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2017

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The Heart Rate as indicator in Relation to Stress in Dairy Cows

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2017

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Chapter 1. Introduction and Aim

In this field study, we investigated heart rate as acute measure of stress in dairy cows milked in five different working farms. Our aim was to evaluate cow's stress responses during the entire milking procedure. We hypothesised lower heart rates of cows in farms working with smaller milking parlours (A and B) and in a farm where a rotary milking system was in operation (E) compared to any other farms either equipped with larger or smaller-sized milking parlours.

This paper reviews and assesses current scientific literature on the effects of various stimuli and situations on changes in heart rate in dairy cattle. These stimuli, which cause stress, are common in the normal farming and husbandry environment of dairy cattle and include changes in ambient temperature, close herding, human contact and milking techniques. An increase in heart rate represents the default response to stressful stimuli.

To accurately quantify a stress response, heart rate was measured in dairy cattle on a Hungarian working farm using a commercial non invasive heart rate monitor.

The consequences of stress on dairy cattle is well documented. It was shown that milk production and efficiency of milk yield dropped in case of heat stress (West, 2003). Grouping new cattle into an established dairy herd also proved stressful and resulted in a reduction in milk production (Philips, 2001). Transportation of cattle, especially overseas has been linked with cortisolemia, a physiological indicator of a stress response. Altered immune function and Increased disease susceptibility, with supporting evidence of a higher incidence of Bovine respiratory disease (West, 2003), has been associated with these stress factors.

By developing a better understanding of the effects of stress in dairy cattle it may be possible to improve and modify our approach to animal husbandry for dairy cattle. It is hoped that this paper and its conclusions will raise awareness of specific environmental conditions that cause stress and minimise their impact. Such improvements in approach may impact on the behavioural, physiological, endocrine and immune response, all of which may result in improvements in reproduction, growth, milk production and immune function. The economic benefits of these could be significant.

Chapter 2. Literature Overview

Definition of Stress

According to Saunders, stress can be defined as "the sum of the biological reactions to any adverse stimulus, physical, mental or emotional, internal or external, that tends to disturb the homeostasis of an organism." Such reactions could potentially be detrimental to the animal, leading to disease states (Blood, 2006).

Disorders of the mind and body are closely linked. A disease state of psychic, emotional or mental origin has the potential to be result in clinical signs, which together represent a pyscho-somatic disorder.

Post-partum dairy cattle in the first six weeks of lactation are prone to abomasal ulcers. Highly stressful conditions including a change of environment and increased production demands leave cattle vulnerable to pyscho-somatic disease. The underlying mechanism for this is unclear. Stress has a role in the stimulation of acid production in the abomasum. This results in a lower gastric pH and a consequential predisposition to the development of abomasal ulcers.

Physiology of Stress

Stress can be further sub divided into two types: **Acute** and **Chronic**.

Acute Stress

Acute stress is defined as an immediate, short-term mechanism, responsible for the activation of the 'fight or flight' reaction.

Mechanism:

The sympatho-medullary pathway governs the following sequence of events:

In response to an external stressful event in which the hypothalamus perceives an acute threat, the sympathetic nervous system is triggered. Neuronal signals are sent from the hypothalamus via the spinal cord to the adrenal gland. These endocrine glands are small and located dorsal to the kidneys. The adrenal medulla is activated and stimulates the release of noradrenaline and adrenaline. Noradrenaline, a hormone and neurotransmitter, is synthesised from dopamine in the adrenal medulla. It is released into the blood where it acts as a hormone. In addition, noradrenaline (also known as norepinephrine), is predominantly produced inside nerve axons and, acting as a neurotransmitter, travels across a synapse in response to generate an action potential. Noradrenaline is released into the circulation continuously at low basal levels. Adrenaline (also known as epinephrine) is structurally similar to noradrenaline from which it is produced, in the adrenal medulla. A small amount, however, can be produced in nerves and act as a neurotransmitter. This hormone is released only at times of stress.

The release of both of these hormones results in a wide range of responses to effectively cope with the given threat. Noradrenaline largely acts to increase and maintain blood pressure levels, providing a more specific role in times of stress. Adrenaline, on the other hand, has non-specific effects including increased heart rate, increased blood pressure, vasoconstriction, increased pupil size, increased breathing rate, increased metabolism, increased blood sugar levels, increased alertness, slower digestion and lower kidney function are all consequential reactions to the release of these hormones. The overall effect is an increase in oxygen and glucose levels to the brain and muscles while suppressing non-emergency bodily processes. Following a successful removal of a stressor, the parasympathetic nervous system returns the body to a stable, homeostatic position.

It was identified that an animal's response to stress can be divided into three forms (1) autonomic, (2) behavioural and (3) neuroendocrine (Hemsworth, 1993). Both the autonomic and neuroendocrine systems contribute towards preparing the behavioural response to a given stress. This includes an increase in heart rate, release of hormones (adrenaline, noradrenaline and catecholamines) and release of energy stores to enable a ‘fight and flight response’.

Chronic Stress

Chronic stress is defined as an ongoing, long-term mechanism, requiring the use of higher brain functions and regulated by the hypothalamic pituitary adrenal system.

Mechanism:

In response to an external stressful event in which the body perceives as a chronic threat, the sympathetic nervous system stimulates the hypothalamus to release corticotropin releasing factor into the bloodstream, marking the beginning of a sequence of events.

Corticotropin releasing factor, a peptide hormone, travels via the bloodstream to reach the anterior pituitary gland at the base of the brain whereby it stimulates the release of adrenocorticotropic hormone. ACTH is produced by corticotroph cells in the pituitary gland and is secreted intermittently into the bloodstream throughout the day, in a circadian rhythm. ACTH travels, via the bloodstream to the adrenal glands, based at the kidneys where it binds to receptors associated with the secretion of cortisol. The adrenal cortex is responsible for the production of cortisol, a glucocorticoid that regulates a wide range of bodily processes, especially in times of prolonged stress. Cortisol provides a source of immediate energy by the release of stored glucose from the liver and ensures the body to maintain stable blood sugar levels. It achieves this by the mobilisation of lipid and protein reserves and stimulates the process of gluconeogenesis for the production of glucose. Cortisol encourages the breakdown of lipids derived from adipose tissue. This results in the formation of fatty acids, which are released into the blood stream and used for the production of ATP by all bodily tissues. Cortisol also has a role in raising the pain threshold and, through inhibition of the immune system, has anti-inflammatory properties. Despite these fundamentals, an increase in cortisol blood levels adversely affects the immune system enhancing disease susceptibility, impairs cognition and raises blood pressure.

The adrenal cortex also releases aldosterone hormone, a mineralocorticoid in response to the presence of ACTH. During an alarm phase, renin is released via the kidneys into the bloodstream. A cascade of events results from this in the formation of angiotensin, a protein, which acts as the promoter of Aldosterone release from the adrenal cortex.

Aldosterone helps maintain blood volume and blood pressure by regulating the secretion of potassium and reabsorption of sodium and water in the kidneys.

The release of cortisol causes a negative feedback response to the hypothalamus. Once blood cortisol levels reach a peak threshold, the secretion of corticotrophin releasing hormone produced in the hypothalamus, and the secretion of adrenocorticotropic hormone produced in the pituitary is blocked. A fall in ACTH levels consequently leads to a drop in cortisol levels. The body has accomplished its purpose to maintain and control an equilibrial state during a stressful event.

An organised, systematic bodily response to stress should sufficiently ensue in full recovery. However, in certain cases where the control mechanisms are not enough, exhaustion and organ damage, including kidney failure can manifest. A long term active response to combat chronic stress may ultimately lead to high blood pressure, a suppressed immune system and a greater tendency to infections such as mastitis. With continuous activation, there is a possibility of heightened potassium loss or high blood sugar levels.

Stimuli of Stress (Stressors) – Definition and Categorisation

According to Saunders, a stressor is defined as, *"any factor that disturbs homeostasis producing stress"* (Blood, 2006). This is a broad term that represents a wide range of factors which generate a stress response.

Stressors can be categorised as psychological and physiological.

