Measuring Stress Level of Dairy Cows during Milking Using by Geometric Indices of Heart Rate Variability

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Abstract
Heart rate (HR) and heart rate variability (HRV) were investigated in cows (n=32, age: 3.86 years, milk production: 35±2.5 kg, DIM: 150±15) milked in a parallel milking parlour. Geometric parameters of HRV (SD1 and SD2) were calculated using Poincaré graphs. HRV indices of resting 1 h after midday milking (reference period) were compared to those measured during the different phases of the evening milking (driving; in the holding pen; udder preparation; milking; after milking in the milking stall). There was no difference between the reference period and the different phases of milking in animal welfare terms. During the reference period SD2 (198.5 ms) was significantly higher (p<0.05) than every other measured period suggesting an increasing parasympathetic tone after milking. This parasympathetic predominance decreased with time of the day (1.5 h after milking). SD2 was significantly affected by parity, by the breeding bull (p<0.01) and by milk production (p<0.05). SD2 was notably higher (102.8 ms) in multiparous cows than in primiparous cows (p<0.017; \( \alpha =0.005 \)) during resting and milking. Results suggested that a conventional milking process is not really stressful for cows. Primiparous cows were more susceptible of milking process than multiparous ones. SD2 is a good marker of vagus activity and affected by several independent factors.

Keywords: dairy cow, geometric measures, heart rate variability, milking, stress

1. Introduction
A fundamental task of applied animal science is to evaluate and improve husbandry and management systems in the context of animal welfare. Important aspects of this are the recognition and evaluation of stress in farm animals. Nowadays, it is of particular interest in intensive cattle farming to identify those physiological characteristics which describe animal’s responsibility in a potentially stressful environment.

Besides sympatho-adrenal [1] and non-invasive measures of cortisol and its metabolites [2,3] as well as simple heart rate (HR) [4,5], recently, an additional parameter of cardiac activity have been investigated for assessing stress and welfare in dairy cattle [6]. This measure is the variability in the successive cardiac cycles (interbeat intervals, IBI) referred to as heart rate variability (HRV) investigating which the activity of the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS) can be monitored separately at the same time [7] allowing detailed interpretation of cardiac activity in terms of autonomic nervous system (ANS) activity.

Acute stress during milking can compromise animal welfare due to the increase in the amount of residual milk [8]. HRV have previously been used in welfare research to gain information about the stress level of dairy cows during milking [6]. Some studies found higher levels of stress in automatic milking system than in a milking parlour [9,10], while others found no such differences [4], or even noted lower stress level than in a herringbone parlour [11]. In most experiments only alterations in HR were monitored [5,8], however, it provides little
information on the underlying physiological mechanisms that govern its modification in many behavioural situations [12]. Several methods have been proposed for the assessment of HRV reviewed in dairy cattle [6,7]. Next to the orthodox methods of HRV analysis quantitative Poincaré measures have been also found to provide useful information on the vagal regulation [13].

We investigated geometric indices of HRV as physiological measures of stress in high producing dairy cows milked in a parallel milking parlour in a Hungarian working farm. The aim of our study was to find out whether a conventional milking means stress for cows. The second aim was to differentiate cows’ stress level in the separated phases of the milking process. Our third interest was with regard to compare the stress levels of primiparous and multiparous cows.

2. Materials and methods

Measurements were carried out between 6:30 and 21:30 during a 2-week period in May 2012 in a Hungarian working farm. 32 focal cows were selected based on age (between 2 and 5 years), milk production (35±2.5 kg) and DIM (150±15) milked in a parallel milking parlour.

Data were collected 3-day continuously from each animal and obtained using video observations of the milkings and continuous HR recordings. Individuals’ HRV values were evaluated for lying used as a baseline in the comparison with values during milking.

Cows’ stress level was differentiated in the separated phases of the milking process. HR was recorded with a monitoring system with two adhesive electrodes that stored RR-intervals for about 15 h continuously (Polar Equine RS800 CX, Polar Electro Oy, Helsinki, Finland). Prior to the study cows were accustomed to wearing this equipment. The RR data were downloaded from the HR receiver onto a computer and inspected for measurement quality and artefacts using the Kubios HRV analysis software [14]. Because the continuous bouts from which we calculated parameters of HRV were of varying length, we calculated all parameters in 5-min time windows following recommendations by earlier studies [11,15]. For HRV analysis geometric parameters (SD1 and SD2) were calculated (Table 1).

For calculation of SD1 and SD2 Poincaré graph were used, which is a map of dots in an XY-diagram. Each dot represents the duration of an IBI plotted against the duration of its preceding IBI (Figure 1).

