

Welfare implication of measuring heart rate and heart rate variability in dairy cattle: literature review and conclusions for future research

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Heart rate (HR) measurements have been used to determine stress in livestock species since the beginning of the 1970s. However, according to the latest studies in veterinary and behaviour–physiological sciences, heart rate variability (HRV) proved to be more precise for studying the activity of the autonomic nervous system. In dairy cattle, HR and HRV indices have been used to detect stress caused by routine management practices, pain or milking. This review provides the significance of HR and HRV measurements in dairy cattle by summarising current knowledge and research results in this area. First, the biological background and the interrelation of the autonomic regulation of cardiovascular function, stress, HR and HRV are discussed. Equipment and methodological approaches developed to measure interbeat intervals and estimate HRV in dairy cattle are described. The methods of HRV analysis in time, frequency and non-linear domains are also explained in detail emphasising their physiological background. Finally, the most important scientific results and potential possibilities for future research are presented.

Keywords: heart rate, heart rate variability, stress, welfare, dairy cattle

Implications

Housing and milking technology, health status and way of handling of animals influence the well-being of animals in intensive dairy farming. Non-invasive methods of assessing stress include heart rate and heart rate variability measurements that involve the monitoring of the autonomic nervous function by digital, high frequency, 24 h multi-channel electrocardiographic recorders. As high-producing cattle breeds are very sensitive to environmental factors, monitoring and decreasing stress is of major importance in terms of both animal welfare and production.

Introduction

In intensive dairy farming, housing and milking systems are main factors in determining the welfare of animals, as adaptation to environmental changes can be challenging for high-producing breeds.

Important aspects in the improvement of dairy cattle management systems in respect of animal welfare are the recognition and evaluation of stress. The stressfulness of the technological environment has been examined in many different contexts. Certain welfare studies proved that for intensively farmed cattle, the *milking technology* (Rushen *et al.*, 2001; Wenzel *et al.*, 2003), *fear* from given routine treatments (Holst, 1998) and *pain* (Broom, 1991; Mellor *et al.*, 2000) mean such load that may cause stress (Dantzer and Mormède, 1983; von Borell, 2001) having a negative impact also on milk production (Rushen *et al.*, 2001). Effects of technology and of social interactions can be described not only by classical descriptive behavioural observations (e.g. Milmann, 2013; Theurer *et al.*, 2013), but with physiological measures as well (Hopster and Blokhuis, 1994; Rietmann *et al.*, 2004). Most dairy cattle studies have focused on the stress reactions of animals expressed in neuroendocrine changes that are considered to be reliable indicators of stress and pain in livestock species (Dantzer and Mormède, 1983; Matteri *et al.*, 2000; von Borell, 2000).

However, stress affects many physiological systems that are controlled by the autonomic nervous system, including

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the cardiovascular system. Monitoring the autonomic nervous system activity in farm animals has recently gained considerable interest worldwide. In farm animals, the vagal component of the autonomic nervous system plays a key role in regulating heart rate (HR) in response to stress (Hopster and Blokhuis, 1994; Hansen and von Borell, 1998). Certain parameters of heart rate variability (HRV) give information about cardiac vagal tone and the *sympatho-vagal balance* (van Ravenswaaij-Arts *et al.*, 1993). Consequently, besides traditional ways of measuring stress – assay of cortisol in plasma or serum and measuring HR – HRV has also been investigated in dairy cattle in veterinary, behavioural and applied animal research (Konold *et al.*, 2011; Kovács *et al.*, 2012b).

HR measurements have been first introduced in the early 1950s in relation with increased feed intake in dairy cattle (Thomas and Moore, 1951). Recently, several HR studies focused on the effect of an altered emotional state of the animals related to separation (Boissy and Le Neindre, 1997), weaning (Hopster *et al.*, 1995), veterinary procedures (Waiblinger *et al.*, 2004), early handling (Stewart *et al.*, 2013), social interactions (Laister *et al.*, 2011) and human contact (Rushen *et al.*, 1999; Schmied *et al.*, 2008a and 2008b).

In the last 15 years, the use of HRV analysis for examining welfare has become prominent in livestock species. In dairy cattle, an increasing number of studies used parameters of HRV to indicate stress caused by diseases (Mohr *et al.*, 2002; Pomfrett *et al.*, 2004; Konold *et al.*, 2011), milking (Hagen *et al.*, 2005; Neuffer, 2006; Gygax *et al.*, 2008; Kovács *et al.*, 2012b) and painful procedures in calf rearing (Stewart *et al.*, 2008a and 2008b and 2010).

Although HR and HRV measurements are not untouched fields in dairy cattle welfare studies, large areas are still left for future research. von Borell *et al.* (2007a) reviewed the methodology related to HRV measurements. In this paper, we aimed to sum up the recently published results and current knowledge in technique, data processing, analysis and evaluation of HR and HRV measurements in dairy cattle – highlighting their efficiency in welfare assessment – and a prospect of the possible areas of future research.

Significance of HR and HRV as non-invasive stress parameters in dairy cattle research

A potential indicator of animal welfare is the presence or absence of stress (Moberg and Mench, 2000). According to von Borell (2001) the adjustment to stress induces a broad range of physiological and behavioural changes. These two classes of responses have mostly been dealt with separately, despite the fact that they are closely related.

In the past, housing technologies and management practices for dairy cattle were mainly assessed by descriptive behavioural methods. Studying the relationship between behaviour and the hypothalamus–pituitary–adrenal (HPA) axis activity (Cole *et al.*, 1988; Mitchell *et al.*, 1988) allows a more precise detection of stress than mere observation (Möstl and Palme, 2002). Till date feedback-free sampling methods for cortisol assays have been developed and validated for *milk* (Fukasawa *et al.*, 2008), *saliva* (Pérez *et al.*, 2004; Weston, 2009), *faeces* (Morrow *et al.*, 2002; Kahrer *et al.*, 2006) and *urine* (Morrow *et al.*, 2000).

These non-invasive techniques provide reliable results (Cook, 2012), however, sample collections limited in loose housed cows (Möstl *et al.*, 2002) and long-term data collection with short sampling intervals is often difficult to carry out.