Physiological Stressors

Physiological stressors involve the excessive strain placed on an animal's body including nutritional, lactational and pregnancy stress. Arduous physical labour and unfavourable environmental conditions also act as physiological stressors.

Psychological Stressors

Psychological stressors evoke negative emotions to a perceived threatening or unfavourable situation. Examples include predation, human contact, absence of food and shelter, isolation, overcrowding, poor ventilation and boredom (Blood, 2006).

Other Categories:

'Absolute' and 'Relative'

Further research has re-defined stressors in terms of '*Absolute*' and '*Relative*'. Absolute Stressors are defined as those that all animals would interpret as stressful. In contrast, Relative Stressors are more subjective

'Acute' and 'Chronic'

Acute stressors are those that induce a short term stress response in animals. More specifically to dairy cattle these include oestrus, calving time, milking time, movement and handling. *Chronic* stressors that induce a prolonged stress response include poor environmental conditions. Heat stress, for example, occurs in the presence of uncomfortably hot weather, high humidity, lack of ventilation and overcrowding.

The relationship between certain stressors and their role in causing disease is dependent on the nature, magnitude and duration of exposure. An animal's vulnerability, including genetics, age, health status as well as situational habituation further determines the ability of a stressor on disease disposition (Schneiderman, 2005).

Animal Stress and Dairy Farm Practice

A newborn dairy calf is usually separated from the cow within the first few days after birth in order to commence the milking process immediately for human consumption.

Colostrum is a vital constituent to a calf's nutrition in the first three days of life. It provides maternal immunoglobulins to ensure immune protection. Depending on farming practices, this can be received either directly from suckling or obtained after cow-calf removal. In this case, the mother is milked for colostrum by the farmer, which is then fed to the calf via a nipple feeder, bottle or bucket.

Scientific research into the stress of calf separation on dairy cows is inconclusive and widely debated in terms of impact on causing stress. Some authors suggest that removal of the calf from the mother induces only a mild stress response. In one investigation, heart rate, plasma cortisol levels and behaviour of multiparous dairy cows, immediately after separation from their calf three days post partum, were examined. Increased heart rate and vocalisation were only observed in the first minutes of separation and was promptly followed by the resumption of feeding. Blood cortisol levels were not influenced (Blokhuis, 1995).

Stress Responses to Calf Separation

Premature breaking of the maternal bond is depicted as an animal welfare matter in another study. The freedom of dairy cattle to express natural maternal behaviour including licking, nursing and protectiveness towards their calf is restricted (Weary, 2007). It is implied that by delaying the separation process until instinctual weaning at around 6 - 8 months, overall health and psychological wellbeing will benefit. On the subject of health, Flower (2001) outlined a number of advantages. Potential risks of retained foetal membranes and disease post partum markedly decreased and improvement of uterine involution were noted. A study by Wagner (2015) at the University of Veterinary Medicine, Vienna, showed that separation of the calf and mother within 24 hours of birth has long term effects on social behavior and 'stress reactivity and the ability to cope with different challenges in various animal species'.

Weary et al. (2002) contradict the conclusions that cows only experience a mild stress reaction in response to calf separation, suggestive of limited emotional distress. On the birth of their calf, free living cows intuitively hide their young from the herd as a protective instinct. Sensory communication between dam and calf, including vocal and visual stimuli, are important in reassuring the calf of its mother's whereabouts. Dairy cows are naturally adapted to long-term isolation from their calf, only reuniting for suckling purposes. In case of no sensory information from the calf and no requirement for suckling, cows remain in the herd and feed. This immediate return to feeding suggests absence of distress to separation in commercial farming (Hopster, 1995). However, this is considered to be a natural response in free living dairy cows and an expression of delayed distress is more significant, when the dam attempts to relocate her calf for suckling (Weary, 2002; Flower, 2001; Compassion in food business, 2013).

Frustration and satisfaction were investigated in another study with strong attention made to eye white visualisation. This is assumed to be another indicator of emotional stress. Dairy cows separated from their calves at four days proved to have greater eye white percentage, a sign of frustration than when reunited with their calves later on, a satisfactory stimulus (Braastad, 2005).

The time at which calf separation occurs may also impact on the magnitude of the stress response. In a study conducted by Flower et al, the effects of calf removal on day 1 and day 14 post-partum were assessed. He concluded that calf separation at a later stage caused greater stress to both the cow and calf than with early separation. This investigation considered dairy cow behaviour and milk production as well as calf behaviour and weight gain. Behavioural alertness to calf removal was evident in all dairy cows. However, a more pronounced response was observed in cows after late calf separation with greater vocalisation, movement and high head posture (Flower, 2001). Similarly, this was observed by Spinka et al. (2008).

Correlating findings is the delayed development of calf social behaviour in late separated calves. These calves appeared more stressed compared with those in the early separation group upon introduction to unfamiliar calves at six weeks. During the two weeks post-partum, cows still in contact with their calves produced less milk. However, this may be a result of greater consumption by the calf who showed increased weight gain than early separated calves. Milk yield of both cow groups were similar after all calves were removed (Flower, 2001).

Parity may also influence how dairy cows respond to calf separation. Flower et al. (2001) rejected this argument in his study.

Isolated from the cow, calves are reared in pens, either individually or placed in groups. EU legislation and the maintenance of current animal welfare standards requires farmers to rear all calves aged 8 weeks old in groups. In most EU farms, animal identification is usually performed by ear tagging at the age of one week. Milk replacer, fed from a bucket or nipple feeder, equips the calf with nutritional satisfaction without the need to consume whole cow's milk provides an economical benefit to the farmer. Clean drinking water must always be accessible. In addition to milk, calves are offered hay, barley straw and eventually dried food until the calves are weaned, usually at 8 weeks old. Post-weaning calves are vaccinated and treated for parasites.

In addition to calving, female calves or heifers may be bought from markets in order to take the place of culled dairy cows, as replacement stock. Male calves born on a dairy farm may either be reared to a breeding bull or sold for veal or beef.

Stress Responses and Calving Cycles

Puberty is influenced by age, weight and breed. Heifers usually reach this at approximately 45% of mature body weight, between 9 - 11 months old. This would allow for the desired breeding age of 13 - 15 months old, in order to ensure first calving at 2 years old. A body condition score of 3 at breeding is favourable. In case of poor farm management, especially concerning heifer nutrition and healthcare, the optimal time period for puberty, breeding and calving may be delayed. As well as a greater potential for unsuccessful mating, undersized heifers calve later, risk birthing complications and produce markedly less milk than a healthy cow.

A common practice for many farms throughout Europe, especially among large dairy farms, is synchronised calving. This is achieved with the use of hormonal injections or intravaginal implant to synchronise cows' oestrus cycles (18 - 24 day cycle). This also facilitates breeding by artificial insemination, a routinely used procedure in the dairy industry. Cows may also be impregnated naturally by a bull.

Impact of high milk production farming practices and coping with stress:

The selection for high milk production has the potential to limit sufficient body resources needed to respond other bodily demands such as coping with stressors (Broom, 2010). When the burden caused by high milk yield leads to a negative energy balance, metabolic stress in dairy cows results. This overall state is indicative of starvation, an animal welfare concern and reactor for a stress response.

There are many advantages to artificial insemination (AI). As well as no maintenance required to keep a breeding bull, AI allows increased conception rates, disease prevention and the selection of genetically desired traits. However, genetic selection for increased milk production in dairy cattle can be detrimental to an animal's health and welfare rights. With increased productivity comes poor reproduction, greater disease risk and declining longevity (Algers, 2005). There has been unfavourable genetic correlations between milk yield and the incidence of ketosis, ovarian cysts, mastitis and lameness. High producing dairy cattle have greater energy requirements. To sustain the high demand, mobilisation of body reserves and functional tissue occurs. Down regulation of energetic processes such as those to maintain health and fertility is also required in order to meet the increased synthesis and secretion of milk.

A disregard to an animal's welfare is also suggested during the process of artificial insemination. Animals are often aversive to veterinary procedures, including AI and pregnancy checks, leading to stress induction.

The gestation period is 283 days. During this time, heifers are housed with the adult herd in order to familiarise themselves with the new environment and milking system. The nutritional requirements to ensure healthy growth rate are monitored.

