Szent István University
Faculty of Agricultural and Environmental Sciences

# Investigating acute and chronic stress using heart rate variability analysis in dairy cows

PhD Thesis (Brief summary)

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## List of abbreviations

| | |
|---|---|
| **IBI** | interbeat interval, the time between two consecutive R-waves on the electrocardiogram |
| **HR** | heart rate |
| **HRV** | heart rate variability |
| **LF** | low-frequency component of HRV |
| **HF** | high-frequency component of HRV |
| **LF/HF** | the ratio of the low- and the high-frequency components |
| **RMSSD** | root mean square of the successive differences between interbeat intervals |
| **SD1** | Standard deviation of instantaneous IBI variability measured from axis 1 in the Poincaré plot |
| **SD2** | Standard deviation of long-term continuous IBI variability measured from axis 2 in the Poincaré plot |
| **SD2/SD1** | ratio of SD1 and SD2 indices |
| **DFA1** | short-term fluctuations in IBIs calculated with detrended fluctuation analysis |
| **DFA2** | long-term fluctuations in IBIs calculated with detrended fluctuation analysis |
| **$L_{MAX}$** | the longest diagonal line segment in the recurrence plot |
| **AUC** | area-under-the-curve |
| **GLM** | generalized linear model |

Cardiac parameters used in the dissertation and the activity of the autonomic nervous system the parameters represent

| Analysis method | HRV parameter | Activity of the autonomic nervous system |
|---|---|---|
| Time domain | HR ($min^{-1}$) | sympathetic tone |
| | RMSSD (ms) | parasympathetic tone |
| Frequency domain | HF | parasympathetic tone |
| | LF/HF | sympatho-parasympathetic balance |
| Poincaré plot | SD1 (ms) | parasympathetic tone |
| | SD2 (ms) | sympathetic tone |
| | SD2/SD1 | sympatho-parasympathetic balance |

# 1. INTRODUCTION

## 1.1. The autonomic nervous system approach of stress

The concept of stress has been formed in the 1930's, when the world famous biologist János Selye has published his stress-concept in Nature. According to his theory based on the concept of „stress-stressor-stress reaction" he defined stress as „the non-specific response of the body to non-specific stimuli". A novel definition of stress, according to studies on animal welfare, is that „it is the attempt of animals to cope with their environment". Yet, in the present dissertation I prefer to use the word 'stress' according to the definition of Stephen W. Porges, the developmental psychologist who is also known for his work in the field of neurology. Porges states that stress-reactivity and stress-sensitivity correlates with the parasympathetic nervous activity. In this manner, homeostasis can be described as the state of the autonomic nervous system in which the inner requirements of an organism not reacting to external stimuli are fulfilled. As this state is characterised by the predominance of the vagal tone, stress can be defined as the state of the autonomic nervous system when homeostasis is disrupted, which entails the decrease of the vagal tone.

## 1.2. The investigation of heart rate variability in cattle

The basis of heart rate variablity measurements is that in healthy animals, the time interval between consecutive heart beats (interbeat interval, IBI) is not constant. The variability of such short time intervals can be described by HRV indices. Changes in the spectral components of HRV are specific either to the parasympathetic or the sympathetic activity of the autonomic nervous system, therefore reliable indicators of stress.

Although in Hungary, the beginning of HRV research in human medicine dates back to 1990, the autonomic nervous system activity has rarely been studied in domestic animals, up until now. In opposite, in the USA and in western countries, this method is frequently used in the detection of stress-related behavioural and physiological changes in cattle, swine, sheep and poultry. Studies on the cardiac function in cattle aimed mostly at testing the reliability of HRV indices and measuring devices designed for humans or race horses. Beyond basic research, the effects of contagious diseases, milking or pain evoking procedures in calves have been evaluted by parameters of the heart function. Most of the recent studies have dealt with the welfare concerns of automated milking systems versus conventional milking methods.

