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Effect of physical activity on cardiac autonomic function of dairy cows on commercial dairy farms

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Interbeat interval data were collected from 219 Holstein cows in 2 smaller-scale farms and 3 larger-scale farms to investigate the effects of posture (standing vs. lying), rumination (rumination vs. no rumination) and feeding on baseline values of heart rate (HR) and heart rate variability (HRV) parameters reflecting vagal and sympathetic activity. A General Linear Model was used for detecting factors (parity, milking technology, herd size) having possible effects on HRV calculated for undisturbed lying posture. Basal values of cardiac parameters were also compared between larger and smaller-scale farms. Neither parity nor milking technology affected HRV parameters. Sympathetic activity increased in the order of lying, ruminating when lying, standing, ruminating when standing and feeding on both sizes of farms. Vagal activity decreased in that order in both lower- and larger-scale farms. Rumination caused an increase in HR and a decrease in vagal tone in both lower- and larger-scale farms and an increase in sympathetic activity during lying in both farm sizes. Basal vagal activity was lower in larger-scale farms compared to smaller-scale farms, while greater sympathetic activity was found in cows housed on larger-scale farms. Our findings demonstrate that reference values of HRV parameters in lactating dairy cows cannot be generally defined for Holstein cattle as they are affected by physical activity and herd size. Higher HR and sympathetic activity at rest in larger-scale farms compared to farms with lower cow population might be associated with higher levels of social stress and therefore should be considered as a potential welfare concern.

Keywords: Cardiac autonomic activity, heart rate variability, physical activity, dairy cows.

According to the task force on the application of the analysis of heart rate variability (HRV) in human medicine (ESC-NASPE Task Force, 1996) and reviews on farm animals (von Borell et al. 2007), parameters of HRV, i.e. the short-term fluctuations in the length of successive cardiac interbeat intervals (IBI) reflect on the dynamic changes of autonomic nervous system (ANS) activity. Heart rate (HR) and parasympathetic nervous system (PNS) indices of cardiac function are reliable measures of animal welfare in dairy cattle (Kovács et al. 2014a). The root mean square of successive differences (RMSSD) between IBIs and the high frequency (HF) component of HRV are used to detect tendencies in PNS activity. Poincaré measure standard deviation 1 (SD1) also represents vagal tone (Tarvainen et al. 2014). The relative power of the low frequency (LF) component and HF (LF/HF ratio) gives information about sympathetic nervous system (SNS) activity and the SNS/PNS balance (van Ravenswaaij-Arts et al. 1993). Earlier HRV studies on cattle reported on the effects of pathological conditions on cardiac function, while current works are available on the physiological and behavioural aspects of pain evoking husbandry procedures (Kovács et al. 2014b). However, no information is available on the effects of common activities (rumination, feeding) on HRV indices in dairy cattle. The lack of data can make interpretation and comparison of results of welfare studies somewhat difficult.

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The aim of our study was the characterisation of cows' ANS function under field conditions by the evaluation of HRV parameters that are extensively used in bovine stress research. We hypothesised that increased physical activity caused by rumination and feeding would have an influence on cardiac function of dairy cows. Considering that earlier studies on short-term physiological and behavioural responses of animals in relation with breed and milking systems (Hagen et al. 2005), milking technology (Kézér et al. 2015), or the effects of responsiveness towards humans (Sutherland et al. 2012; Frondelius et al. 2015; Kovács et al. 2015a) were carried out on small-scale farms, we decided to measure HRV of Holstein cows either on larger- and smaller-scale commercial dairies differing in management, milking technology and production to provide valuable information not only for research but also for professionals working in dairy husbandry. We further hypothesised that herd size can have an effect on HRV parameters, namely that baseline sympathetic activity is higher on large-scale farms (with increased stocking density) compared to farms with a lower cow population.

Materials and methods

Farms, animals and housing

Measurements were carried out on five commercial dairy farms in Hungary on multiparous Holstein Friesian cows. Two farms were of smaller-scale and medium production (Farms 1 and 2) and three were larger-scale intensive production farms (Farms 3, 4 and 5). Cow population, housing conditions and feeding regime of the farms involved in our study are summarised in Supplementary Table S1. Focal animals were selected from clinically healthy cows. Cows that were in oestrus were not involved in the study. Averages for focal animals were similar in age parity, days in milk and body condition score for all farms (Supplementary Table S2). The body condition of the animals was scored using the 5-point scoring system (Hady et al. 1994). In order to avoid any effects of high ambient temperature on cow's cardiac activity, measurements were made in autumn 2013. The study was ethically approved by the Department of Epidemiology and Animal Protection of the Directorate of Food Chain Safety and Animal Health at Central Agricultural Office (Permit Number: 22.1/1266/3/2010).