<table>
<thead>
<tr>
<th>Table 1. HRV parameters calculated in study</th>
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<tbody>
<tr>
<td>Parameter</td>
</tr>
<tr>
<td>HR (beats/min)</td>
</tr>
<tr>
<td>SD1 (ms)</td>
</tr>
<tr>
<td>SD2 (ms)</td>
</tr>
</tbody>
</table>

ms: millisecond

Figure 1. Analysis of Poincaré plot

HRV indices of resting 1 h after midday milking (reference period: 1) were compared to those measured during the midday milking (2) and the different phases (different behavioural categories) of the evening milking: 1.5 h before evening milking (3); driving (4); in the holding pen (5); stepping into the milking parlour (6); udder preparation (7); milking (8); after milking, in the milking stall (9); after letting out of the milking stall (10); lying after milking (11). In statistical analyses, we used generalised linear mixed-effects models in R 12.2.1. For comparisons between resting and the different phases of milking process, individuals’ HRV values were compared with paired T-tests.
3. Results and discussion

HR was significantly lower (90.32±5.69) in primiparous cows than in multiparous cows (105.11±10.31) during the entire milking process (p<0.05). There was no difference in the values of HR, SD1 and SD2 between resting and the different phases of milking process (driving, being in the holding pen, udder preparation, milking, being in the milking stall after milking, after letting out of the milking stall) suggesting that milking process in a parallel milking parlour is acceptable in animal welfare terms. Namely, neither sudden moving of the animals nor crowding in the parlours’ holding pen meant such load for cows which was detectable in HRV. In contrast to this an earlier study reported of higher stress level during waiting for letting out the milking stall after milking [16].

During the reference period SD1 (198.5 ms) was significantly higher (p<0.05) than every other measured period suggesting an increased vagal tone after milking. This parasympathetic predominance decreased with time of the day (1.5 h after milking) reflecting a slow re-establishment of the sympathetic-vagal balance.

Effect of parity was only calculated on SD2 but all three parameters were significantly affected by different phases of milking process (behavioural categories) and breeding bulls (P=0.0001). Effect of milk production was proved in two cases (HR: P=0.018, SD2: P=0.044) (Table 2).

<table>
<thead>
<tr>
<th>Source</th>
<th>Mean HR (beats/min)</th>
<th>SD1 (ms)</th>
<th>SD2 (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>0.0001</td>
<td>0.135</td>
<td>0.001</td>
</tr>
<tr>
<td>Parity: 1,2</td>
<td>0.050</td>
<td>0.632</td>
<td>0.002</td>
</tr>
<tr>
<td>Behavioural categories: 11</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>Breeding bulls: 21</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>Age, year (covariate)</td>
<td>0.776</td>
<td>0.058</td>
<td>0.346</td>
</tr>
<tr>
<td>Milk production, kg (covariate)</td>
<td>0.018</td>
<td>0.297</td>
<td>0.044</td>
</tr>
</tbody>
</table>

SD2 value of Adept’s daughters (239 ms) was higher in 7 cases than the daughters of other bulls. The results of O-Man’s daughters and Zenit’s daughters on SD2 differed not to the SD2 values of the daughters of other bulls (Figure 2). Corresponding to others [13] SD1 was significantly higher (102.8 ms) in multiparous cows than in primiparous cows (p<0.017; α=0.005) all the day and during the entire milking process reflecting higher PNS activity and lower levels of stress in multiparous cows than primiparous ones.

Based on the literature, it can be stated that the effects of milking systems on the cardiac activity are well documented. Most of recent studies evaluated CMS and AMS in respect to animal welfare.

Till now, none of the studies evaluated cow’s stress responses in parallel milking parlours, several experiments were carried out on conventional herringbone parlours, however. PNS predominance was reported during milking in a conventional milking system using the low-frequency component of HRV [16] interpreted with the release of oxytocin during udder preparation and the pleasant sense of the udder’s emptying, concealing the effects of technology.

In line with our results in conventional milking systems considerable stress was not detectable neither by HR [17] and HRV [18], in cows milked during waiting for letting out the milking stall after milking [16].

Figure 2. SD2 by parity and behavioural categories.
in automatic milking systems HR increased significantly during a 5-min period prior to entering the milking stall, however. HR rapidly decreased in the first 5 min of milking [9]. This slight stress diminishes through the period of adaptation to the novel milking environment as proved by investigating HR [4,11] and HRV [18]. This can explain that in our experiment multiparous cows had lower levels of stress during the entire milking process than primiparous cows. In our opinion it was maybe a result of the longer time spent in producing and habituation of milking technology. More research is needed to find the exact reasons of this difference.

4. Conclusions

Results suggested that milking process is not really stressful for cows, primiparous cows were more susceptible of milking technology than multiparous ones in this experiment, however. SD1 seems to be a reliable geometric measure of PNS activity and physiological stress load. SD2 is affected by several independent factors such as breeding bull, parity and milk production.

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