### 1.3. Objectives

By interpreting the results of own dairy cattle field studies, I have aimed to justify the reliability and applicability of HRV measures in the field of behavioural physiology, animal welfare and primarily animal husbandry. My major research objectives were as follows:

1. determining baseline values of heart function in dairy cows during different body positions in herds of different population and housing conditions,
2. detecting possible relations between temperament or fear of humans and baseline cardiac parameters,
3. investigating diurnal or seasonal changes in HR and HRV,
4. investigating the effects of conventional milking (the most commonly used technologies) and automated milking (milking robot) on cardiac function and behavior,
5. investigating the effects of short-term stress induced by transrectal palpation on the cardiac function in lactating and non-lactating dairy cows,
6. investigating cardiac function of cows suffering from lameness-related chronic stress at rest and during feeding,
7. evaluating cardiac and endocrine responses of lame sound dairy cows to acute stress (transrectal palpation, parlour milking)

# 2. OWN STUDIES

## 2.1. Basic measurements on the cardiac function of dairy cows (Study 1)

2.1.1. Materials and methods

In Study 1 two smaller-scale farms with a lower number of cattle and medium production [Józsefmajor (n=51), Nóráp (n=43)] and four large-scale farms with a higher number of cattle and intensive production [Jászapáti (n=36), Ödön-major (n=55), Csípőtelek (n=58), Lászlópuszta (n=64)] were visited. The study aimed at evaluating the relationship between HRV parameters and body position, rumination, feeding, temperament and fear of humans on multiparous dairy cows (n=243) and the seasonal and diurnal rhythm of HRV in non-lactating dairy cows (n=64).

8-12 animals were examined each day. IBIs were recorded by a Polar Equine RS800CX (Polar Electro Oy, Kempele, Finland) recorders, which consist of a special signal transmitter a HR-receiver unit and two electrodes. One electrode was positioned on the cardiac region, the other was secured above the right scapula and the contacting surfaces were lubricated with ample ultrasound gel (Aquaultra Blue, MedGel Medical, Barcelona, Spain). The electrode belts were fitted by specially designed leather girths.

IBIs were recorded during lying, ruminating while lying, standing, ruminating while standing, and during feeding. HRV values were determined in time- (RMSSD) and frequency domain (HF, LF/HF), and by Poincaré-plot (SD1, SD2/SD1) with the help of the Kubios 2.1 software. The temperament of cows was assessed at the time of fixing the girth – as an unusual environmental stimulus – by a score of 1–3 (calm, active, restless). Reactions of fear were evaluated during feed by an approach test. I have used the following parameters: approachability (A; 0 cm: 1point, 1–40 cm: 2 points, more than 40 cm: 3 points), the type of the reaction (R; not moving: 1 point, turns head away: 2 points, backs away: 3 points, flees: 4 points), the type of contact (C; the animal can be stroked, smells/licks the approaching hand). To characterize fear of humans (FOH), the following formula was used on the basis of the assessed parameters: $FOH = M \times 0.6 + R \times 0.4 - K$.

Seasonal rhythm of the cardiac function was assessed in summer (n=30, June–July) and in winter (n=34, November–December) in non-lactating cows. As only those HR and HRV values were involved in the latter analysis that were recorded during lying, the body position of the animals was observed 24–hours a day (2–4 samples/hour) with the help of two night vision cameras (Vivotek IP8331, VIVOTEK Inc., Taiwan). Data were continuously recorded for 4–7 days for each animal.

From dairy cows, fecal samples were taken two times a day, on all farms except for Nóráp and Jászapáti. Fecal samples were measured for the level of cortisol metabolites via triciated (3H) cortisol RIA assay.

Statistical analysis was done by the SPSS 18 software package (SPSS Inc., Chicago, IL). Spearman rank correlation, the GLM method, the Tamhane post hoc method, the Kruskal–Wallis test and AUC analysis were used.

### 2.1.2. Results of Study 1

The HR and the index of the sympatho-parasympathetic balance (LF/HF) were higher in a standing than in a lying position, on both smaller and large-scale herds. In parallel, parasympathetic measures (RMSSD, HF, SD1) were lower when standing, compared to lying.