Calving patterns differ depending on the dairy farm. Seasonal calving is the most commonly exercised technique, whereby cows are calved at a certain time of year. This has several advantages. For example, Spring coincides with plentiful food supply - grass growing period. Farm costs are lower because labour is only required at peak times. Another calving pattern is all year round calving, whereby milk production is more evenly distributed throughout the year (Compassion in World Farming, 2012).

Stress and the Lactation Cycle

As in the case of intense milking practices, which cause a significant reduction in body reserves to fight stressors, inappropriate management of the lactation cycle may cause a similar scenario.

Lactation Cycle

Post calving, cows enter the early lactation stage. Cattle Site Journal describes four phases of the milk production cycle - the early, mid and late lactation and the dry period. Numerous physiological changes occur in the cow between each phase including: changes in milk yield, nutrient requirement, body condition and gestation period.

Early lactation is defined as the period between calving and peak milk yield, lasting approximately 120 days. The optimum service period for a cow is approximately 60 days in order to achieve a desired calving interval of 12 - 13 months. This interval between calving and successful conception serves as a recuperation period following the stress of calving while allowing reproductive organs to fully recover. Consequently, lactation and pregnancy will occur simultaneously.

Milk production is a highly demanding activity. Therefore, appropriate feeding during early lactation is important in order to attain an early high peak milk yield while avoiding an excessive depletion in body condition. The impact of poor feeding management not only reduces peak milk yield but also results in reproductive problems including, infertility, delayed oestrus and poor rate of conception.

Following peak yield, cows enter mid to late lactations, both of which span for approximately 120 days. Overall milk production declines and energy demands necessary to maintain high milk yield decrease. However, energy reserves are still essential in order to support pregnancy and an appropriate body condition required for subsequent lactations.

The dry period of the lactation cycle is the interval between milk stasis and the subsequent calving. Generally, the recommended length is 45 - 50 days. Optimising milk production along with maximising economical returns are the ideal objectives of a dairy farm. A dry period longer or shorter than the proposed number of days could adversely affect milk yield of the coming lactation. As well as ensuring udder recovery in preparation for lactation, the dry period acts as an opportune to treat possible mastitis-inducing pathogens. An optimal body condition of 3.25 and adequate body reserves are critical prior to calving. Excessive or under conditioning at calving can result in dystocia, reduced milk production, reproductive failures and metabolic problems including milk fever (Managing Cow Lactation Cycles, 2015; Keown, 1993).

On average, the lifespan of a dairy cow in a commercial system is 6 years when economical loss is assumed and culling is deemed appropriate. Dependent on farm management, this typically means a cow will undergo four lactation periods in their lifetime.

Lactation Cycle – Related to Malnutrition and Starvation as Physiological Stressors

Post calving and cow-calf separation, milking commences. Depending on the dairy farm practice, cows are milked one to three times a day. Primiparous Holstein cows were studied to compare the effects of twice and thrice milking. Productivity increased with milking frequency. As feed intake remained the same as that of twice milked cows, this is indicative of larger quantities of functional tissue required for milk production (Pearson, 1990).

Malnutrition and starvation induce stress on the body and increase the susceptibility of further diseases. In another study, a reduction in milking frequency was found to influence a decline in milk quality and elevate somatic cell count and polymorphonuclear leukocyte content (Foley, 1998). As previously mentioned, a higher somatic cell count is indicative of an immune response to mastitis causing pathogens. As well as being economically unfavourable, poor health and mastitis exert additional stress upon dairy cows.

Stress and Management of Milking Parlours

Prior to entering the milking parlour, cows are detained in the holding area. Minimising waiting time before milking is essential to avoid unnecessary stress. Suitable design of these holding areas are important.

Heat Stress and Capacity of Holding Areas

It is recognised that the shape and size of the pens should be appropriate to accommodate herd capacity, to avoid heat stress. Rough textured concrete flooring to required prevent slipping. In a study conducted by the University of Wisconsin School of Veterinary Medicine, the relationship between heat stress and lameness was examined. Higher animal density results in an increase in temperature and humidity, especially in confined holding areas. According to Nordlund, the natural cooling system of a cow is more efficient while standing. This was supported by the heat stress investigation in which core body temperature was measured to be higher at lying down than while standing. An exception to this relates to cows waiting in a holding area. An explanation for this relates to the idea that holding areas provide greater heat stress potential. In theory, increased core body temperature in the holding area necessitates longer pen standing time to dissipate heat. This results in a shorter resting time, significantly increasing the risk of lameness. Appetite and milk production may also be adversely affected. For these reasons, it is considered important that a competent cooling system is installed in the holding area. Natural ventilation and supplementary cooling via circulation fans and low pressure sprinklers are considered acceptable (Hoard’s Dairyman, 2016; Atkins, 2016).

Somatic Cell Count, Udder Cleanliness and Stress

Admission into the milking stall and udder preparation is the second stage of the milking process. An integral part of the milking routine is udder hygiene. Preparation is an important step in controlling and reducing the incidence of mastitis in the dairy herd. Routines differ between farms but the overall objectives are the same. In a simplified exposition of the procedure, the teats are washed and dried, fore milk is stripped, pre dip is applied and after allowing sufficient onset of action, the teats are dried. Hygiene and maintenance of milking equipment is also fundamental in minimising pathogenic disease. Gleeson (2009) studied the effects of pre-milking teat preparation procedures on the microbial count on teats. Although they found no statistical differences between any of the disinfectant treatments applied, including chlorhexidine, chlorine and Iodine, there was a strong implication that the use of only washing and drying with paper or the total absence of preparation resulted in a higher incidence staphylococcal and streptococcal pathogens. Ultimately a lower bacterial count influences a lower somatic cell count, a general indicator of udder health (Gleeson, 2009).

Somatic cell count has also been evaluated in relation to stress (Gleeson, 2009). Six clinically healthy dairy cattle were subjected to stressful conditions involving isolation from the herd and being chased by a dog. Quarter milk samples revealed a significant increase in cell count with the most substantial rise found in cows with a previous history of mastitis (Whittlestone, 1969).

Supporting this, a similar study conducted by Wegner et al. (1976) proved that when dairy cows were confined to a heat-humidity chamber, exposed to environmental heat stress or injected with corticotrophin, high blood leukocyte level and milk somatic cell count were observed. This substantiates the importance of udder health and stress management (Wegner, 1976).

Paape et al. (1973) challenged this understanding and rejected the effect of stress on high cell count following his investigations, whereby administration of corticosteroids and ACTH failed to increase milk leukocyte concentration. Somatic cell count is subject to the influence of many factors, not only mastitis or stress. Meek (1982) reported that counts may also be affected by the time of milk collection with a higher number at evening milking compared with morning.

Elevated somatic cell count levels due to sub-clinical mastitis adversely affects milk production and milk composition. Although the magnitude of production loss varies among literature, according to Meek (1982), it may frequently 20% of a cows production potential. Another consequence of high cell count is a reduction in milk fat and lactose content and a rise in sodium, chloride and free fatty acid levels. An increase in FFAs has been reported to produce rancid flavoured milk (Meek, 1982).

The interval between pre-milking udder preparation cluster attachment has been associated with variable milking efficiency, milk quality and udder health. Milk production was found to be higher in dairy cows of a standardised milking practices compared to those subjected to a prolonged interval period. The percentage of milk fat throughout lactation was also favourably higher in standard milking. Milk flow rate was higher in delayed milked cows. The course of actual milking was therefore shorter, requiring less time attached to the machine. The implication of this may be favourable in controlling stress levels and meeting welfare standards. However, overall time spent in the milking parlour was longer (Rasmussen, 1990).

Reduced milk production and quality are supported by other authors. Investigations were carried out in three different dairy breeds of varied lactation stages. Similarities in milk yield and fat percentage were identified in all cows, regardless of genetics and age (Petersson, 1992). Although no direct reference has been made to stress, it may suggest that disrupted milk production and efficiency are a consequence of a stress response.

