings indicate that the milking process itself (preparation and main milking) is not stressful for cows. Decreased parasympathetic activity during driving might be the result of the physical activity of the cows, whereas waiting in the holding area and in the milking stall after milking caused stress for animals.
Key words: heart rate variability, dairy cow, milking stage, stress

Short Communication

In intensive cattle farming, it is of particular interest to identify physiological indicators that describe the animal's responsiveness to stressors. Besides hypothalamus–pituitary–adrenal axis measures and heart rate measurements, heart rate variability (**HRV**) is an alternative measure that has been used recently for the evaluation of stress responses in dairy cows (Konold et al., 2011; Kovács et al., 2012b). Heart rate variability is defined as the oscillation in the length of the time interval between consecutive heart beats (interbeat interval, **IBI**), which are nonuniformly separated over time.

Stressful stimuli are known to cause a decrease in parasympathetic activity (Porges, 2003) and changes in the sympatho-vagal balance (von Borell et al., 2007). With certain parameters of HRV, the activity of the sympathetic (**SNS**) and parasympathetic (**PNS**) nervous system can be monitored separately (ESC-NASPE Task Force, 1996). The effects of technological factors on the autonomic nervous system (**ANS**) have been reported in dairy cattle using parasympathetic and sympatho-vagal indices of HRV (Mohr et al., 2002; Hagen et al., 2005; Kovács et al., 2012b). Alterations in HRV measures sensitive to PNS modulation or representing sympatho-vagal balance allow a detailed interpretation of stress in different situations of dairy management.

Several methods have been proposed for the assessment of HRV as reviewed by von Borell et al. (2007) in farm animals and by Kovács et al. (2012a) in dairy cattle, specifically. Power spectral analysis of HRV helps to identify components that characterize the sympatho-vagal balance. The low frequency component (**LF**) depends on the baroreceptor modulation of both vagal and sympathetic impulses, and has been used as an indicator of stress in dairy calves (Stewart et al., 2008) and cows (Mohr et al., 2002; Hagen et al., 2005; Konold et al., 2011).

The high frequency (**HF**) component reflects the vagal control of the heart (Akselrod et al., 1985) and has been used in studies involving dairy cows (Hagen et al., 2005; Konold et al., 2011). In itself, measuring the heart rate does not allow us to distinguish the effects of the individual branches of the ANS (Hainsworth, 1995), because an increase in heart rate may originate from reduced vagal or increased sympathetic activity, or possibly from simultaneous changes in the activity of both branches (von Borell et al., 2007). Nonlinear Poincaré measures and time domain parameters of

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[1]Corresponding author: kovacs.levente@mkk.szie.hu

HRV—especially root mean square of successive IBI differences (**RMSSD**)—have recently been used in dairy cattle studies (Minero et al., 2001; Kovács et al., 2012b) and found to be successful in assessing the vagal regulation of cardiac dynamics, which plays a key role in response to stress (von Borell et al., 2007).

Heart rate variability has been used in welfare research to gain information about the stress dairy cows might experience during milking. Gygax et al. (2008) found higher levels of stress (lower parasympathetic tone during milking compared with resting) in cows milked in an automatic milking system (**AMS**) than in cows milked in an auto-tandem milking parlor, whereas others noted lower stress levels in an AMS than in a herringbone milking parlor (Hagen et al., 2005). No research has been done in evaluating stress-related changes of HRV in parallel milking parlors with nonvoluntary exit (a commonly used system in central Europe) during each of the stages involved in the whole process of milking. Thus, the objective of the present study was to investigate the changes of HRV in dairy cows during milking in a conventional system with nonvoluntary exit.