HR and sympathetic measure LF/HF values ranked in an ascending order in parallel with increasing physical activity: lying < ruminating while lying

< standing < ruminating while standing < feeding, while vagal parameters HF and SD1 were shown to decrease in that order. In smaller-scale herds, HR, LF/HF and SD1/SD2 were lower, while HF values were higher than in large-scale herds. Fecal cortisol concentrations also differed between farms, the lowest concentrations were measured on the farms equipped with robotic milking.

The baseline parasympathetic activity of restless animals was lower (lower HF, $P<0.001$), while the sympathetic activity was higher (higher HR and HF/LF, $P<0.05$) compared to calm animals. The cortisol concentration in the faeces of restless animals was higher than that of calm animals ($P<0.05$). Animals that were not shy (could be touched and got in contact with the approaching person) had a higher baseline heart rate than shy animals ($P<0.05$). Animals that were not afraid of humans had a higher baseline sympathetic activity (higher HF/LF, $P<0.001$), and a lower baseline parasympathetic activity (lower HF, RMSSD, SD1, $P<0.001$, $P<0.05$, $P<0.05$, respectively) than very shy animals.

HR and HF showed a diurnal rhythm in summer. HR-values were higher in the afternoon period, than in the morning ($P<0.01$) or in the evening ($P<0.05$). HF values were higher in the evening and night-dawn hours than during the day ($P<0.01$, in both periods). Comparing summer and winter measurements, daytime HR (7:00–12:00 and 13:00–18:00) and night-time HF (19:00–24:00 and 01:00–06:00) showed seasonality.

### 2.1.3. Conclusions of Study 1

HR and HRV are influenced by body position, rumination, feeding and cow population. In view of methodology, the body position and rumination are important influential factors. The results may provide help in the methodology of studies that use HR and HRV to investigate the stress-physiology of technological stimuli, comparing the induced changes to baseline values.

Baseline cardiac parameters differed from farm to farm. In smaller scale herds, the parasympathetic tone measured during lying was higher, while the sympathetic tone was lower compared to large-scale herds. The differences are possibly due to frequency of milking and different production levels. Baseline differences show that though a breed-specific 'HRV-profile' may exist, such standards cannot be generally described due to varying cow population, housing and other conditions among farms.

On the basis of results, the fecal cortisol concentration is in correlation with temperament, that is the activity of the hypothalamus-pituitary-adrenal axis influences behaviour. It could be also concluded that fear of humans is physiologically influenced, and it can be an innate characteristic, not only the consequence of cruel treatment. If so, targeted selection and attention can improve production parameters.

Seasonality in HR and HF suggests that the high ambient temperature characteristic of summer afternoons is a severe load for non-lactating cows.

## 2.2. Investigating acute stress around milking (Study 2)

### 2.2.1. Materials and methods

In Study 2, cardiac parameters of dairy cows (n=219) were assessed in conventional and automatic milking systems. I have collected data from conventional milking parlours from Jászapáti (n=36), Ödön-major (n=50), Csípőtelek (49), Józsefmajor (n=27). Data on the effects of robotic milking was collected from 20 animals in Józsefmajor, and from 37 animals in Nóráp. IBIs were recorded after the morning milking (7:30-9:30). Data recording has continued for 60 minutes after the evening milking has finished.

Baseline cardiac parameters were recorded in the 2 hours before milking during standing (in animals that were not ruminating during milking, 2–4 samples/animal) and during ruminating while standing (in animals that were ruminating during milking, 2–4 samples/animal). In the time around milking the cardiac function and the behaviour (number of steps/min) were examined in parallel. The phases preceding main milking (moving to the milking parlour, waiting in the holding pen, and the last minute before entering the milking parlour) were distinguished by visual observation and a watch synchronized in time with the HR receivers. Phases of milking after entering the milking parlour (preparation, main milking, waiting after milking) have been distinguished by the video recordings of cameras synchronized with the HR-receivers.

HR and HRV indices measured during the different phases of milking have been evaluated by the GLM method in respect of each farm, the number of steps were compared by the Tamhane post-hoc test between the phases of active milking.