Preparation of IBI data collection

IBIs were recorded using a mobile recording system, which included a Polar Equine T56H transmitter with two electrodes, a Polar H2 heart rate sensor and a Polar RS800 CX heart rate monitor (POLAR, Kempele, Finland). After soaking the body surface under the electrodes with tap water, transmitters and the electrodes were positioned on the thoracic region, one electrode on the cardiac area, and one over the right scapula, as advised by von Borell et al. (2007) in their review. Electrode sites were covered with ultrasound transmission gel (AquaUltra Blue; MedGel Medical, Barcelona, Spain) without shaving the skin.

On each farm, devices were fitted to cows after the morning milking (between 8·00 and 8·45 according to the milking schedule of the different farms). IBI recording started after a 2-h acclimatisation period and lasted until returning from the evening milking (between 19·30 and 21·00 depending on farm).

Behavioural observations

In parallel with IBI recordings, behaviour was continuously recorded by visual observation and classified with regard to posture (lying, standing) and with regard to activity (ruminating, feeding). The following levels of physical activity were determined: (1) lying, (2) lying and ruminating, (3) standing, (4) standing and ruminating, (5) feeding. Criteria of lying were the following: (1) the cow is lying comfortably in the cubicle/on the pasture without any disturbance from her herd mates; (2) the cow finished feeding or walking up to 10 min before the start of data recording. Criteria of standing were the followings: (1) the cow is standing in any site of the barn/pasture without any disturbance from her herd mates; (2) the cow finished feeding or walking up to 5 min before the start of data recording. For both postures during the last 2 min before data recording and throughout the period of interest any kind of disturbances (presence of stockmen, sudden noise) were recorded.

We used the same protocol on each farm. After fixing the HR receivers, focal cows were released back to production groups. Animals were identified by the numbers on their hind legs and backs drawn on at the time of fixing the HR monitors. The cows were observed by a maximum of four persons at a time, to whom the cows were accustomed. A maximum of eight cows were observed at a time (max. 2 animals for each observer). Observers stood at least 6 m from the cows. They used watches which were synchronised with the HR receivers to register the exact starting and end points of the animal's actual behaviours or activity. Observers also recorded any disturbances occurring (e.g. a stockperson walked close by an experimental cow, interactions with group mates either as a performer or a receiver).

Processing of IBI data

The segments of IBI recordings matching the periods of uninterrupted display of the studied posture/activity were used for HRV analysis. A 2-min interval after any kind of disturbance or social interaction and a 5-min interval after changing posture were excluded from analysis. We examined periods of 5 min as recommended for analysis of HRV in earlier reviews (ESC-NASPE Task Force, 1996; von Borell et al. 2007). As cardiac activity has a circadian rhythm in animals (Piccione et al. 2005), IBI samples for each level of physical activity were chosen balanced for morning and afternoon periods for individuals on each farm. Number of IBI samples was also balanced for levels of physical activity and for farm. Longer periods of recording were subdivided into several 5-min segments.

The Kubios HRV software (version 2.2, Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland) was used for HRV analysis. Artefacts were corrected by using the custom filter, which identified IBIs differing from the previous IBI by more than 30 % as artefacts. After abnormal interval removal, the algorithm of the program substitutes detected errors with interpolated IBIs calculated from the differences between previous and next accepted IBIs. For removing slow nonstationary trend components, the 'smoothness priors' based detrending method was chosen ($\lambda = 1000$, $f_c = 0{\cdot}029$ Hz). Besides time domain measures (HR, RMSSD) for computing frequency-domain HRV, IBI data were subjected to Fast Fourier Transformation of power spectrum analysis (Akselrod et al. 1981). The HRV spectrum was calculated with the Welch's periodogram method set to 256 s overlapping segments with 50 % window overlap. The interpolation rate of IBI series was 4 Hz. Spectral parameters included the normalised power of HF and LF/HF ratio. Recommendations of von Borell et al. (2007) were considered by setting the limits of the spectral components (LF: 0·05–0·20 Hz and HF: 0·20–0·58 Hz). For graphical representation of the correlation between successive IBIs, where each interval in the time series (IBI_{i+1}) is plotted against its successor (IBI_i) SD1 was calculated by Poincaré plot analysis as described earlier (ESC-NASPE Task Force, 1996; von Borell et al. 2007).