Thirty-six (primiparous, n = 18 and multiparous, n = 18) lactating Holstein-Friesian cows (mean ± SD; parity = 2.42 ± 1.12 lactation; daily milk production = 38 ± 6.2 kg; DIM = 150 ± 10 d) were selected from clinically healthy animals (mean ± SD; BCS = 2.48 ± 0.32, locomotion score: 1.52 ± 0.36) for this study. Cows were housed in modern freestall barns with individual stalls bedded with straw in a large dairy farm in Jászapáti, Hungary. Cows were fed TMR twice a day at 0700 and 1600 h and had ad libitum access to water. Cows were milked 3 times a day in a 2 × 24 Bou-Matic parallel milking system (Bou-Matic, Madison, WI) at approximately 0400, 1200, and 1900 h. The experiment was carried out in May 2012 during a 2-wk period (each day from 0630 to 2130 h) under normal weather conditions (mean ± SD; temperature: 16.4 ± 6.7°C).

Each cow was in the trial for 3 consecutive days. The trial was split into 3 periods as follows: (1) habituation: the heart rate monitoring systems that stored IBI for about 15 h continuously (Polar Equine RS800 CX, Polar Electro Oy, Helsinki, Finland) were attached to the cows by using a specially designed girth on d 1; and the cows were given a 1-h habituation period before starting the experiment (starting time: 0730 h); (2) baseline: from d 1 to 3, each morning from 0830 to 1130 h, baseline HRV data were recorded during lying (resting bouts); and (3) milking process: from d 1 to 3, HRV data were collected twice a day during the midday and evening milkings for each of the milking process stages (Table 1). Two video cameras (Legria HF M36, Canon, Tokyo, Japan) were installed, one in the holding area and one in the parlor to help match milking stages and HRV recordings. Information on the length of milking stages was collected based on the video time signal. Focal cows were identified by the numbers on their hind legs and backs drawn on at the time of fixing the girths.

Heart rate variability parameters during lying (baseline data) were recorded by direct observation in 5-min time windows, following recommendations of earlier studies (Mohr et al., 2002; Hagen et al., 2005). Moments of lying down, standing up, and any kind of disturbance (e.g., sudden noise, handler walking close by) were perceivable in the heart rate data; thus, undisturbed bouts (2–5/cow per day) could be evaluated, starting from 5 min after the cow had lain down. Because it is advised to use data bouts of the same length for the comparison of HRV measures (ESC-NASPE Task Force, 1996), the first 5 min of recordings during each milking stage were used, following the practice of Gygax et al. (2008). In case of stages exceeding 10 min in length (e.g., driving and being in the holding pen), the last 5 min of recordings were also included in analysis. Because of the large number of animals being milked simultaneously (2 × 24), preparation and waiting times were, in most cases, sufficiently long for reliable HRV analysis.

The IBI data were imported into the Kubios HRV 2.0 software (Niskanen et al., 2004) for artifact inspection and HRV analysis. The errors in IBI data during resting and milking bouts were corrected using the algorithm provided by the software.

A total of 4 to 6 milkings per cow were analyzed in the course of the experiment. Data loss was encountered because of technical problems (e.g., damaged belts, dried out electrodes, or flat batteries in heart rate receivers).

We calculated parameters of HRV in time and frequency domains and by nonlinear methods (Table 2). For frequency-domain analysis, a power spectrum method developed by Akselrod et al. (1981) was used based on fast Fourier transformation. Recommendations of von Borell et al. (2007) were considered by setting the limits of the spectral components as follows: LF: 0.05–0.20 Hz, and HF: 0.20–0.58 Hz, which were calculated in normalized units ($\mathbf{LF_{norm}}$ and $\mathbf{HF_{norm}}$). Poincaré plots were calculated according to the methodology described by Minero et al. (2001).

A generalized linear model (SPSS 18, SPSS Inc., Chicago, IL) was used to determine the effects of milking stages (factors), parity, and milk production (possible confounding effects) on HRV parameters (response variables). Data for a given cow were compared with the baseline data of the same cow. To account for the repeated milkings of the cows, an individual cow was included as a random effect in the model. The residuals