### 2.2.2. Results of Study 2

In larger milking parlours, stepping was more frequent in the preparative phase of milking, than in the latter phases ($P<0.001$, in all 3 large-scale herds). The difference in spectral parameters of HRV measured during the time spent in the milking stall (the period of time between entering the milking stall and stepping out from it) and baseline values reflect the differences between smaller and larger-scale milking systems. The rate of decrease in HF was statistically higher in larger sized milking parlours (Ödön-major, Csípőtelek, Jászapáti) than in Nóráp and Józsefmajor ($P<0.01$, in both milking systems). Values of the LF/HF ratio reflected stress during main milking in milking parlours with a higher number of stalls (Ödön-major, Jászapáti) compared to Józsefmajor and Nóráp, as confirmed by a marked increase in the sympathetic tone.

During waiting in the holding pen/area before milking, in smaller scale milking systems, we have measured lower LF/HF and higher HF values, while in larger sized milking parlours, the opposite was observed. In larger units, LF/HF decreased during preparation, yet – in contrary to smaller herds – it was higher than baseline values in the first minute of milking (Jászapáti: $P<0.001$, Ödön major, Csípőtelek: $P<0.01$), during main milking ($P<0,01$, on all 3 farms) and in the last minute of milking ($P<0.01$, on all 3 farms). During waiting after milking, the LF/HF ratio – similarly to HF – indicated an increase in sympathetic tone in Jászapáti and Ödön-major, however, it has not been observed in Csípőtelek, where animals are milked in a 72-stall rotary milking parlour.

The ejected milk yield and the speed of ejection were not in correlation with HRV parameters during the time spent in the milking stall. The length of preparation was not in correlation with any of the cardiac parameters during milking. The length of time spent in the holding area did not have a significant influence on cardiac function, either.

### 2.2.3. Conclusions of Study 2

The higher number of steps in larger milking parlours are possibly due to the quick and intensive udder preparation and the presence of 2–3 milkers at a time. The registered number of steps did not refer to intense stress, though. HRV analysis, however, indicated that milking means a greater stress to cows in a large sized milking parlour, compared to smaller milking systems, and the difference is most pronounced during waiting in the holding area before milking. The crowding in the holding area is the main reason of the decrease in the parasympathetic tone in larger milking parlours.

Based on the results it could be concluded that the parlour size- or milking technology-related differences in stress levels cannot be attributed to merely the type of milking system, but also to other elements of the milking process (e.g. number of animals in the holding area, number of animals milked at the same time), which are in some way related to the size or capacity of the milking parlour. Rotary milking is less stressful for cows than milking in conventional milking parlours.

## 2.3. Effects of palpation per rectum on cardiac activity (Study 3)

### 2.3.1. Materials and methods

Cardiac responses to palpation per rectum were evaluated in lactating (n=11) and non-lactating animals (n=12) in Csípőtelek. 3–3 cows from each group were involved each day. Heart rate monitors were fixed on the cows in the barn. The preparations were done 18 h before the start of the measurements to allow time for habituation. Self-locking headgates in the barn allowed that the experiment could be easily fit to the regular management practice of check-ups and treatments, ensuring the studied animals for easy identification, without moving or separating them. HR-recordings started 10 min after the animals have finished feeding, having returned from the morning milking. After a baseline period of 40 min, the palpation was performed.

The examination lasted 5 min, and was done with care, following the routine clinical practice. The examiners were unknown for the cows. HR recordings have continued for 120 min after the examination has finished. The animals remained standing throughout the whole length of the recording period and had a neighboring cow on each side. All other cows in the group were released from the headgates and moved to the lying cubicles at the time the palpation per rectums were done.

HRV indices were calculated in equal time windows of 5 min in the following periods: 1) in the 40 min prior to palpation per rectum; 2) during the 5-min of the examination; 3) during the 120 min following palpation per rectum. As baseline, the mean values in the 15 min prior to the examination were used. The analyzed time domain measures were mean HR and RMSSD. As a frequency domain parameter, HF was chosen. Any kind of disturbance (sudden noise, presence of people) or any unnecessary contact with animals throughout the whole experimental period was avoided.