In the last decades – complementary to behavioural and physiological stress measures – HR and HRV enable continuous data recording by monitoring autonomic nervous system activity (Marchant-Forde *et al.*, 2004; von Borell *et al.*, 2007b). Rapid changes in HRV parameters allow a precise identification of stressors therefore HR and HRV are often viewed as more detailed and immediate stress indicators than HPA measures (Stewart *et al.*, 2008a; Mialon *et al.*, 2012). Although the suitability of using HRV parameters to estimate sympathetic nervous system activity is still controversial, HR and vagal tone indices of HRV seem to be reliable indicators of both technological stress and the level of the animal’s activity (Figure 1). As supported henceforth, recent HR and HRV studies proved that stress responses can be quantified by the relative changes in vagal activity, which allows the monitoring of stress

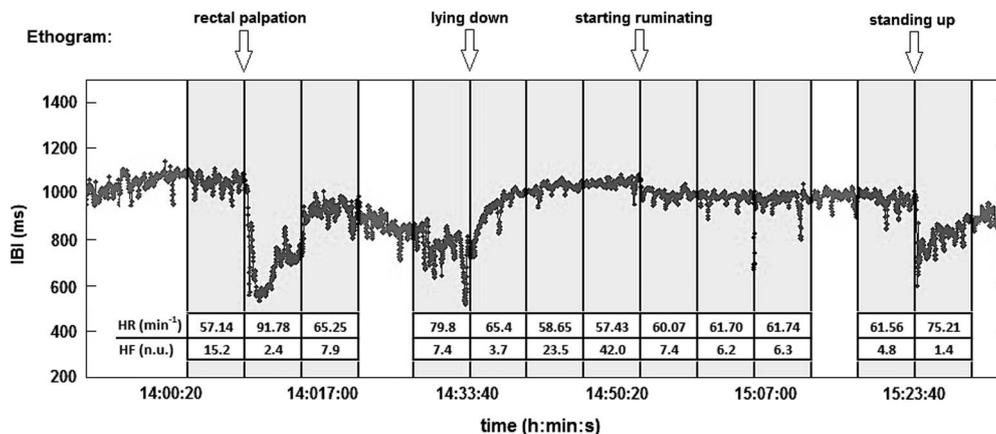


Figure 1 Changes in interbeat intervals (IBI, ms), heart rate (HR, min⁻¹) and the high frequency component (HF, normalised units (NU)) of HRV during short-term stress (rectal palpation) and during different activities of a lactating dairy cow. HRV was analysed in 5-min time windows following recommendations of von Borell *et al.* (2007b) using Kubios 2.0 HRV analysis software.

levels and coping mechanisms in different situations of dairy management and the comparison of technologies and handling methods in terms of animal welfare.

Interpretation of HR and HRV: the relation of autonomic regulation of the cardiovascular function and stress

HR is defined as the number of heart beats per min and used widely to explore inner short-term events occurring parallel to behavioural changes (Lefcourt *et al.*, 1999). Interpretations have often been based on the assumption that HR reflects the activity of the sympathetic branch of the autonomic nervous system and therefore it is an indicator of the stress response (Hopster *et al.*, 1995; Sgoifo *et al.*, 1999) and the animal's emotional reactivity (Wolf, 1970; Obrist, 1981). However, it provides little information on the underlying physiological mechanisms that govern its modification in many behavioural situations (Sayers, 1973). Increased HR can occur in a state of pleasure or in response to a negative stimulus (Waiblinger *et al.*, 2006). The complex interplay of the two branches of the autonomic nervous system is not always comprehensible when cardiac activity is measured only by HR (Porges, 1995; Marchant-Forde *et al.*, 2004), as a rise in HR is due to an increase in sympathetic activity (Hainsworth, 1995), the decrease of vagal tone or the simultaneous changes in both regulatory systems (von Borell *et al.*, 2007b). In a physiologically and neurally intact heart, successive cardiac cycles (R–R intervals) are non-uniformly separated in time domain (Lewis *et al.*, 2007). This beat-to-beat variability is referred to as HRV. With certain parameters of HRV, the sympathetic and vagal activity can be monitored separately at the same time (Malik *et al.*, 1996), moreover it is possible to measure the balance between the two (Porges, 2003; von Borell *et al.*, 2007b).

Alterations in HRV derive from the dynamic interplay between the multiple physiologic mechanisms that regulate the instantaneous HR (Kleiger *et al.*, 1995). The sympathetic and the parasympathetic nerves of the heart constantly modulate electrical dynamics mediated by the pacemaker cells of the sinoatrial (SA) node (Levy and Martin, 1979). The vagus nerves provide rich innervations to the SA node, atrio-ventricular conducting pathways, the atrial myocardium (Hainsworth, 1998) and possibly the ventricular myocardium (Johnson *et al.*, 2004). When both vagal and sympathetic inputs are blocked pharmacologically, HR is higher than the normal value at rest (Cacioppo *et al.*, 1994). This fact supports the Poly-Vagal theory of Porges (Porges, 1995 and 2003), namely that HRV is predominantly determined by the vagus.

Both left and right vagus nerves stimulate the SA node (in 0.2 to 0.6 s) and tend to decrease HR within 5 s (Hainsworth, 1995) but overall effects are relatively short lived (von Borell *et al.*, 2007b). Response to sympathetic activity is relatively slower (few seconds), followed by an increase in HR lasting for ~20 s (Berntson *et al.*, 1997). Activity within the right *Ansa subclavia* (right sympathetic nerve) mainly influences HR, whereas left *A. subclavia* activity impacts stroke volume

(Levy and Martin, 1979). According to von Borell *et al.*, 2007b, besides several anatomical disparities between the autonomic branches the differences in response times are a consequence of the differences between the release times of sympathetic-regulator noradrenaline (slower) and vagal regulator acetylcholine (faster).

Besides the non-additive activity originating from the individual branches of the autonomic nervous system and the tonic changes in the central nervous system activity (mainly from the medulla oblongata), HRV is also affected by several physiological control and feedback mechanisms that can provide quick reflexes (von Borell *et al.*, 2007b). Both sympathetic and parasympathetic activities are influenced by baroreceptor stimulation through the four pathways of the arterial baroreflex (Rompelman, 1993). The Bainbridge reflex acts in a reciprocal way and as the baroreflex. The increase in HR caused by the Bainbridge reflex is followed by an increase in arterial pressure, which stimulates the baroreceptors of the carotid sinus and aortic arch that in turn trigger the baroreflex mechanism to decrease HR (Lanfranchi and Somers, 2002). The renin–angiotensin system (Kanters *et al.*, 1996), the thermoregulation (Akselrod *et al.*, 1985; Malliani *et al.*, 1991), the sympathetic vasomotor tone, the cyclic variation in intrathoracic pressure and the Hering–Breuer reflex (van Ravenswaaij-Arts *et al.*, 1993; Cerutti *et al.*, 1995) are also taking part in HR regulation.

Methods of measurement and analysis of HR and HRV in dairy cattle

Data collection

Measuring HR is based on electrocardiography (ECG) or the count of the arterial pulse, as in human medicine. Different types of Holter recorders (Konold *et al.*, 2011) and fixed (Little *et al.*, 1996; Pomfrett *et al.*, 2004) or telemetric systems (Lefcourt *et al.*, 1999) as well as portable HR monitors have been used to investigate HR and HRV in dairy cattle (Table 1). The latter ones were originally developed for human athletes and sport medicine research (von Borell *et al.*, 2007b) but these models do not record and store the whole electrocardiogram, only the interbeat intervals (IBIs) (Hopster and Blokhuis, 1994). It was shown that a portable HR monitoring system is valid and reliable in measuring HR in animals compared with ECG (Essner *et al.*, 2013). Twenty-four hour recordings are also possible with the newest models (Polar R–R Recorder and Polar Equine) in field studies (Marchant-Forde *et al.*, 2004; Kovács *et al.*, 2012b).