Statistical analysis

Statistical analysis was performed using the SPSS 18 (SPSS Inc., Chicago, IL) statistical software. The analysis included two steps. At first, a General Linear Model was used for detecting factors having possible effects on baseline HR and HRV values calculated for lying posture. To avoid pseudo-replication, the averaged values of single samples collected for individuals were used for analysis. The model included cardiac parameters as dependent variables, while parity, milking technology (herringbone, parallel and rotary parlours) and herd size (smaller-scale farms, larger-scale farms) were fixed factors. Age, days in milk, body condition score and daily milk yield were entered into the model as covariates, with farms and experimental days as random factors. The evaluation of main effects was adjusted by the Bonferroni *post hoc* test. The level of significance was set at 0·05. As HR and HRV indices were affected only by herd size (see Section Results and discussion), in the next step, HRV values were compared between different levels of physical activity separately for larger and smaller-scale farms. The averaged values of single samples collected for each animal were used for analysis. Due to the non-normal distribution of data (Kolmogorov–Smirnov test) a log transformation was used before analysis of the frequency domain measures of HRV (HF and LF/HF ratio). Then the Repeated Measures ANOVA was followed for all cardiac parameters. Statistical significances between levels of physical activity were calculated with Tukey's post-hoc test for each HRV parameter at the significance level of 0·05.

Results and discussion

None of the covariates had a significant effect on HRV parameters. Parity had no effect on HR ($F_{1,1278} = 2{\cdot}1$, $P = 0{\cdot}120$) and basal ANS activity (RMSSD: $F_{1,1278} = 3{\cdot}4$, $P = 0{\cdot}092$; HF: $F_{1,1278} = 2{\cdot}8$, $P = 0{\cdot}164$; LF/HF ratio: $F_{1,1278} = 2{\cdot}8$, $P = 0{\cdot}164$; SD1: $F_{1,1278} = 1{\cdot}9$, $P = 0{\cdot}235$). Similar to the present data, Minero et al. (2001) found no difference between intensively housed multiparous and primiparous cows either for RMSSD or for SD1, however, in that study the large interindividual variation due to small number of animals involved prevented the exact interpretation of results. In concordance with Hagen et al. (2005) we found no effect of milking system on cardiac activity (HR: $F_{4,1278} = 2{\cdot}3$, $P = 0{\cdot}232$; RMSSD: $F_{4,1278} = 3{\cdot}0$, $P = 0{\cdot}125$; HF: $F_{4,1278} = 2{\cdot}9$, $P = 0{\cdot}205$; LF/HF ratio: $F_{4,1278} = 1{\cdot}9$, $P = 0{\cdot}352$; SD1: $F_{4,1278} = 1{\cdot}7$, $P = 0{\cdot}425$). However, in contrast to that study all of our farms employed conventional milking parlours, with milkers familiar to the cows, not partially or fully automatic systems.

Herd size affected all HRV indices (HR: $F = 8{\cdot}2$, $P = 0{\cdot}020$; RMSSD: $F = 12{\cdot}4$, $P = 0{\cdot}006$; HF: $F_{4,1278} = 56{\cdot}8$, $P = 0{\cdot}002$; LF/HF: $F_{4,1278} = 56{\cdot}8$, $P = 0{\cdot}002$; SD1: $F_{4,1278} = 6{\cdot}9$, $P = 0{\cdot}035$). On smaller-scale farms, HR was lower compared to larger-scale farms ($67{\cdot}6 \pm 0{\cdot}5$ vs. $75{\cdot}3 \pm 0{\cdot}4$, $P = 0{\cdot}005$). Differences in RMSSD and in SD1 between smaller and larger-scale farms ($24{\cdot}7 \pm 0{\cdot}8$ vs. $15{\cdot}7 \pm 0{\cdot}6$, $P = 0{\cdot}004$ and $16{\cdot}3 \pm 0{\cdot}6$ vs. $10{\cdot}8 \pm 0{\cdot}4$, $P = 0{\cdot}002$, respectively) showed cows' greater basal PNS activity in smaller-scale farms. Correspondingly with time-domain measures, LF/HF ratio was lower ($1{\cdot}5 \pm 0{\cdot}2$ vs. $2{\cdot}5 \pm 0{\cdot}2$, $P = 0{\cdot}002$), while HF was greater ($47{\cdot}9 \pm 1{\cdot}0$ vs. $34{\cdot}6 \pm 0{\cdot}8$, $P = 0{\cdot}001$) in smaller-scale farms compared to larger-scale farms. Our results are indicative of a greater basal PNS activity parallel with a lower SNS activity in smaller scale farms than those in larger-scale farms. The differences between basal HRV parameters calculated for smaller- and larger scale farms are possibly related to space allowance for individuals. Reduced space allowance is considered a source of social stress (Kondo et al. 1989; Fregonesi & Leaver, 2002) and in our study, on Farms 4 and 5 it was less than approved standards of space allowance requirements for dairy cows for bedded lying area per cow (AWAS for Dairy Cattle and Calves, 2014). There is, however, one problem with our assumption, as we were not able to determinate the exact space allowance for individuals, as during the experiment they were housed in different barns with different cow distributions on large-scale farms. It is also possible that basal HRV was influenced by factors other than herd size, milking system or parity. In our recent studies, we found that individual traits, such as behavioural reactivity to humans or