Heart rate, RMSSD and HF were analyzed with a GLM procedure with penalised quasi-likelihood. The Bonferroni adjustment was used for post hoc comparisons of HR and HRV values within the groups. A value of $P<0.05$ was considered significant. Changes in HR and HRV parameters were calculated as area under the curve (AUC). Such parameters included: baseline and maximum values of HR, RMSSD and HF, amplitude (the maximal alteration compared to baseline) of the HR, RMSSD and HF response, and long-term measures of cardiac responses to the examination (AUC response and time to return to baseline). The area under the response curve was determined for the first 40 min following the examination for HR and for the first 80 min following palpation per rectum for RMSSD and HF.

### 2.3.2. Results of Study 3

During the examination, HR suddenly increased ($P<0.001$, in both groups), and in the first 5 min after the examination it suddenly decreased, and then returned back to the baseline values. Though baseline and maximum HR were significantly higher ($P<0.001$, in both cases) in lactating than non-lactating group, the amplitude of HR did not differ between groups. The AUC analysis did not detect any effect of the lactation either before or following palpation per rectum. After returning to baseline, HR was relatively stable in both studied groups, with an average slightly below the physiological level in lactating, and slightly above that in non-lactating cows.

RMSSD – similarly to HR – did not change considerably before the palpation per rectum in either group. Baseline HR was lower in lactating group, compared to non-lactating (P<0.001). AUC before the examination was similar in both groups. During the examination, RMSSD dropped in both lactating and non-lactating cows (P<0.001 and P<0.01, respectively) reflecting the decrease in vagal activity. RMSSD in the first 5 min following the palpation highly exceeded baseline in both groups (P<0.001). The exact rate of the increase was on average 24.39% and 41.74% in the lactating and non-lactating groups, respectively. The maximum and amplitude of RMSSD were lower in the lactating group, compared to non-lactating (P<0.001 and P<0.01, respectively). More time was required for RMSSD to return to baseline in lactating cows (P<0.001). AUC was greater in lactating cows after the examination (P<0.01).

Similarly to RMSSD, baseline HF was lower in lactating than non-lactating cows (P<0.001). During the examination, HF decreased in both groups (P<0.001) indicating high stress levels. The rate of decline was on average 28.47% in lactating and 38.12% non-lactating cows, respectively. AUC parameters of short-term stress-responsiveness indicated a higher level of stress in non-lactating cows (higher maximum in the 5 min following the examination, P<0.001; higher amplitude, P<0.05). Yet, AUC analysis confirmed that the stress reaction was more prolonged in lactating cows measured in HF (greater AUC, P<0.001; longer time to return to baseline, P<0.001).

### 2.3.3. Conclusions of Study 3

Palpation per rectum causes stress for both lactating and non-lactating cows. Lactating cows exhibited lower short-term cardiac responsiveness to palpation per rectum than non-lactating animals, whereas in terms of magnitude and duration cardiac responses mirrored by PNS indices of HRV were more intensive in lactating cows than non-lactating ones. Short-term vagal responses were higher in non-lactating cows, compared to lactating animals.

As non-lactating animals had pregnancy diagnosis done 8 months before the time of our experiment, and lactating animals had likely undergone pregnancy testing for 3 to 4 times in the preceding two months, our results seem to confirm that habituation to a stressor can reduce the intensity of stress-response when the stressor is repeatedly occurring. However, our results indicate a longer duration of stress in lactating cows (45 min vs. 90 min). Based on our findings, it is worth investigating whether the differences owed to lactation have a physiological or a management-related background.

## 2.4. Cardiac activity of chronically stressed (lame) cows (Study 4)

### 2.4.1. Materials and methods

Study 4 was carried out in Ödön-major on lame cows (n=56) and on healthy controls (n=52) between October 2012 and April 2013. This time interval allowed the expert evaluation of chronic stress, as elevated ambient temperature could not have an effect on experimental animals' cardiac activity. Based on their locomotion score two groups of cows (non-lame: scores 1–2; lame: scores 3–5) were formed.

On each experimental day heart rate and heart rate variability of 7–10 individuals were recorded, around the half of them were healthy and the half of them were lame. The cardiac activity and the behaviour of the two groups were evaluated during the following test periods: 1) palpation per rectum, 2) at the feeding bunk, 3) during standing and lying in the barn and 4) during milking.