In dairy cattle practice, the recording of IBIs is carried out with two separate electrodes with a specific transmitter and a HR monitor. It is recommended to place one of the electrodes next to the sternum, on the left side of the chest (cardiac area) and the other one on the right scapula (von Borell *et al.*, 2007b). In some cases, electrode sites were shaved before attaching the electrodes (Després *et al.*, 2002; Mohr *et al.*, 2002), whereas others did not consider this important (Hagen *et al.*, 2005; Schmied *et al.*, 2008a; Kovács *et al.*, 2012b).

Table 1 Summary of selected HR and HRV research in dairy cattle

Authors of study	Study objective	Experimental animals	Investigation farms	Equipment used	Reported parameters		Procedures and other factors
					Time domain	Frequency domain	
Després <i>et al.</i> (2002)	Evaluation of the <i>sympatho-vagal balance</i> using autonomic blockers	Holstein–Friesian bull calves ($n=8$)	Experimental farm	Fixed ECG system	HR (bpm) SDNN (ms) RMSSD (ms)	Total Power (ms^2) LF (ms^2): 0.00 to 0.15 Hz HF (ms^2): 0.15 to 1.00 Hz LF/HF ratio	
Stewart <i>et al.</i> (2010)	Assessment of stress level during castration	Holstein–Friesian bull calves ($n=30$)	Working farm group housing outdoor system	Polar S810i	HR (bpm) RMSSD (ms)	LF _{norm} (NU): 0.04 to 0.30 Hz HF _{norm} (NU): 0.30 to 0.80 Hz LF/HF ratio	Eye temperature Blood cortisol assay Epinephrine assay Norepinephrine assay
Stewart <i>et al.</i> (2013)	Effect of previous handling on responsiveness during husbandry procedure	Holstein–Friesian heifers ($n=40$)	Research farm	Polar S810i	HR (bpm) RMSSD (ms)		Respiration rate Blood cortisol assay Behavioural observation
Mohr <i>et al.</i> (2002)	Assessment of external (insect harassment and high temperature) and internal load (BVD)	Calves ($n=52$) cows ($n=31$)	Intensive working farm individual calf igloos	Polar Vantage NV (calves) Par Port/M (cows)	HR (bpm) RMSSD (ms) SDNN (ms) SDANN (ms)	LF _{norm} (NU): 0.04 to 0.30 Hz HF _{norm} (NU): 0.30 to 0.80 Hz LF/HF ratio	Behavioural observation
Konold <i>et al.</i> (2011)	Cardiac activity in BSE-infected and healthy cows	Pure or crossbred Holstein–Friesian cows ($n=428$)	Experimental farms	24 h Holter monitor	HR (bpm)	LF (NU; ms^2): 0.04 to 0.16 Hz HF (NU; ms^2): 0.16 to 0.70 Hz LF/HF ratio	Behavioural observation Postmortem test
Hopster <i>et al.</i> (1995)	Effects of cow–calf separation on behaviour	Holstein–Friesian multiparous cows ($n=8$)	Working farm experimental conditions	Polar Sport Tester	HR (bpm)		Behavioural observation Blood cortisol assay
Hagen <i>et al.</i> (2005)	Evaluation of welfare of cows in automatic and conventional milking systems	Austrian Simmental cows ($n=12$) Brown Swiss cows ($n=12$)	Experimental farm	Polar S810i	HR (bpm) RMSSD (ms) SDNN (ms)	LF _{norm} (NU): 0.04 to 0.25 Hz HF _{norm} (NU): 0.30 to 0.80 Hz (calves) HF _{norm} (NU): 0.25 to 0.58 Hz (cows) LF/HF ratio	Behavioural observation

Table 1: (Continued)

Authors of study	Study objective	Experimental animals	Investigation farms	Equipment used	Reported parameters		Procedures and other factors
					Time domain	Frequency domain	
Gygax <i>et al.</i> (2008)	Restlessness behaviour in automatic and tandem milking systems	Holstein–Friesian and Brown Swiss cows ($n = 120$)	Intensive working farms ($n = 12$)	Radio receiver and oscillator	HR (bpm) RMSSD (ms)		Behavioural observation SCC measurement
Hopster <i>et al.</i> (1998)	Individual characteristics during milking	Holstein–Friesian and Holstein \times Dutch Friesian cows ($n = 89$)	Semi-extensive working farm	Polar Sport Tester	HR (bpm)		Behavioural observation
Rushen <i>et al.</i> (1999)	Fear of people by cows and effects on behaviour and residual milk	Holstein–Friesian primiparous ($n = 5$) multiparous ($n = 9$) cows	Experimental farm	Polar Sport Tester	HR (bpm)		Fear tests Behavioural observation
Rushen <i>et al.</i> (2001)	Stress reducing effects of human contact during milking	Holstein–Friesian multiparous ($n = 18$) cows	Experimental farm	Polar Sport Tester	HR (bpm)		Behavioural observation Milk cortisol assay Oxytocin assay
Waiblinger <i>et al.</i> (2004)	Effects of positive handling on behaviour during rectal palpation and insemination	Austrian Simmental ($n = 10$) cows and Brown Swiss ($n = 10$) cows	Veterinary medical teaching farm	Polar Sport Tester	HR (bpm)		Behavioural observation
Mialon <i>et al.</i> (2012)	Stress level during handling, restraint and rumenocentesis	Holstein–Friesian cows ($n = 20$)	Research farm	Polar HR monitor	HR (bpm)		Behavioural observation Blood cortisol assay
Schmied <i>et al.</i> (2008b)	Effects of handling on avoidance and behaviour	Austrian Simmental ($n = 30$) cows Brown Swiss ($n = 30$) cows	Experimental farm	Polar S810i	HR (bpm)		Behavioural observation

The contacting surface was cleaned before attaching in every case. In one experiment, electrode sites were dampened with 38°C salt water for better conductivity (Janžekovič *et al.*, 2006). However, especially in experiments longer than 2 h using ample electrode gel is essential (Kovács *et al.*, 2012b) not only because of the optimal electrode–skin contact, but for the adhesion of the electrodes to the skin surface (Després *et al.*, 2002; Hagen *et al.*, 2005; Schmied *et al.*, 2008a).

In calves, electrode belts can be easily fixed around the thorax with an elastic strap (Després *et al.*, 2002; Mohr *et al.*, 2002; Clapham *et al.*, 2007; Stewart *et al.*, 2008a and 2008b), whereas in cows strong girths must be used (Minero *et al.*, 2001; Hagen *et al.*, 2005; Gyğax *et al.*, 2008; Kovács *et al.*, 2012b). Signal receivers are usually fixed on the outside of girths. Another method is to fix receivers on the site of the observation, instead of the animals. It has the advantage of continuous monitoring of data collection, yet, in case of uncoded signal transmission it often occurs that the studied animal move too far from the signal receiver and the receivers start to record the signals of the closest animals. Also, implanted devices were used in a recent experiment (Warren *et al.*, 2008), but their application is not widespread.