Table 1. Cardiac parameters of dairy cows during different levels of physical activity on smaller- and larger-scale farms

	HRV parameters§ on smaller-scale farms				
	HR (min^{-1})	RMSSD (ms)	HF (n.u.)	LF/HF ratio	SD1 (ms)
Statistics†	F = 117·1, P < 0·001	F = 8·6, P < 0·001	F = 172·7, P < 0·001	F = 126·1, P < 0·001	F = 8·4, P < 0·001
Physical activity					
Lying	63·8 ± 0·8^{A}	29·5 ± 2·1^{Aa}	50·0 ± 1·5^{A}	1·4 ± 0·7^{A}	20·9 ± 1·5^{A}
Lying and ruminating	69·6 ± 0·9^{Ba}	27·1 ± 2·8^{Ab}	36·8 ± 2·0^{B}	3·3 ± 1·0^{B}	15·8 ± 2·0^{Ba}
Standing	73·0 ± 1·0^{Bb}	25·1 ± 2·3^{Ab}	26·9 ± 1·8^{C}	5·9 ± 0·9^{C}	16·2 ± 1·8^{B}
Standing and ruminating	74·4 ± 0·9^{Bb}	22·9 ± 2·6^{Ab}	25·4 ± 1·6^{C}	6·5 ± 0·8^{C}	17·7 ± 1·6^{Bb}
Feeding	82·7 ± 0·9^{C}	17·2 ± 2·4^{C}	15·6 ± 1·7^{D}	13·5 ± 0·8^{D}	12·1 ± 1·7^{C}
	HRV parameters on larger-scale farms				
	HR (min^{-1})	RMSSD (ms)	HF (n.u.)	LF/HF ratio	SD1 (ms)
Statistics	F = 54·3, P < 0·001	F = 12·8, P < 0·001	F = 141·9, P < 0·001	F = 67·5, P < 0·001	F = 12·8, P < 0·001
Physical activity					
Lying	76·9 ± 0·4^{Aa}	19·2 ± 0·8^{Aa}	31·5 ± 0·7^{A}	2·5 ± 0·6^{A}	13·7 ± 0·6^{Aa}
Lying and ruminating	81·2 ± 0·5^{ABb}	16·9 ± 1·0^{Ab}	19·2 ± 0·9^{Ba}	5·8 ± 0·9^{B}	12·1 ± 0·7^{ABb}
Standing	82·3 ± 0·6^{Bb}	15·8 ± 1·0^{Bc}	16·3 ± 1·0^{Bb}	9·0 ± 0·9^{C}	11·6 ± 0·7^{Bb}
Standing and ruminating	85·2 ± 0·6^{BCc}	12·4 ± 1·0^{C}	13·0 ± 0·9^{C}	13·0 ± 0·8Da	10·1 ± 0·6^{Bc}
Feeding	87·6 ± 0·5^{C}	8·7 ± 0·9^{D}	10·1 ± 0·7^{D}	15·1 ± 0·7^{Db}	8·8 ± 0·5^{Bd}

†Descriptive statistics are based on means (±SEM) of non-transformed data. Statistical significances for frequency domain measures (LF, HF, and the LF/HF ratio) are based on log-transformed data. F-statistics are based on the output of ANOVA. Means with different superscripts within a column are significantly different (^{abcd}P < 0·05, ^{ABCD}P < 0·01). Statistical significances between levels of physical activity are based on the Tukey's post hoc test.