During this experiment blood samples were also taken three times a day: 1) baseline: during calm standing, 2) between 10 and 15 min following rectal palpation and 3) between 10 and 15 min after milking, before the removal of the HR-monitors. Faeces were collected two times: 1) after rectal palpation (between 8:30 and 10:00) and 2) after evening milking before the removal of the HR-monitors (between 18:30 and 21:00).

Blood was collected by veterinarians by caudal venepuncture into heparinized tubes (approximately 7 mL) for serum cortisol measurement. Samples were placed on ice and then centrifuged at 4°C and 3000 g. The average duration of sampling was 20 s. Cortisol assay was done using a radioimmunoassay (RIA). Fecal samples were measured for the level of cortisol metabolites via triciated (3H) cortisol RIA assay. Statistical evaluation of data was performed using the Mann–Whitney test, the GLM and the Wilcoxon test.

### 2.4.2. Results of Study 4

Vagal determined time- (RMSSD, $P<0.01$) and frequency-domain (HF, $P<0.001$) parameters of HRV and Poincaré indices (SD1, $P<0.05$) were higher, while sympatho-vagal indices (LF/HF and SD2/SD1: $P<0.001$, in both parameters) were lower in lame cows than in sound ones. This suggests an elevated parasympathetic activity in the affected animals and the shift in sympatho-vagal balance towards vagal tone.

All of the non-linear indices ($L_{MAX}$, DFA1, DFA2) were statistically lower in lame animals than in non-lame ones ($P<0.001$, $P<0.001$, $P<0.05$, respectively) suggesting a greater stress load in lame cows.

HF and SD1 values measured during feeding were higher ($P<0.001$, $P<0.05$, respectively), while indices of sympatho-vagal balance (LF/HF, SD2/SD1) were lower in lame cows than in sound animals (P<0.001, P<0.05, respectively).

Heart rate did not increase significantly during palpation per rectum neither in lame nor in non-lame cows. 15 min after the examination HR was similar to baseline values in both groups. HF decreased significantly only in lame cows during the palpation (P=0.017) when compared to basal values. LF/HF values increased during the examination in lame cows (P=0.022), while in sound animals this phenomenon was not detected. Restlessness behaviour, the number of steps per min, vocalization and rumination frequency did not differed between groups during palpation (P>0.05, regarding each behaviour).

Heart rate did not differ between healthy and lame cows during milking (P=0.138). HF was lower in lame animals than in healthy ones when measured in the holding pen (P=0.039), while LF/HF was higher in affected animals (P=0.013). These results suggest the higher stress level in the lame group, which could be the result of the pain associated with moving towards the milking parlour. LF/HF was higher in lame cows during the phase of milking than in sound cows (P<0.05) reflecting a higher stress load in this period. The observed stepping rate confirmed this, however the number of steps did not differ statistically between groups registered either udder preparation and during the phase of waiting after milking.

Serum cortisol concentration gave similar result as the spectral parameters of HRV regarding the stress sensitivity of the experimental groups. The cortisol concentration of the lame animals exceeded baseline levels (107%, P=0.012), while in healthy cows it differed not statistically from those (P=0.637). After milking similar cortisol values were found in the two groups (P=0.988), which is in line with the minimal alterations in HF and in LF/HF.

### 2.4.3. Conclusions of Study 4

Chronic stress caused by lameness resulted in a shift in the balance of the autonomic nervous system activity towards vagal tone. However, the lower resting HR of the affected animals is not necessarily indicative of chronic stress, rather than a consequence of the altered behaviour and the decreased daily activity of lame cows. According to our findings non-linear HRV parameters are valuable in the assessment of chronic stress in dairy cows, especially when measured during lying posture.

Based on these results lame cows shows higher sensitivity (higher cortisol levels, higher sympathetic and lower parasympathetic activity) to acute stress accompanied by pain such as palpation per rectum than healthy ones, therefore I suggest that chronic stress increases hormonal and autonomic responsiveness to acute stressors. Since the behavior did not differ significantly between the two groups it can be concluded, that acute stress responsiveness is better evaluated when using physiological (hormonal or cardiac) parameters instead of using only behavioral observation in cases of studying chronically stressed animals.