When interaction of animals and crowding cannot be avoided, especially in the milking parlour and in the pre-milking waiting area in loose housing systems, girths have to be set tight enough to prevent the shifting of electrodes (Kovács *et al.*, 2012a) because, checking the device's position and fastening is difficult to implement further on. However, sudden pressure on the cattle's chest can also induce bradycardia (Clabough and Swanson, 1989). Therefore, an adaptation period of 1 h is needed for the animals to get accustomed with the equipment (Rushen *et al.*, 2001; Mohr *et al.*, 2002; Wenzel *et al.*, 2003; Waiblinger *et al.*, 2004; Stewart *et al.*, 2008a), whereas others recommended 5 days in calves (Clapham *et al.*, 2007) or 7 days in cows (Janžekovič *et al.*, 2006). However, after fixing the girth, visible reactions occur only occasionally for about 5 to 10 min (Mohr *et al.*, 2002) and cows show no obvious signs of being hampered by the equipment (Kovács *et al.*, 2012b).

To eliminate biases in results, it is also recommended that preparations on animals shall be done by the same person each time to avoid stress that might influence results (Minero *et al.*, 2001; Mohr *et al.*, 2002). On working farms, the protocol should fit into the daily operations of the farm and it is important to avoid conditions that would overly distort data recordings (Kovács *et al.*, 2012a).

The periodicity of HR must also be taken into consideration during recording. Several authors found that HR shows a circadian rhythm. A lower peak in the morning hours (0600 to 0800 h) and a higher peak in late afternoon (1630 to 1800 h) were demonstrated (Yamamoto, 1989; Aharoni *et al.*, 2003; Brosh, 2007). Others reported HR increase in the early evening hours (Wenzel *et al.*, 2003; Hagen *et al.*, 2005; Kovács *et al.*, 2012b), which was most likely caused by increased metabolic activity in the evening. These findings suggest that it is advised

to record baseline HR parameters close to (30 min) the event to be studied or calculate an average HR value at rest from data recorded at different times of the day.

The movements of free ranging animals has to be controlled – especially when elastic belt is used – to prevent the shifting of the electrodes on the body surface, as much as possible (Rushen *et al.*, 1999; Mohr *et al.*, 2002), as the non-sufficient skin–electrode conductance may cause errors in IBI signals (Minero *et al.*, 2001; Hagen *et al.*, 2005), most often because of the electrodes getting dry (Gyğax *et al.*, 2008).

The noises caused by the action potential of the muscles, environmental electromagnetic interference, stress-related sinus arrhythmia (Bernston and Stowell, 1998) or mis-identification of R-peaks of the ECG and ectopic beats (Kamath and Fallen, 1995) can also lead to artefacts.

HRV parameters are highly sensitive to measurement errors (Marchant-Forde *et al.*, 2004); therefore, selection of IBI data and correction criteria must be rigorous. Each 5-min time window of the recordings involved in HRV analysis must be inspected separately for artefact correction (Kovács *et al.*, 2012a). It is recommended to exclude data segments if the error rate is larger than 5% or it contains three or more consecutive erratic IBIs (von Borell *et al.*, 2007b). IBI correction is usually done automatically by special algorithms of the software installed. IBI data are transmitted wirelessly in an encoded form, for undisturbed recording of data deriving from different animals. The IBI signals are stored by the HR monitor and can be downloaded onto a computer wirelessly for later analysis (Niskanen *et al.*, 2004).

Traditional methods of HRV analysis

Among several methods, conventionally the linear dynamics of HRV is assessed using time-domain and frequency-domain techniques. Time-domain measures are simple descriptive-statistical procedures, whereas with frequency-modeling approaches the total variability of IBIs is attributed to a spectrum of different frequency components.

The most informative parameter in time-domain analysis is the root mean square successive differences (RMSSD) in milliseconds (ms). The RMSSD is determined by calculating the difference between consecutive IBIs before squaring and after summing them the values are averaged and square root obtained (von Borell *et al.*, 2007b). This reflects the short-term variability of cardiac activity and represents vagal tone (Kleiger *et al.*, 1995; Kanters *et al.*, 1996; Malik *et al.*, 1996).

The standard deviation of the normal-to-normal intervals (SDNN, ms) and the standard deviation of the average normal-to-normal intervals (SDANN, ms) reflect the long-term variability and are typically measured over 24 h. The SDNN is the standard deviation of all IBIs over a 24-h period or the standard deviation of IBIs of a single 5-min period, whereas the SDANN is the standard deviation of all the 5-min IBI means (von Borell *et al.*, 2007b). The pNN50 calculates the percentage of differences between successive IBIs over 24 h that are >50 ms and measures short-term HRV. These parameters are highly correlated with each other

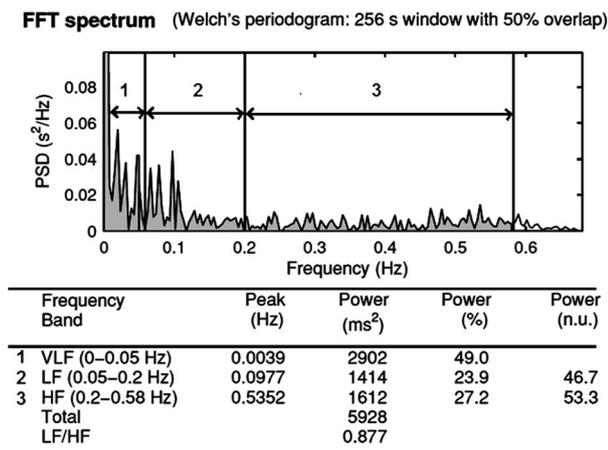


Figure 2 Power spectral density (PSD) of heart rate variability (HRV) performed by Fast Fourier Transformation (FFT) using the Welch Periodogram method corresponding to the 256 values of analysis selected previously. Spectral frequency bands are obtained at very low (VLF: 0 to 0.05 Hz), low (LF: 0.05 to 0.20 Hz) and high (HF: 0.20 to 0.58 Hz) frequencies, in absolute (ms²) and normalised units (NU). LF/HF is expressed as the ratio between the absolute areas of low and high frequency bands. Data were calculated with Kubios HRV analysis software.

and their values are determined by both sympathetic and parasympathetic regulation (Malik *et al.*, 1996; von Borell *et al.*, 2007b).

Various spectral methods have been proposed for the calculation of cardiovascular measures of sympathetic and parasympathetic control in frequency domain, which can be used to separate the constituent band-limited components of the IBI signal (Stein *et al.*, 1994).