Delayed Milking and Stress

A reduction in milk fat percentage of delayed milked cows may be associated with stress. Raised cortisol concentrations in heat stressed cattle was found to adversely impact production potential and milk composition. As well as an endocrine misbalance, heat stress may reduce feed intake and a depletion in udder health. Ultimately, these all have the ability to negatively impact milk quality. With an elevated core body temperature, mammary gland lipogenesis declines with a subsequent reduction in milk fat content (Pragna, 2017; Das, 2016).

Familiar Environment and Milking Routine

Alterations in routine milking were made to assess its impact on behaviour and productivity in Tunisian dairy camels. Lag time between preparation and cluster attachment was varied. Delayed milking negatively affected milk yield and flow rate and significantly worsened with increased postponement. Behaviour was also influenced with further delay. At two minutes, camels showed signs of alertness and escape efforts. At four minutes, the maximal experimental wait, acute stress was evident. The importance of pre-stimulation on milking efficiency was also examined. In its absence, optimal milk removal and milk flow were disturbed. In conjunction with these assessments, consciously made loud sounds during the course of milking was performed. As expected, the camels indicated distressed behaviour. Milk ejection and peak milk flow rate were disrupted and there was an increase in residual milk. Although this study was conducted on camels, similar results are predictable for dairy cattle. Dairy animals require a familiar, efficient and relaxing environment in order to optimally perform and fulfil their milking responsibilities (Atigui, 2014).

Milk Let Down - Calf Suckling, Hand and Machine Milking

Stimulation of milk let down has changed over time with intensification of dairy farms and the demand for milk for human consumption. In place of calf suckling, hand or machine milking can induce milk ejection via tactile stimulation of the mammary gland.

Mechanism of Milk Let Down

> The milk ejection reflex involves the neuroendocrine regulation of oxytocin, produced in the hypothalamus and released in the posterior pituitary. Once released, oxytocin influences myoepithelial cell activity in the mammary alveoli which results in an increased intraalveolar pressure leading to milk expulsion into the cisternal system. The efficiency of this mechanism is critical for complete and rapid milk removal during milking.

Stress Consequences of Interrupted Milk Let Down

Inhibition of oxytocin release or the magnitude of poor milk ejection can be connected to many factors, more specifically stress. Milking by suckling resulted in higher oxytocin release than machine milking (Akers, 1982; Tancin, 1994).

An explanation for this is a stress response. Primiparous cows immediately post partum subjected to unfamiliar surroundings and unconditioned to mechanical mammary gland stimulus (hand or machine induced) had poor milk ejection. Compared with older dairy cows, oxytocin release was considerably lower (Lefcourt, 1991).

Bruckmaier, (1993 and 1997) studied the effects of milk ejection after relocating cattle to an unfamiliar environment. Secretion of oxytocin and milk yield were reduced. Elevated oxytocin levels are thought to be an indicator of emotional stress. In an animal experiment, administration of oxytocin in rats resulted in sedation - a decreased sympathetic response, lowered blood pressure and inhibit cortisol secretion (Uvnäs-Moberg, 1994). This strongly implicates the important role of oxytocin in minimising the stress response and restoring homeostasis (Taylor, 2006).

However, calf separation post-partum, did not effect oxytocin release or milk ejection. It was shown, however, that later removal of a calf resulted in a greater negative impact on milk yield. This may be because older calves require more milk - i.e higher intensity of sucking and lower amount of milk stored in the udder (Tanèin, 2001).

Attachment of the cluster unit concludes this stage and milking commences.

Design of Milking Parlours and Impact on Stress

There are many different types of milking parlour systems in use across different farms, varying from conventional or automatic, and parlour size and layout. The choice of a milking parlour and its suitability for a dairy farm is dependent on many factors including herd size, milk production potential, milking routine, other parlour uses and farmer preference (work environment).

As part of this paper, I will discuss the five main milking parlour layouts used among dairy farms.

Side opening or tandem parlours

Figure 1 illustrates a side opening or tandem parlours. These are functionally more appropriate in smaller herd sized dairy farms and in farms where individual cow care is practiced. This system allows cows to enter the parlour from the holding area and access any empty stall. Stall number varies between 4 to 12, dependent on the number of operators (one or two). On completion of milking, the cow exits its stall and the parlour via a single or dual return lane. An advantage of this system is that the parlour is organised in such a way that prevents the throughput of cows to be disrupted by a slow milking cow.

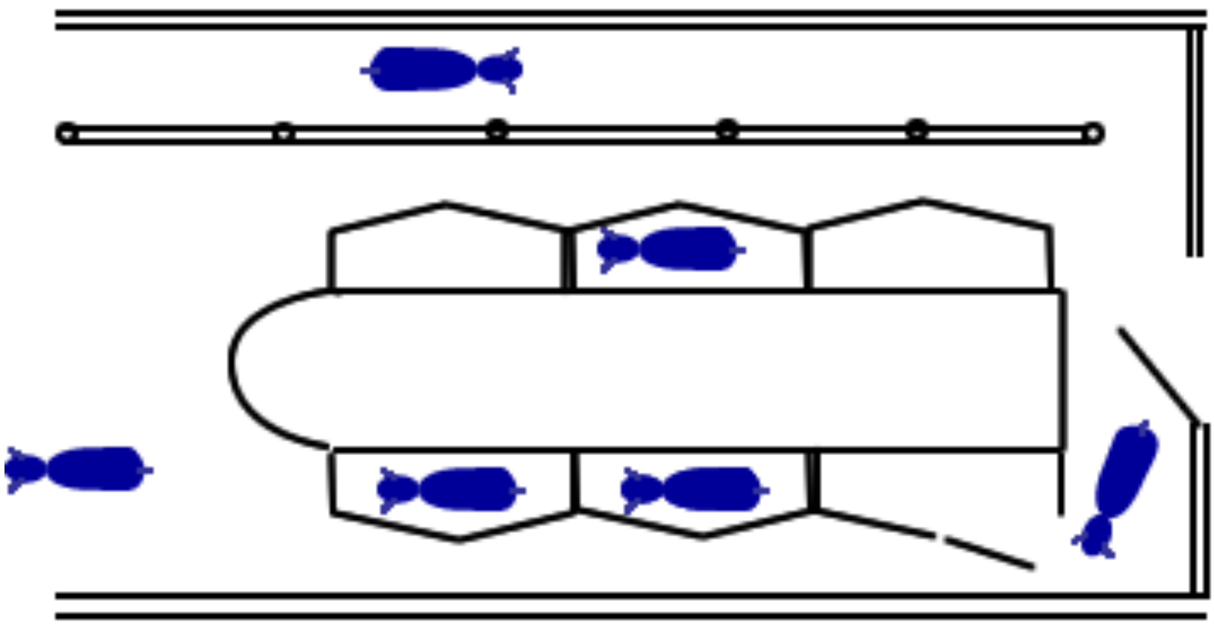

Figure 1: Side Open Milking Parlour with Single Return Lane. Adapted from Reinemann (2003).

Herringbone or Fishbone Parlour

Figure 2 illustrates a herringbone or fishbone parlour. It is identifiable due to its typical shape, giving it its name. Like most systems, this parlour facilitates direct access from the holding area, allowing cows to enter, in groups (dissimilar to side opening parlours where cows enter individually, when an empty stall opens). There are usually on average 12 number of stalls on elevated platforms each side of the operator's area. In this system, cows stand with there back half closest to the operators, exposing their udders for easy attachment of milking equipment at the cows side. On completion of milking, the cows exit the parlour in a group via a return lane.