HE and HRV indices measured during the separated phases of the milking process (udder preparation, milking, waiting after milking) showed no obvious differences between cardiac activity of lame and sound animals. Based on this results we concluded that milking cause no serious stress for lame cows, which results in a serious impairment of animal welfare.

## 3. CONCLUSIONS

The monitoring of cardiac parameters in complementation with other stress assessment tools can give a clear picture on the relationship between the production environment and animal welfare. We can state that conventional analytical methods of HRV make it possible to detect and assess short-term stress in dairy cattle. Body position, population of herds, temperament, fear of humans, time of day, season and size of the milking parlour all have an influence on cardiac function. Non-linear HRV-parameters – not used before in production animal studies – have proved to be useful for investigating chronic stress. Monitoring cardiac function can be useful in studies of behavioural physiology in dairy cattle. Expertise gained from results and practical experience can provide even more precise answers to issues in dairy cattle welfare.

Though it is difficult to integrate HRV measurements in routine practice, results provide help in identifying and resolving stress-inducing elements of the management system or the way of handling the animals. Results may serve as a basis of further investigations on the evaluation of certain elements of management technology with respect to animal welfare.

## 4. NEW SCIENTIFIC RESULTS

1. Posture influences the cardiac function of dairy cows. Heart rate and sympathetic activity are higher, while the parasympathetic activity is lower in a standing position, compared to lying.

2. Rumination – while standing or lying – increases heart rate, decreases the parasympathetic tone and shifts the sympatho-parasympathetic balance towards sympathetic predominance.

3. Baseline HRV values differ with herd size. Sympathetic activity measured at rest is lower, the vagal activity is higher in smaller scale herds than in larger ones.

4. Animals with a restless temperament have lower baseline parasympathetic activity, and higher baseline sympathetic tone and heart rate than calm ones.

5. Fecal cortisol concentrations are in correlation with temperament. Cortisol in the faeces of restless animals is found in higher concentrations than in calm ones.

6. Average fecal cortisol concentrations vary with herd size and milking technology. The lowest values were found on farms operating with robotic milking systems.

7. Animals that are not afraid of humans have a higher baseline heart rate and sympathetic activity and lower parasympathetic activity than that of shy animals.

8. Heart rate and parasympathetic activity have a diurnal rhythm in the summer. The heart rate show seasonality in the daytime hours, the vagal tone in the night hours.

9. In conventional milking systems, the crowdiness of holding pens in the milking parlour imposes a severe stress load for dairy cattle. In larger sized milking parlours, sympathetic and parasympathetic measures of HRV reflect greater stress than in smaller milking units and automatic milking systems.

10. Transrectal palpation induces the decrease of the vagal tone, such reaction is more prominent in non-lactating cows, compared to lactating ones. The decrease in the vagal tone is more persistent in lactating animals.

11. Results have confirmed that geometric and non-linear HRV parameters are suitable for the detection of long-term stress. In lame cows, these measures are significantly lower than in sound ones. The difference could be detected at resting (during lying).

12. The parasympathetic tone of lame cows during feeding is higher, while the sympathetic tone is lower than that of sound animals.

13. Transrectal palpation is more stressful for lame cows, than for sound animals. It is reflected in hormonal and autonomic nervous system indices.

## 5. The author's papers published concerning the dissertation

### Book chapter in Hungarian

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### Monograph

**Kovács L.,** Kézér F.L., Tőzsér J. (2014): A szívritmus-variancia vizsgálata tejelő szarvasmarhákon. Szent István Egyetem Kiadó, 128 pp.

### Publications in peer-reviewed scientific journals with an impact factor (first author)

**Kovács L.,** Nagy K., Szelényi Z., Szenci O., Tőzsér J. (2012): A szívritmus-változékonyság elemzésének biológiai háttere, módszertani kérdései és eredményei szarvasmarha stresszvizsgálataiban – Irodalmi összefoglaló. Magyar Állatorvosok Lapja, 134. 515-523.

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