§RMSSD, root mean square of successive IBI differences; HF, normalised power of the high-frequency band; LF/HF ratio, the ratio between the low frequency (LF) and the HF band; SD1, standard deviation of instantaneous IBI variability measured from axis 1 in the Poincaré plot.

temperament (Kovács et al. 2015a) as well as chronic lameness (Kovács et al. 2015b) could also have an effect on HRV parameters calculated for lying. It should be noted that temperamental animals were excluded either when visiting lower- or larger-scale farms and cows suffering from clinical health disorders were also not involved.

Based on the analysis of effects of farm- and individual-related factors on HRV, we provide fundamental data on ANS activity separately for smaller- and larger-scale farms (Table 1). We found that HR and ANS-related HRV were influenced by physical activity both on smaller- and larger-scale farms. In accordance with a previous observation (Hagen et al. 2005) it seems that the physical activity during standing is reflected in HRV measures, and – in the present study – in HR as well. Frondelius et al. (2015) observed 2·5 beats/min greater HR during standing than lying in Holstein Friesian and Ayrshire cows, both recorded while animals were tethered. As expected, HR and LF/HF ratio calculated for standing were greater while HF and SD1 were lower than during lying in the recent study on both smaller- and larger-scale farms, suggesting a greater SNS activity when animals were standing. Such a difference was observed on every farm, and it was most pronounced in HR and spectral components of HRV (HF, LF/HF ratio). Studies on the welfare implications of different milking systems have found HR to be on average 10 beats/min greater during milking, compared to resting in a lying posture (Gygax et al. 2008), which the authors have interpreted as the effect of milking being a stressor. Based on our results, standing without any other physical activity has caused a 8·2 beats/min and a 6·8 beats/min increase in the average of estimated means of HR compared to lying in smaller and larger-scale herds, respectively. In line with our results, Hagen et al. (2005) found greater HR (+8·1 beats/min) and LF/HF ratio (+4·5) during standing than during lying, whereas RMSSD (−5·5 ms) and HF (−4·5 normalised units) were lower while standing compared to lying in Brown Swiss and Simmental cows.

Rumination was accompanied by an increase in HR and a decrease in PNS tone (lower HF and SD1) when lying, and a greater LF/HF ratio reflected on a shift of the SNS/PNS balance to the SNS activity on both types of farms (Table 1). Such effects of rumination have not been previously described. When animals were in a standing posture, rumination did not have any influence on cow's ANS activity on smaller-scale farms (Table 1); however, on larger-scale farms, a decrease in HF and RMSSD with a parallel increase in HR and LF/HF ratio were observed suggesting an increased SNS activity caused by rumination (Table 1). Although the explanation of the difference between smaller and larger-scale farms requires further investigation, the effect of posture and rumination on HRV parameters has a great importance with respect to methodology, particularly when setting baseline values for comparisons in HRV studies. Earlier publications did not distinguish between lying and rumination during lying when determining baseline (Hagen et al. 2005; Gygax et al. 2008; Frondelius et al. 2015). It also seems important to compare HRV values of animals ruminating during e.g. milking to values recorded when animals are ruminating during standing, especially when calculating spectral parameters.

Our results emphasise the importance of physical activity on HR and HRV, which has to be considered in further studies when investigating stress. Baseline HRV values should be set in the same level of physical activity (e.g. the effects of milking should be compared to baseline values during standing). Cows prefer to rest in a lying position (Tucker et al. 2004; Drissler et al. 2005), thus it is difficult to record 5-min IBI segments in a standing position without any other activity occurring. Therefore, it is crucial to plan a sufficiently long recording period (from morning to evening, or a few days) for studying the effects of technology on HRV and use values calculated while animals are standing as baseline.

As the effect of herd size was significant both for time-domain and frequency domain measures, the differences between smaller- and larger-scale farms point out that reference values of HRV parameters in lactating dairy cows cannot be generally defined for the Holstein Friesian breed. The reasons for the pronounced differences in cardiac activity on smaller- and larger-scale farms were not explored, however, the higher baseline sympathetic activity on farms with higher stocking density should be considered as not just a technological but a potential welfare concern.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/S0022029917000607.

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