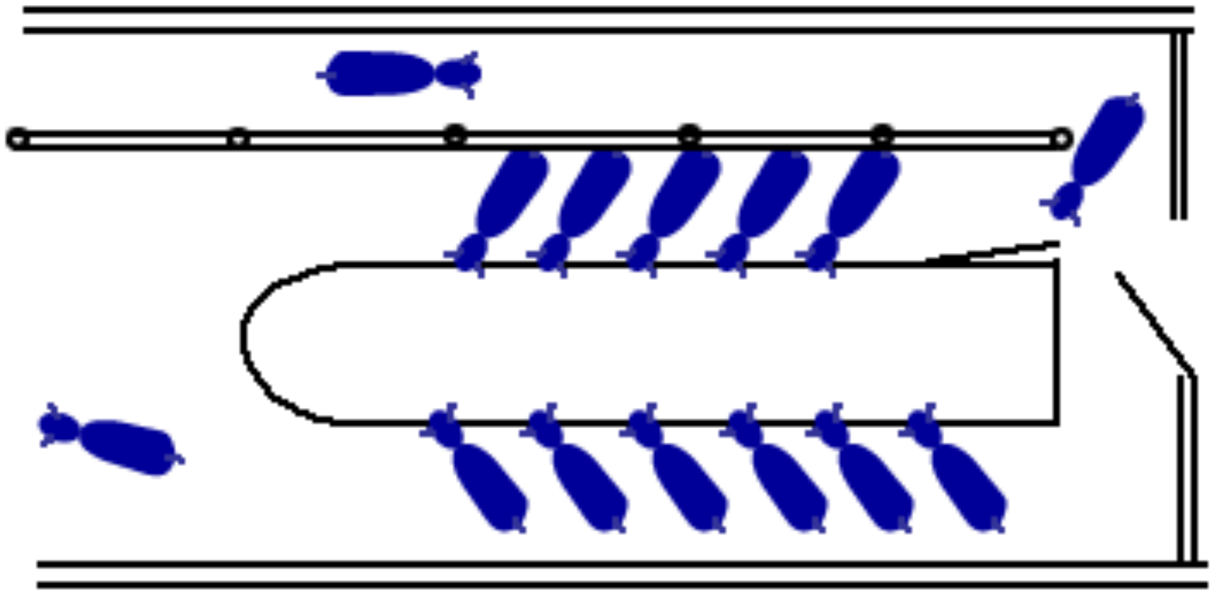

Figure 2: Herringbone Milking Parlour with Single Return Lane. Adapted from Reinemann (2003).

Parallel (or Side by Side) Parlour

Figure 3 illustrates the parallel (or side by side) parlour. Similar to the herringbone layout, this parlour operates based on the same theory, with a group of cows assembling together on raised platforms in individual stalls and depart together via return lanes once milking has been achieved. Cows stand at a 90 degree angle to the operator's area with their rear side more proximal. Attachment of milking units occurs between the cow's rear legs and thus found to be more difficult than in the herringbone parlour system.

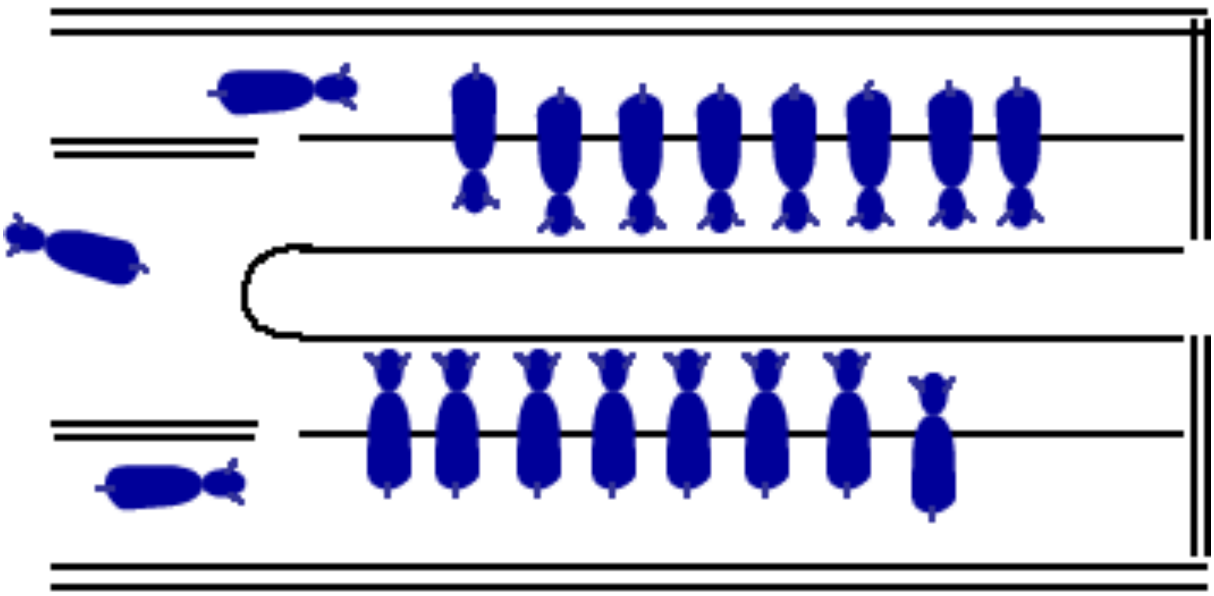

Figure 3: Parallel Milking Parlour with Rapid Exit and Two Return Lanes. Adapted from Reinemann (2003).

Rotary or Carousel Parlour,

Figure 4 illustrates a rotary or carousel parlour. Cows stand facing inwards on a circular raised platform, that continuously rotates slowly. Cow movement is largely automated into and out of the parlour, allowing three farmers to perform unit attachment, unit detachment and another on hand in case of milking problems. This system is usually more advantageous in large dairy herds due to the cost of installation.

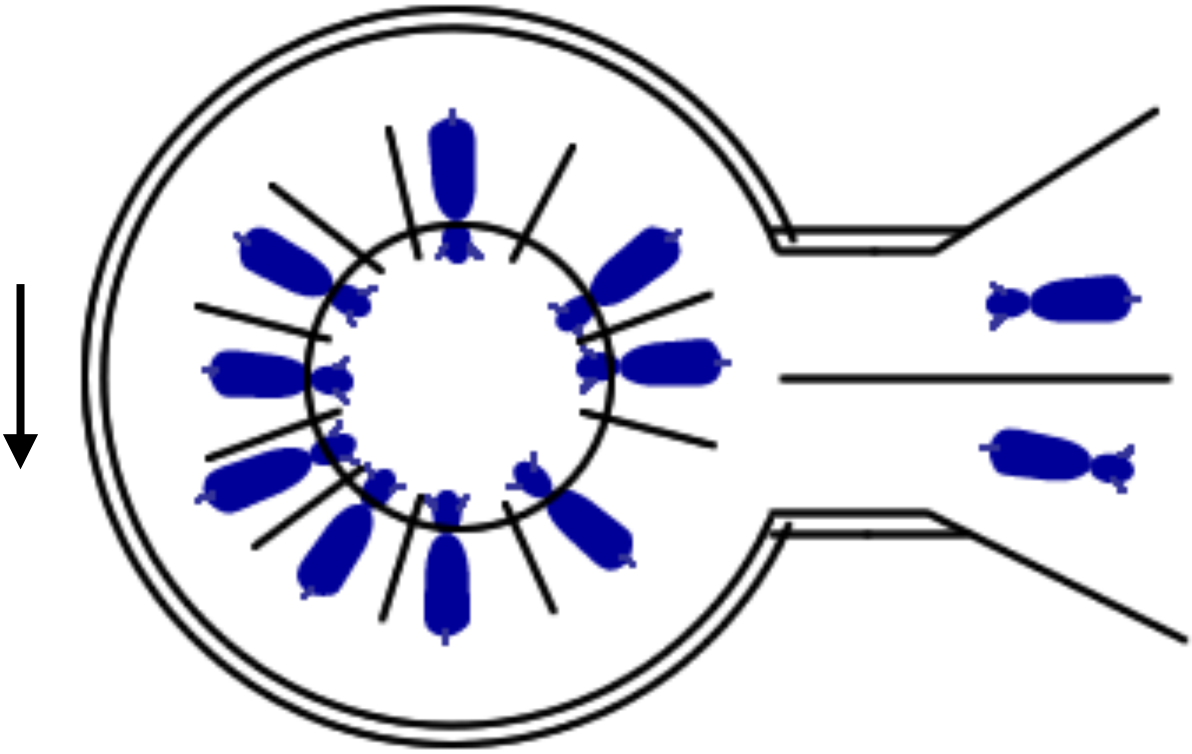

Figure 4: Rotary, Carousel Turnstile Milking Parlour. Adapted from Reinemann (2003).