A fundamental hypothesis supporting the use of various spectral methods in HRV studies postulates that the two branches of the autonomic nervous system influence HR in a frequency-dependent way. Power spectral analysis provides basic information of how power (i.e. variance of IBIs) is distributed in function with frequency (Akselrod *et al.*, 1981). The 'power' of a signal can be estimated within a finite frequency range by calculating its power spectral density (PSD) that may be integrated to determine the power within a defined bandwidth (Lewis *et al.*, 2007). In dairy cattle research, spectral analysis – as developed by Akselrod *et al.* (1981) – is used, in which the PSD estimation is carried out using the Welch's periodogram method (Welch, 1967) based on Fast Fourier Transformation (FFT; Cooley and Tukey, 1965; Figure 2).

The FFT's algorithm separates the HRV to its harmonic components and describes it in frequency domain, making it possible to assign the power of different bands to different physiological functions. The accuracy of the R-wave occurrence time estimates is often required to be 1 to 2 ms and, thus, the sampling frequency of the ECG is recommended at 500 to 1000 Hz (Malik *et al.*, 1996). If the sampling frequency of the ECG is <500 Hz, the errors in R-wave occurrence times can cause critical distortion to power spectrum analysis results (Merri *et al.*, 1990).

Short-term variability of the IBI signals can be described by three spectral bands of the HRV spectrum.

The high frequency (HF) band is regulated by the vagus, because it can be almost completely abolished by parasympathetic blockade with atropine (Akselrod *et al.*, 1985), whereas the administration of β -blockers (Aronson and Burger, 2001) or rennin–angiotensin blockade (Pomeranz *et al.*, 1985) had no effect on HF component. Consequently, HF is a reliable measure of the parasympathetic activity (Akselrod *et al.*, 1985; Porges, 1995; Slangen *et al.*, 1997).

It has been shown that the modulation of SA node activity via respiratory frequency and volume is the main mechanism influencing the fast periodicities in the HF range of HRV (Porges, 1986; Fleisher, 1996; Malik *et al.*, 1996); therefore, species-specific respiratory rates must be taken into account when locating the HF band during HRV analysis (Slangen *et al.*, 1997). In mature cattle, according to von Borell *et al.* (2007b) HF power is defined as 0.20 to 0.58 Hz (corresponds to a respiratory rate between 12 and 35 min⁻¹) while in calves as 0.5 to 0.83 Hz (corresponds to a respiratory rate between 30 and 50 min⁻¹).

The other characteristic oscillation in HRV spectrum is the low frequency (LF) band, which is thought to be closely associated with the fluctuations of the peripheral vasomotor tone and reflects the 10-s periodicities, or so-called Mayer waves of the blood pressure (Berntson *et al.*, 1997; Hamner *et al.*, 2001) because of the baroreflex regulating the slow, respiratory-induced blood pressure oscillations (Kitney *et al.*, 1985; Kardos and Gingl, 1994).

In cattle LF, power is defined as 0.05 to 0.20 Hz (von Borell *et al.*, 2007b). Although, some researchers have suggested the LF component to be mainly of sympathetic origin (Malliani *et al.*, 1991) – in horses it is used as the indicator of sympathetic outflow (Physick-Sheard *et al.*, 2000; Rietmann *et al.*, 2004) – in cattle it does not represent the changes in sympathetic activity (Després *et al.*, 2002; Mohr *et al.*, 2002; Hagen *et al.*, 2005). It has been confirmed, that the LF component – besides other physiological mechanisms (Malik *et al.*, 1996; Kuwahara *et al.*, 1999) – arises from the interaction of sympathetic and vagal responses (Akselrod *et al.*, 1985; Houle and Billman, 1999) and can be reduced with either sympathetic or parasympathetic antagonists (Cacioppo *et al.*, 1994). These findings are consistent with the observations in humans that the parasympathetic nervous system is able to modulate HR effectively at all frequencies between 0 and 0.5 Hz, whereas the sympathetic modulates HR only below 0.1 Hz (Berntson *et al.*, 1997).

It has been suggested that the relative power of the LF and HF components (LF/HF ratio) characterises the *sympatho-vagal balance* (Solan *et al.*, 1994; Malik *et al.*, 1996) – a concept reflecting the dual opposing effect of the two autonomic systems on heart activity – and is an accurate indicator of increased sympathetic activity (Yamamoto *et al.*, 1991; Marchant-Forde and Marchant-Forde, 2004).

The very low frequency (VLF) band (≤ 0.05 Hz) represents sympathetic or sympathetic plus vagal activity (Cerutti *et al.*, 1995) associated with the thermoregulation of vasomotor

tone and with the renin–angiotensin–aldosterone system (Akselrod *et al.*, 1985), however, its physiological relevance is not fully clear in cattle.

Instead of the absolute values (ms^2) of each power, generally the normalised units of LF and HF power are reported in dairy cattle studies, describing the relative value of each power in comparison with total power minus VLF power. Normalisation tends to minimise the effect of the changes in total power (Malik *et al.*, 1996) and inter-individual differences (von Borell *et al.*, 2007b).

Non-linear characteristics of HRV

The non-linear components of HRV are determined by hemodynamic, electrophysiological and humoral interactions (Giuliani *et al.*, 1998). Since the first publication of the HRV standards (Malik *et al.*, 1996), a number of non-linear methods has been proposed to quantify HRV, which are derived from chaos theory and non-linear system theory (Voss *et al.*, 2009). Non-linear measures differ from the conventional HRV methods as they are not designed to assess the magnitude of variability but rather the quality, scaling and correlation properties of the HR dynamics (Cervantes *et al.*, 2009; Melillo *et al.*, 2011). Human HRV studies focus on mainly non-linear methods that are implemented in Kubios, a free software for HRV analysis (The Biomedical Signal and Medical Imaging Analysis Group, Department of Applied Physics, University of Kuopio, Finland).

Till date, a series of mathematical approaches have been applied for the estimation of non-linear characteristics in various physiological time series data (Theiler *et al.*, 1992).

The Poincaré Plot is a graphical representation of the correlation between successive IBIs, where each interval in the time series ($\text{IBI}_i + 1$) is plotted against its successor (IBI_i). A common approach to analyse the Poincaré plot of RR series consists in fitting an ellipse oriented according to the line-of-identity and computing the standard deviation of the points perpendicular to and along the line-of-identity, referred to as SD1 and SD2, respectively (Figure 3).

SD2 reflects intermediate-term variability and sympathetic activity, whereas SD1 reflects short-term variability and parasympathetic activity (Minero *et al.*, 2001).