Stress - Impact of Parlour Layout and Design

Gygax et al. (2008) have studied the differences in restlessness behaviour, heart rate and heart rate variability in automatic milking systems and auto-tandem milking parlours. Restless behaviour was determined by calculating stepping rates and foot lifting during milking.

Automatic Milking Systems (AMS) allow the milking process to be fulfilled without the need of manual labour. As mentioned previously, in contrast auto–tandem (side opening) milking parlours (ATM) permit the automated entrance and exit of a cow into a milking stall but requires staff to carry out and supervise milking. Gygax et al. (2008) focused their evaluation of two different automated milking system models. In each case, milking boxes were used. AMS 1 comprised of a stationary service arm on which teat cleaning brushes and cups were attached. AMS 2 involved the use of a dynamic multi purpose arm that obtained the teat cleaning equipment and cups from an area adjacent to the milking stall. The auto tandem milking parlours were evaluated on four different farms, in which cows were milked in the presence of humans. Another difference between these systems included the mechanism of teat cup removal. Automatic milking system models involve the removal of teat cups quarterly, while in auto tandem milking parlours, milking clusters were removed all at once. Dairy cattle of similar age, parity, health and physiological demands, such as milk yield, were monitored. Although feeding regimes and living conditions of the different farms were not controlled, there were no systematic differences between the milking systems. Twenty lactating cows of similar age and lactation stage, considered to be healthy and trouble free, were investigated on each farm (Gygax, 2008).

Variation in Heart Rate between different Milking Parlour Types

Gygax et al. (2008) concluded that in all three milking systems, heart rate values increased during milking compared to that at rest. A lower overall heart rate was observed by dairy cows of auto tandem milking parlours, suggesting that automatic milking system models are more stressful for cattle. A higher overall stepping rate and tendency for foot lifting and kicking was observed in cows of AMS. As indicators of increased restlessness, these results suggest that cows milked in AMS were subjected to greater levels of stress than cows of auto-tandem milking parlours. Another study, carried out by Wenzel et al. (2003) evaluated heart rate in dairy cows of AMS and ATM systems. The comparison presented similar results supporting the conclusion of Gygax et al. (2008) study.

In contrast, Hagen et Al noted lower stress levels in an AMS than in a herringbone milking parlour (Waiblinger, 2005).

A similar investigation conducted by Kovács et al. (2014), dairy cattle were observed during both conventional milking and automatic milking systems. Heart rate and heart rate variability were the focal comparable parameters during the different phases of the milking process. These phases included time spent in the holding area prior to entering the milking parlour, admission time into the parlour and udder preparation and thirdly, the main milking operation. The latter is defined as the interval between the attachment and removal of the last teat cup. Both heart rate and heart rate variability during conventional milking, were higher in all three phases compared with that in the automatic milking system, especially during the holding area. Consequential to this, it was concluded that conventional milking causes greater stress to dairy cattle. Further to this, stress levels were evaluated to be higher during the holding area phase compared with admission into the parlour, udder preparation and the main milking operation (Kovács, 2013).

Stress and Handling of Dairy Cattle

The size of farms and the use of automated, feeding and animal cleaning milking systems have steadily increased throughout Europe in the last decade. This has been accompanied by a reduction in the ratio of handler to head of cattle. The impact of these changes on the interaction between dairy cattle and handlers has encouraged considerable interest and research especially with regard to their effects on productivity and welfare in the dairy industry.

Positive and Negative Human / Animal Interaction

The interaction between dairy cattle and humans has been defined into two groupings '*positive*' and '*negative*' (Seabrook, 1994).

> **Positive interaction** is considered to include close proximity, tactile stimulus and soft talking. Close proximity is especially significant during times of milking, heating and animal cleaning.
>
> **Negative interaction**, on the other hand, refers to events where the animal handler is aversive, loud and inflicting rough or physical abuse (such as hitting the animal). Non-abusive interactions, which are in the best interest of the animal, chemical stress. These include ear tagging, dehorning and vaccinations.

Each approach has a direct effect on stress and influence productivity and welfare (Hemsworth, 1993). Physiological stress responses are heightened in those animals that experience negative interactions (Hemsworth, 1993). In circumstances where an animal is 'roughly handled' it has been shown to inflict fear, whereby the animal may perceive danger and demonstrates avoidance behaviour (Boissy, 1995). Avoidance may appear as increased kicking and agitation in the milking parlour. Other behavioural features of fear and stress include increased defecation and animal vocalisation. Higher residual milk correlates well with negative interactions (Rushen, 1999; Hemsworth, 2003). Hemsworth (1993) has identified that persistent stress for an animal has a negative impact on immune function, reproduction and feeding.

Several studies have been performed to assess fear in farm animals (Welp, 2003; Breuer, 2000; Munksgaard, 2001; Waiblinger, 2003). The main stress indicator used to access the level of fear in these studies was animal observance. There was a direct correlation between the level of animal vigilance and fear.

Several studies by Brewer (2000) and Rushen (2001) indicate that significant improvements in dairy productivity can be achieved by positive interaction between handler and animal. Such measures include include brushing in advance of the milking procedure and ensuring that the handler is familiar to the dairy cow. It is noted that these particular studies have been based largely on small dairy holdings in the true impact of changing handling methods for large commercial farming remains unclear.

Figure 5: 'Flight Zone' effects of an unfamiliar handler during herding of sheep at market. The pattern of sheep avoidance from the handler is reflected on the other side of the barrier (Grandin, 1984).

Figure 6: Effects of crouching position of handler and stroking. The pigs appear calm and comfortable in being close to the handler (Grandin, 1984).

The primary measure used in most studies to assess dairy productivity in relation to handling and stress is milk yield. A number of studies have been conducted to quantify the impact on both negative and positive interactions on milk yield. Unexpectedly the conclusions are not definitive. One study by Rushen (1999) concluded that negative interaction reduced milk yield by 70%, whereas Munksgaard's study (2001) indicated no change. The disparity between both these studies may be related to the level of intense handling by the handler (Rushen, 1999; Munksgaard, 2001).

The composition of milk in terms of protein and fat content has also been used as a measure of productivity. Breuer's study in 2000 concluded that both fat and protein content increased when the handle was familiar (Breuer, 2000).

Heart rate variability parameters provide useful non-invasive means to assess the autonomic responses to acute stress in cattle (Kovács, 2014). Kovács et al. investigated heart rate variability in both non-lactating and lactating cows in response to per-rectal palpation. They concluded that lactating cows registered lower short-term cardiac responsiveness between these two groups.

Other Veterinary Management Procedures which Induce Stress

Rectalisation (palpation of the uterus via rectum) and insemination of cattle. These routine management procedures that may result in increasing corticosteroids (Nakao, 1994). These veterinary procedures represent the most common and regular procedure for dairy cattle.

Rough handling of cows during artificial insemination has been shown to cause an increase in body temperature, increase in plasma adrenaline and lowered conception rates (Grandin, 1984).

A study by Anderson and Gantt (1966) showed that changes in heart rate was less significant in dogs when they were stroked regularly by a familiar person. Waiblinger (2003) notes that dairy cattle can be calmed in this way following a veterinary procedure.

Waiblinger's study (2003) set out to identify if positive human animal interaction affects direction of cows during a common bedroom procedure and to identify whether an animal stress response can be diminished by gentle handling. The study was based on 30 cows aged 3 to 11 years old. The handlers were instructed to calm the cows by stroking her at the neck and speaking to the cows in a soothing way. The behavioural responses from the animals were scored based on observations on the restlessness (head shaking, movement in the milking parlour) and calmness (such as standing calm returning the head away). The animal response was also monitored in regard to leaking from the hand and scored appropriately. Heart rate is recorded throughout all the test sessions using a non-invasive heart rate monitor. The results of the study support the theory a positive gentle in directions do reduce stress in cows. Specific regions of a dairy cow (the withers, neck ventral, and lateral chest) which are stroked have been studied by Schmied (2007) to determine the most effective impact on reducing stress. The study noted a particular decrease in heart rate when the animals were stroked on the ventral side of the neck.