The procedure of recurrence quantification analysis (RQA) was developed by Zbilut and Webber (1992) and ideally suited for physiological systems characterised by non-homoeostatic transients and state changes (Zbilut *et al.*, 2002). This method was performed for the measurement of the complexity of the time series in a symmetrical K -dimensional square matrix, which is calculated by computing the Euclidean distances of each vector X_i from all the others (Trulla *et al.*, 1996). The vectors represent the RR interval time series as a trajectory in m dimensional space. The recurrence plot (RP) is the representation of the matrix as a black (for ones) and white (for zeros) image (Niskanen *et al.*, 2004) in which L_{\max} is the longest diagonal line segment in a continuous row within the plot. A shorter L_{\max}

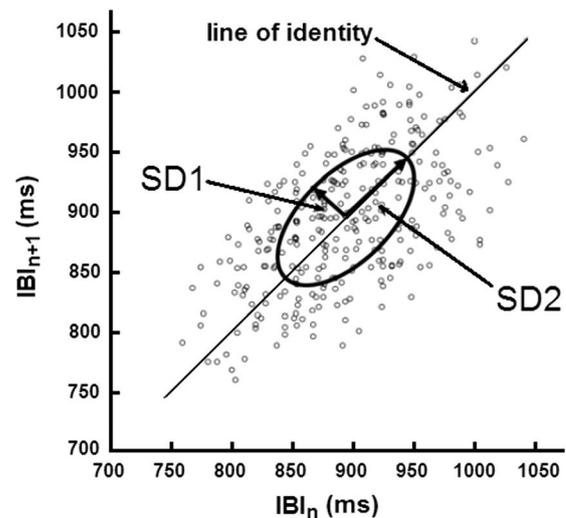


Figure 3 The Poincaré plot, in which each dot represents the duration of an interbeat interval (IBI) in milliseconds (ms), plotted against the duration of its preceding IBI. SD1 (ms) and SD2 (ms) are the standard deviations of the points perpendicular to and along the line-of-identity, respectively.

is a hallmark for a large amount of chaos in time series data (von Borell *et al.*, 2007b).

The percentage of recurrent points forming upward diagonal lines in the RP referred to as %Determinism (%DET), whereas the percentage of recurrent points in the RP as % Recurrence (%REC). Both %REC and %DET are parameters of the regularity of HRV (von Borell *et al.*, 2007b). The Shannon entropy (Entropy, Ent) of the deterministic line segment length distributed in a histogram and corresponds to the richness of deterministic structuring of the series (von Borell *et al.*, 2007b).

A detrended fluctuation analysis (DFA) enables the detection of long-range correlations within the signal and reflects the amount of randomness in the HR time series (Voss *et al.*, 2009). According to Kleiger *et al.* (2005), the IBI time series usually comprise two distinct areas of scale invariance, covering short-term (DFA1, computed for 3 to 11 IBIs) and long-term fluctuations (DFA2, computed for 12 to 20 IBIs).

The complexity and irregularity of HR fluctuations can be calculated by several methods of non-linear HRV. Approximate entropy (ApEntr) and its improved version, sample entropy (SampEntr) quantifies the unpredictability of HR time series (Richman and Moorman, 2000), while correlation dimension (D2) gives information of the strangeness of the time series (Niskanen *et al.*, 2004).

Although a series of human studies described that DFA, ApEntr, SampEntr and D2 are promising approaches for measuring non-linear characteristics of HRV (Tulppo *et al.*, 2005; Casties *et al.*, 2006; Blasco-Lafarga *et al.*, 2010), the physiological relevance of these measures is not well understood in animals.

In dairy cattle research non-linear HRV has been described by Poincaré plot (Minero *et al.*, 2001; Kovács *et al.*, 2013) and RQA, demonstrating differences in connection with lactation (Mohr *et al.*, 2002), breed or bodyweight (Hagen *et al.*, 2005).

HR and HRV as indicators of stress in dairy cattle in applied animal research

Recent publications on HR and HRV outline four main topics of research activity in dairy cattle: (1) disease-induced pathological loads; (2) stress caused by husbandry and veterinary procedures; (3) animal's behavioural and physiological stress reactions during milking and (4) short-term cardiac responses to fearful situations (novelty, weaning and isolation). Each set of literature is relatively small in number but demonstrates an increasing interest in the area of HR and HRV measurements within the last 15 years (Table 1).

Effect of pathological disorders

Relatively few studies have used HRV as a measure of the effect of pathological conditions in dairy cattle. Research demonstrated a significant shift in the activity of the autonomic nervous system reflected in HRV in relation with certain infectious diseases. Studies detected the predominance of the vagal tone experiencing the radical increase of RMSSD (Pomfrett *et al.*, 2004) or HF power (Little *et al.*, 1996), whereas others described bradycardia (Winter *et al.*, 1989; Austin *et al.*, 1997) in cows positive for bovine spongiform encephalopathy (BSE).

In contrast, there was a study that did not find any disease-related changes in HRV (Konold *et al.*, 2011), which suggests that the BSE agent has an effect on the dysfunctions of both the sympathetic and the parasympathetic nervous system at the same time.

Results in calves indicated a loss of complexity in HRV in response to pathological load (Mohr *et al.*, 2002). All non-linear parameters calculated with RQA were significantly higher in calves infected with bovine viral diarrhoea (BVD) compared with healthy calves. In the BVD-infected group, both parasympathetic (lower RMSSD and HF power) and sympathetic activity decreased (lower SDNN and SDANN), which suggests that a strong pathological load decreases both vagal and sympathetic tone. Furthermore, in this study researchers determined that parameters calculated both in frequency or time domain are more affected by pathological load, than heat stress or disturbance by external parasites.

Studies on diseases underline the efficiency of HRV in measuring the effects of pathological loads. The decrease in HR associated with an increased parasympathetic tone in case of BSE highlight the impact of chronic stress on cardiac activity. Parallel decline of both sympathetic and vagal activity and a rise in overall non-linear HRV parameters in case of BVD indicate a severe impairment in animal welfare.

Effect of pain

As pain experienced in specific circumstances can induce a stress response when the animals are re-exposed to those circumstances (Boissy and Bouissou, 1994), increases in HPA axis activity (i.e. cortisol levels) have been widely used for indicating stress associated to pain in calves during treatments such as disbudding (Stafford and Mellor, 2005 and 2011) or castration (Coetzee, 2013).

Recently, the physiological effects of painful husbandry procedures causing tissue damage have been studied by using HR and HRV in dairy calves and in cows focusing on the test the efficacy of analgesics.

In adult cattle, a painful veterinary procedure (rumenocentesis) was found to be no more stressful than restraint preparatory to the intervention (Mialon *et al.*, 2012). No changes in HR and behaviour were found during surgery carried out either with or without local anaesthesia.

These results were recently confirmed by other authors who evaluated the effects of local anaesthesia during handling procedures in dairy calves. Increased HR after ear-tagging was found in calves (Stewart *et al.*, 2013) similarly during the 5 min following hot-iron disbudding both with and without local anaesthesia (Stewart *et al.*, 2008a). However, in the latter case, prolonged HR elevation was found only without local anaesthesia (up to 3 h) and sympathetic measure (LF/HF ratio) only increased following disbudding without local anaesthesia suggesting the efficiency of analgesia.

Contradictory, in another experiment Stewart *et al.* (2008b) found no effect of local anaesthesia on the autonomic nervous system proved by calves' acute HRV response to pain. In this research, following disbudding with local anaesthesia reduced vagal tone (lower HF power) and increased sympathetic activity (higher LF power and LF/HF ratio) was detected verifying that stress stimuli cause a progressive rise in sympathetic tone along with a decrease of parasympathetic tone, especially when accompanied by pain (Porges, 1995).