This particular region of the cow is a common area for licking between animals. Social licking in cattle plays a significant role in strengthening and maintaining social bonds, in relieving social tension an enhancing social cohesion (Sambraus, 1969).

Grandin (2000) demonstrated that cortisol levels were lowered in trained animals in contrast to trained.

Animal Welfare Standards – Legal Obligations and Stress

The five freedoms for animal welfare include:

- Freedom from hunger and thirst
- Freedom from discomfort
- Freedom from pain, injury or disease
- Freedom to express normal behaviour
- Freedom from fear and distress

EU Directives and UK legislation recognise the importance of minimising distress and fear when caring for livestock, especially with regard to environmental design, nutrition, animal handling, animal transport, slaughter and general farm management.

Legal background:

The first legal act that was established in the UK, which offered limited protection of farm animals, was Crawl of Cattle Act 1822. In 1839-year-old is updated to the offence to ill-treatment of cattle such as beating. More substantive legislation to protect animals was introduced in 1911, the Protection of Animals Act. The main offences that were set out in this legislation included the following: Beating, kicking, ill-treating, over-riding, over-working, torturing or frightening any captive animal. 75 years later the legislation was changed again when the Animal Welfare Act 2006 was introduced. This new act included the provisions of the previous welfare laws as well as introducing a duty of care on all animal owners to provide essential basic needs, nutrition and health care, - to provide the animal with a suitable environment, diet, shelter and protection from pain, suffering, disease and injury. The act refers to Promotion of Welfare and Codes of practice. In conjunction with European law and jurisdiction relating to animal welfare the care of dairy cattle is enshrined in a code of practice document called 'Code of Recommendations for the Welfare of Livestock – Cattle'. This code of practice covers stockman-ship, health, accommodation, equipment and animal management. With regard to the care of dairy cattle handling and the design and implementation of milking parlours is specified. Special attention is paid to a need to reduce distress in dairy cattle as much as possible. A definition of distress, however, is not given.

The document supports the evidence given in studies mentioned above. In particular it encourages animals to be treated gently, to be moved at their own pace, avoidance of pressure or striking the animal in any sensitive part and the avoidance in the use of electric goads. Animal behaviour should be monitored carefully throughout. Handling pens should be efficient and easy-to-use, which allow adequate space to move.

It is especially pertinent to the codes of practice recommend the daily milk yield is recorded at least once a month for each lactating dairy cow. These figures and other available data should be used as a management tool to identify possible welfare problems at an early stage.

The code also recognises that animal distress may be caused by an acceptable waiting times in the milking parlour, failures to milk lactating dairy cows at the right time, the use of automatic backing gates that pushed the cattle rather than encourage movement towards the milking parlour, the unacceptable use of alarms used in robotic milkers, failed attachments, and incomplete milking.

Animal welfare in the UK is carefully monitored by many independent organisations and charities. The RSPCA (Royal Society Protection Cruelty against Animals) is especially prominent and it has set out clear guidance and documentation which it expects all dairy farm owners to follow and is entitled 'RSPCA welfare standards for dairy cattle (June 2011)'. In terms of handling, it specifically states that the 'behavioural characteristics of cattle must be taken into account when they are being moved, so as to avoid unnecessary fear and distress.' The document identifies that dairy cattle have poor vision for distance and detail. They are easily startled. Their hearing is similar to humans and therefore they should not be subjected to sudden loud noise. In particular their herd instinct is strong and therefore they should not be isolated. The document emphasises that animal handlers must be properly 'trained, understand the likely stress factors cattle may be subject to and to appreciate how they react towards other cattle, towards man and strange noises, sights, sounds and smells'.

A study by Rushen et al. (1999) specifically looked at the effects of social isolation as an acute stress in dairy cattle. Cattle that are socially isolated in unfamiliar surroundings have increased cortisol, prolactin, endorphin concentrations, increased heart rate, increased vocalisations and reduced oxidation responses to milking (Veissier, 1992).

Chapter 3. Materials and Methods

Farms, Animals and Housing

The study was approved by the Department of Epidemiology and Animal Protection of the Directorate of Food Chain Safety and Animal Health at Central Agricultural Office (Permit Number: 22.1/1266/3/2010). All procedures involving animals were approved by the former Ethics Committee of the Faculty of Veterinary Science, Szent István University. Measurements were carried out on five commercial dairy farms in autumn 2014 on mid-lactation multiparous Holstein–Friesian cows that were adapted to the milking systems applied. Cow population and housing conditions of the investigated farms are summarised in Table 1. Two lower-scale farms were visited, Farm A (47°27'21.7"N, 17°46'01.7"E) with a 2×4-stall herringbone milking system (DeLaval International AB, Tumba, Sweden) called hereinafter HMS1, and Farm B (47°66'30.7"N, 19°62'41.7"E) with a 2×5-stall parallel milking system (System Happel, Friesenried, Germany) called hereinafter PMS1. Large-scale farms included Farm C (47°51'21.2"N, 20°14'23.3"E) with a 2×2×12-stall herringbone milking system (Bosmark, Biatorbágy, Hungary) called hereinafter HMS2, Farm D (47°26'35.4″N 18°43'44.4″E) with a 2×2×12-stall parallel milking system (BouMatic, Madison, WI, USA) called hereinafter PMS2, while on Farm E (45°47'15.9"N, 18°25 ′56.4"E) a 72-stall rotary milking system (RMS) was operated (BouMatic Excaliber 360, BouMatic, Madison, WI, USA).

The investigated farms were similar in feeding and milking routine. In all farms, TMR was provided twice daily and water was available ad libitum. Morning milkings took place between 0430 and 0730 h, while evening milkings took place between 1700 and 2000 h on each farm.

Focal animals were selected from non-lame and clinically healthy cows with optimal BCS (means ± SD; PMS1: 2.6±0.4, PMS2: 2.8±0.5, HMS1: 2.9±0.4, HMS2: 2.7±0.3, RMS: 3.0±0.4) for their stage of DIM (means ± SD; PMS1: 153 ± 21, PMS2: 148 ± 14, HMS1: 160 ± 24, HMS2: 157 ± 18, RMS: 154 ± 9). Mean parity was similar across groups with balanced number of cows with 2, 3, 4 and 5 lactations for each milking system. Cows that were in oestrus were not involved in the study.

Heart rate data collection

Heart rate was recorded using a Polar Equine T56H mobile recording system, which included two electrodes, a Polar H2 heart rate sensor and a Polar RS800 CX heart rate monitor (POLAR, Kempele, Finland). Electrodes were covered with ultrasound transmission gel (AquaUltra Blue; MedGel Medical, Barcelona, Spain) without shaving the skin and positioned on the thoracic region as advised by von Borell et al. (2007). The electrode belt was protected against external impacts by an own designed girth made from bovine leather that was strapped around the cow's thorax behind the forelimbs. On each farm, devices were fitted to cows after animals finished with the morning milking, between 0630 and 0800 h. Heart rate recording started after a 6-h acclimatisation period and lasted until the arrival of the cows from the evening milking to the barn (between 1900 and 2030 h).

The Kubios HRV software (version 2.2, Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland) was used for analysis. Heart rate was analysed during baseline (lying calm) and during five stages of the evening milking procedure (i.e. driving, being in the holding area, udder preparation, milking and waiting after milking, *Table 1*). Selection of baseline data (lying) was based on human observation (resting). For this aim, observers recorded any disturbances occurring. Baseline date were determined by calculating the average of the HR parameter (as number of contractions of the heart per minute) spent in lying during a day.

In case of stages exceeding 10 min in length (e.g. being in the holding area) recordings were subdivided into 5-min segments where the first sample was fit to the first and the second to the last 5-min of the period of interest ("holding area 1', "holding area 2"). Start and end points of pre-milking stages (i.e. "driving" and being in the "holding area") were recorded based on direct visual observations.

Artefacts were corrected by using the custom filter, which identified IBIs differing from the previous IBI by more than 30% as artefacts. After abnormal interval removal, the algorithm of the program substitutes detected errors with interpolated IBIs calculated from the differences between previous and next accepted IBIs. In addition, a visual inspection of the corrected data was performed to edit out any artefact still existing.