Using no local anaesthetics, parasympathetic indices (HF power and RMSSD) increased after surgical castration in dairy calves compared with baseline period, opposite to sympathetic HRV parameters (LF power and LF/HF ratio) that were lower following surgery (Stewart *et al.*, 2010). HR increased during the first 2 min following castration and then declined rapidly to baseline levels. These data suggest that somatic pain from disbudding caused acute sympathetic responses and prolonged HR elevation, whereas following castration a deeper visceral pain conveyed by parasympathetic nerves from the testicles caused a short-lived increase in HR and in vagal tone parallel with a decrease in sympathetic activity.

In general, reports support that pain caused by surgery can be reduced poorly by using local anaesthesia, which may have major implications for animal welfare. However, studies clearly demonstrate that pain-evoking husbandry procedures clearly had an effect on HR and HRV both in calves and cows.

Short-term cardiac responses to fearful situations

An increasing number of studies using HR and HRV suggest that not only pain, but also fear during routine handling and management procedures reduces animal welfare in dairy cattle (Boissy and Bouissou, 1995 and 1998; Rushen *et al.*, 1999). Investigations have been mainly focused on the examination of factors affecting welfare, including emotional states based on their novelty or irregularity for animals.

Hopster *et al.* (1998) described individual characteristics in cows under challenging conditions. Cows showing side preference in a tandem parlour reacted with a 1-min increase in HR and associated decreases in HRV to milking in their non-habitual side. These individual characteristics of cows showing side preference were explained with the restlessness of animals. This short-term effect did not reduce the milk yield in this particular study, opposite to other experiments (Bruckmaier *et al.*, 1993; Rushen *et al.*, 2001), in which lower milk yield and reduced oxytocin release were detected in cows milked in novel milking stalls separated from their herd mates. However, in those experiments social isolation could also increase stress accompanied by an increase in HR (Bruckmaier *et al.*, 1993; Rushen *et al.*, 2001).

Another study found, that the way cattle are handled can also affect HR during milking (Rushen *et al.*, 1999). When an aversive handler was present during milking, HR was higher than in the presence of a gentle handler. These effects were most obvious among the cows that could best distinguish the two handlers showing that cows that can recognise individual people are more affected by stress caused by fear. Sutherland *et al.* (2012) report a relationship between exit speed and parasympathetic activity during milking in a familiar and a novel environment. Novelty caused a decrease in RMSSD in high responder cows, but not in low responder ones (defined based on exit time from a restraint device); however, this difference was not distinguished regarding HR suggesting that vagal tone is more representative in determining stress level in challenging situations than HR. However, a higher basal HR of high responder cows compared with low responder cows suggested that basal HR can be used to distinguish between different temperaments in dairy cows.

It has been proven that human-animal relationship has an impact on animal stress (Hemsworth and Barnett, 2000) and welfare (Hemsworth and Biovin, 2011). Recent studies suggest that handler-cow relationship can be improved by gentle human interaction. Stroking the neck before examination decreased HR (Schmied *et al.*, 2008a) and flight distance of an approaching person (Schmied *et al.*, 2008b). A 4-week period of positive handling before the test period has resulted lower HR compared with untreated control cows during transrectal palpation and artificial insemination (Waiblinger *et al.*, 2004) demonstrating that handling does have positive effects in reducing stress during veterinary procedures. The stress-moderating effect of contact with a familiar person, as shown by decreased HR following removal from the herd, was also reported by Rushen *et al.* (2001). With no human contact, social isolation in both Holstein-Friesian heifers (Boissy and Le Neindre, 1997) and cows (Hopster and Blokhuis, 1994; Hopster *et al.*, 1995) caused a rapid and prolonged increase in HR possibly due to stress evoked by separation anxiety (Forkman *et al.*, 2007).

Significant stress was found in calves also in a weaning-model study by Clapham *et al.* (2007) using HRV. Following isolation from the mother, yet maintaining visual contact, calves showed decreased parasympathetic tone (lower RMSSD) lasting for a 12-day period. Weaning on the 3rd day after calving –

without contact with the calf – caused only moderate HR increase lasting only for 1 min in cows (Hopster *et al.*, 1995), suggesting that isolation of the calf does not mean long-term stress for the cow.

Overall, results from these researches suggest that novelty and separation can impair emotional state both in cows and calves. HR and short-term HRV are effective to assess welfare implications of human-cattle relationship and its possible stress reducing effects.

Emotional challenges caused by milking

HRV analysis in cattle appears to be a sensitive measure of stress responses to milking process. Most HRV studies evaluated conventional and automatic milking systems in terms of animal welfare. Some experiments have compared different milking technologies, whereas others have compared different phases of a technology.

We recently found no difference in HR – neither in Poincaré measures (SD1 and SD2) – between the different phases of milking process (udder preparation, milking and waiting in the milking stall after milking) in a parallel milking parlour (Kovács *et al.*, 2013).

Contradictory, in our earlier work we found that despite parasympathetic predominance (increase in HF power) during milking, reduced HRV and elevated stress level was exhibited by cows during waiting to exit from the milking stall in a herringbone milking parlour (Kovács *et al.*, 2012b). Results let us to conclude that a rise in vagal tone during milking was possibly the consequence of the release of oxytocin during udder preparation (Bruckmaier, 2005) being in association with increased parasympathetic activity (Uvnäs-Moberg and Petersson, 2005) which decreased with time spent in the milking stall and by anticipation to be released resulting in lower vagal activity.

While previously published research have documented no considerable stress on the basis of HR in conventional milking parlours during milking (Hopster *et al.*, 1998; Rushen *et al.*, 1999), in cows milked in an automatic milking system HR increased significantly during a 5-min period before entering the milking stall, but rapidly decreased in the first 5 min of milking (Wenzel *et al.*, 2003). Becoming adapted to the novel milking environment, this pattern could not be observed as proved by HR (Hopster *et al.*, 2002; Weiss *et al.*, 2005) and HRV studies as well (Neuffer *et al.*, 2004; Neuffer, 2006; Gyax *et al.*, 2008). Results suggest that automatic milking did not cause a higher load than conventional milking. However, vagal activity were lower (lower RMSSD and HF power) and LF/HF ratio was higher during lying in cows milked in an automatic milking system compared with those milked in a herringbone milking parlour (Hagen *et al.*, 2005) reflecting higher levels of stress. Yet, in the same study, there were no differences in the HRV parameters of animals during milking. It indicates that the difference in parasympathetic tone detected during lying can be attributed not to the difference in the milking system; but rather to the housing conditions.

Furthermore, Gyax *et al.* (2008) showed that the physiological reactions of cows measurable in HRV could

be different in different types of automatic milking systems. The findings of Ketelaar-de Lauwere *et al.* (1998) suggested that cows in automatic milking systems with forced cow traffic (used by Hopster *et al.*, 2002) spent less time lying in the cubicles than cows in systems with partially forced cow traffic (Hagen *et al.*, 2004 and 2005) or free cow traffic (Wenzel *et al.*, 2003; Gyax *et al.*, 2008).

It seems that the relationship between HR and HRV reactivity to milking is extremely complex. According to the afore mentioned research findings, cow traffic strategies may have measurable effects on HR and HRV. However, due to the different methods and functions of the milking robots used in these studies the comparison of the results is hardly possible.

Summary of research findings

The studies in dairy cattle suggested that: (i) the effects of technological stress and pathological loads on the autonomic nervous system are predominantly manifested by changes in the activity of the nervus vagus as represented by RMSSD and HF parameters in calves and in mature cattle; (ii) the index of *sympatho-vagal balance* (LF/HF ratio) has been found to be a reliable indicator of external stress; (iii) the effect of disease-induced physiological challenge in calves was obvious in non-linear HRV parameters, especially in L_{maxi} ; (iv) short-term stress caused by pain-evoking interventions in calves are detectable in HRV parameters; (v) HR proved to be effective in detecting short-term stress (isolation, fear of veterinary or husbandry procedures, rough or aversive handling and novelty); (vi) stress-reducing effects of regular and positive handling were confirmed by HR; (vii) conventional and automatic milking are both acceptable in terms of animal welfare.

Further opportunities in HR and HRV in dairy cattle research

Pathological conditions, pain, fear and technological stress – especially milking – have been the most commonly investigated factors affecting HRV in dairy cattle. Hereinafter we tried to identify some areas for further research in order to enhance the better understanding of cardiac activity in relation to stress and welfare of dairy cattle providing guidelines on how HR and HRV measurements might be enhanced.

Improvement of evaluating milking as a source of stress

Although several studies have shown an effect of milking technology on HRV (e.g. cow traffic and management), differentiating between the potential sources of stress seems to be difficult as research failed to find exact explanations of their results. Contradictory findings could be partly explained by the fact that handling and technological processes around milking may have also an effect on stress load of animals. Studying the phases before (and if relevant, after) the main milking itself helps to make reliable comparisons between

different milking systems, and reveal possible influential effects of pre-milking events on the cardiac activity measured during milking. Evaluating HR and HRV during the whole milking process may allow optimisation of the milking systems and reduction of stress and consequently the improvement of dairy management and cow's welfare.

Contradicting results in comparing stress levels induced by conventional and automatic milking systems suggest that chronic stressors may influence the cardiac function of animals (Kovács *et al.*, 2012a) and the reliability of linear parameters of HRV in assessing acute stress. Moreover, chronic stress induced immunosuppression could also reduce tolerance to stressful situations cows experience during milking (Moberg, 2000). Evaluating factors that interact in the stress-related changes in cardiac activity lead to a more precise identification of the technological sources of stress and make the comparison between different milking technologies easier.

Attention on the role of chronic stress

As mentioned above, research on HRV in dairy cattle has generally focused on acute stressors, whereas chronic stress, which is more likely to be encountered in intensive housing conditions, has received little attention.

Although many reports have proved HRV to be an effective tool in studying certain infectious diseases, less attention was given to measuring autonomic nervous system responses to non-infectious metabolic diseases (Forslund *et al.*, 2010) or painful claw horn disorders and leg injuries (Walker *et al.*, 2008) inducing chronic stress, which can suppress reproduction and decrease milk yield (Dobson and Smith, 1995; von Borell *et al.*, 2007a) and possibly leads to a pathological condition (Moberg, 2000).

Although stressors rarely occur unaccompanied and only once, and chronic stress resulting from experiencing a series of acute intermittent stimulation is thus more frequent (Dantzer and Mormède, 1983), information regarding the acute stress sensitivity of chronically stressed animals is still lacking. Therefore, considerably work is still necessary to concerning the questions of chronic stress and its interactions with acute stress manifesting in changes in HR and HRV. However, the advance in this field is hampered by the lack of suitable physiological criteria to assess long-term physiological changes in the animal body.

In humans, recently developed non-linear techniques have revolutionised the evaluation of the effects of chronic stress exposure. Research mainly focused on understanding the changes in HRV related to long-term stressors on autonomic nervous system activity. Schubert *et al.* (2009) reported that certain non-linear HRV parameters were useful to detect chronic stress while others found that chronically stressed individuals show decreased HR (Furlan *et al.*, 2000; Lucini *et al.*, 2005).

The study of Mohr *et al.* (2002) involving dairy calves suffering from BVD showed that RQA parameters in describing the response to a pathological load.

The application of the above mentioned non-linear parameters is promising, however, further research is required to better understand the welfare impact of chronic stress

and disease-indicated loads by finding clearer relations with non-linear HRV.

On the base of associations of suppressed immune state and chronic stress, another way to interpret long-term effects of challenged situations is the study of HRV together with, for example, somatic cell count (SCC) investigation and immunological tests, hereby chronic stress may be more extensively studied. Consequently, a more integrated view of stress reactions in farm animals is needed for the expansion of approaches how chronic stress can be conceptualised in research on HRV.

HRV in characterising individual traits

Previous research presumed HRV-related temperamental differences between Austrian Simmental and Brown Swiss cows (Hagen *et al.*, 2005). Till date, few studies have evaluated temperament in parallel with cardiac responses in cattle. As behavioural responsiveness to humans and milking technology can affect the behaviour, physiology and productivity of dairy cows (Sutherland *et al.*, 2012), it can be an important area of further research. An HRV-based selection of animals adapting better to the daily occurrence of challenging technological factors could improve management and farm profitability as well.

There is evidence that behavioural characteristics – similarly to temperament – are also consistent over time and individual animals exhibit different coping styles towards a stressor (Koolhaas *et al.*, 1999). Changes in the autonomic nervous system activity of animals differing in stress reactivity are suggested to be highly different (Koolhaas *et al.*, 2010); therefore, HR and HRV may effectively represent immediate autonomic responses of animals differing in natural individual variation in quantitative (stress reactivity) and qualitative (coping styles) dimensions in respect of stress. Studying HR and HRV in relation with stress reactivity and coping might also provide more detailed information on the physiological background of the human–cattle relationship as well, the importance of which has been shown earlier by several studies, on health and welfare (Rousing *et al.*, 2004) and milk production (Breuer *et al.*, 2000; Dodzi and Muchenje, 2011).

Final conclusions

On the strength of their sensitivity, HR and HRV measurements may improve our ability to assess and interpret autonomic nervous system responses to short-term and chronic stress and have significant potential to provide valuable insight also on pathological conditions of dairy cattle.

In summary, HR and HRV may have benefits when investigating acute responses to environmental challenges constituting an immediate and detailed index of stress and welfare in dairy cattle. Studying long-term effects of stress focusing especially on the non-linear components of HRV can be a suitable procedure to complement classical behavioural and HPA activity measures.

Following this integrative utilisation of HRV focusing on the aspects of stress that are most relevant to the animal's welfare

may provide valuable information on how dairy cattle handling and housing can be improved in the near future.

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