In time domain, we quantified heart rate. We examined equal time periods of 5 min as recommended for analysis (ESC-NASPE Task Force, 1996; von Borell, 2007).

Statistical analysis

The HR baseline data and the deviations from the baseline at five stages of the evening milking procedure were analysed by single trait general linear models that included season, parity and farm as fixed effects, and milk yield on the day of the investigation as covariate. As result, the Least Squares Mean (LSM) and the Standard Error of Mean (SE) will be given. To measure eventually differences the Tukey's post hoc method has been used. Variance components were also calculated for effects.

In graphical presentation of the results scatterplots of HR deviations according to farms will be shown with help of lowess curve fitting. The alteration of the absolute HR values within the period of time investigated will also be displayed.

All data processing was carried out by use of program Statistic ver. 13 (Dell Inc., 2015).

Table 1 The investigated stages of the evening milking procedure

Stage	Definition	Number of analyzed 5-min heart rate samples/ cow
Driving	Time lag between letting out of the stable's gate and entering the holding area	1-2
Being in the holding area	Time lag between entering the holding area and stepping into the milking parlor	1-2
Preparation	Admission + udder preparation Admission: time lag between entering the milking stall and beginning of udder preparation Udder preparation: first contact between animal and milker until attachment of all teat-cups	1
Main milking	Time lag between the attachment and the removal of the last teat cup	1
Waiting after milking	Time lag between the removal of the last teat cup and stepping out of the milking stall with all four legs	1

Table 2 Characteristics of the investigated farms and milking systems

Farm/milking system[1]	Farm A/HMS1	Farm B/PMS1	Farm C/HMS2	Farm D/PMS2	Farm E/RMS
Number of examined cows	20	27	51	33	49
Herd size	75	80	1150	1200	1900
Housing conditions	pasture, fold	old barn, for 100 cows	5 modern barns, for 250 cows each	4 modern barns, for 350 cows each	2 modern barns, for 1000 cows each
Bedding	fold bedded with straw	rubber mattress	straw	straw	sand
Group size	No grouping	35–40	110–140	130–150	230–245
Average space allowance (m^2/cow)[2]	55.0	14.6	8.7	8.5	6.2
Milking system	2×4-stall herringbone milking parlor	2×5-stall herringbone milking parlor	2×2×12-stall herringbone milking parlor	2×2×12-stall parallel milking parlor	72-stall rotary milking parlor
Number of milking a day	twice	twice	twice	three times	three times

[1]PMS = parallel milking system, HMS = herringbone milking system, RMS = rotary milking system.
[2]Calculated for the whole barn area, including feeding place.

Chapter 4. Results and Discussion

There are statistically significant differences ($P < 0.001$) in the baseline of HR among the dairy farms. The smaller farms show lower HR (67.0 to 68.3) than the larger dairy operations (75.6 to 80.8) as it is presented in *Table 3*.

Also *Table 3* informs us that during driving the increase of heart rate was the lowest in a pasture-based system (lower-scale farm A, LSM ± SE = 20.7 ± 3.3) and the highest in lower-scale farm B with mattress-equipped cubicle system (LSM ± SE = 37.5 ± 2.9). Heart rate of cows in larger-scale farms showed similar increments during driving up to the milking parlour without any statistical differences among farms (LSM ± SE; farm C: 24.2 ± 1.7, farm D: 25.0 ± 2.5, farm E: 28.5 ± 2.3 and $P > 0.05$ for all comparisons).

Table 3: Average HR in lying and its elevation at driving up (in number of beats a minute)

Effect	Number of cows	At lying P-value VC LSM	SE	At driving up P-value VC LSM	SE
Season		P= 0.117 8.93%		P= 0.975 0.30%	
- cold (months 1-4)	65	73.2	0.97	26.9	1.90
- getting warmer (months 5-8)	73	72.6	0.84	27.5	1.62
- getting cooler (months 9-12)	42	75.8	1.17	27.1	2.27
Lactation		P= 0.362 4.19%		P= 0.083 28.90%	
First	46	72.8	1.06	24.9	2.03
Second	65	74.2	0.76	26.6	1.48
Third and more	69	74.6	0.72	30.0	1.38
Farm		P< 0.001 82.53%		P= 0.002 50.99%	
Farm A	20	67.0[a]	1.68	20.7[a]	3.26
Farm B	27	68.3[a]	1.48	37.5[c]	2.85
Farm C	51	77.6[bc]	0.87	24.2[a]	1.70
Farm D	33	80.8[c]	1.28	25.0[ab]	2.46
Farm E	49	75.6[b]	1.19	28.5[bc]	2.31
Daily milky yield, kg		P= 0.809 0.24% 0.1796*		P= 0.395 8.35% 0.1138*	
Error		4.10%		11.47%	
Overall mean	180	73.9	1.30	27.2	2.52

a, b, c – different letters mean significant (P< 0.05) differences (Tukey's post-hoc test)
* – regression coefficient
VC – variance components
LSM – least squares means
SE – standard error

In *Table 4 - 5*, it is visible that on farms A (pasture-based system with a small 2×4-stall milking parlour) C and D (cubicle housing systems and 2×2×12-stall milking parlours) similarly low heart rate changes were found either in the holding pen, during udder preparation, during milking or during waiting after being milked ($P < 0.05$ for all comparisons during all phases).

Partly controversial to the hypothesis, also farm B (small 2×5-stall milking parlour) - besides farm E (rotary milking system) - heart rate changes were significantly higher in the holding pen or during the phases of milking compared to Farms A, C and D with around twofold and threefold higher values in cases of Farm B and Farm E, respectively.

Season and parity had no effect on heart rate either during lying ($P = 0.117$ and $P = 0.362$, respectively) or during driving ($P = 0.975$ and $P = 0.083$, respectively; *Table 3*). Season and parity affected heart rate changes from baseline measured neither in the holding area ($P = 0.884$ and $P = 0.960$, respectively) nor during any phase of the milking process (preparation, milking and waiting after milking, $P > 0.05$ in all cases; *Table 4 - 5*).

Covariate daily milk yield had effect on heart rate change only during milking and during the time spent with waiting after milking in the milking stall ($P = 0.011$ and $P = 0.003$, respectively; *Table 4*).

Table 4: Change of HR in waiting area and in preparation for milking (in number of beats a minute)

Effect	Number of cows	At waiting area P-value VC LSM SE		At preparation for milking P-value VC LSM SE	
Season		P= 0.884 0.84%		P= 0.624 4.51%	
- cold (months 1-4)	65	13.3	1.81	15.1	1.93
- getting warmer (months 5-8)	73	14.5	1.59	17.0	1.65
- getting cooler (months 9-12)	42	14.1	2.18	14.4	2.33
Lactation		P= 0.960 0.28%		P= 0.607 4.78%	
First	46	13.6	2.00	16.1	2.15
Second	65	13.9	1.44	14.3	1.50
Third and more	69	14.3	1.35	16.1	1.42
Farm		P< 0.001 70.24%		P< 0.001 58.58%	
Farm A	20	8.3[a]	3.13	11.7[a]	3.29
Farm B	27	28.3[b]	2.91	26.5[b]	3.04
Farm C	51	7.6[a]	1.61	10.6[a]	1.73
Farm D	33	9.1[a]	2.37	10.3[a]	2.52
Farm E	49	16.5[b]	2.21	18.4[b]	2.32
Daily milky yield, kg		P= 0.074 21.87% 0.2002*		P= 0.126 22.59% 0.2001*	
Error		6.77%		9.54%	
Overall mean	180	13.9	2.45	15.5	2.58

Abbreviations, as before.

Table 5: Change of HR at milking and at standing after milking (in number of beats a minute)

Effect	Number of cows	At milking P-value VC LSM	 SE	At standing after milking P-value VC LSM	 SE
Season		P= 0.647 2.05%		P= 0.383 3.33%	
- cold (months 1-4)	65	9.3	1.57	7.2	1.32
- getting warmer (months 5-8)	73	10.7	1.37	9.2	1.19
- getting cooler (months 9-12)	42	11.7	1.90	9.9	1.62
Lactation		P= 0.971 0.14%		P= 0.965 0.12%	
First	46	10.6	1.71	8.5	1.48
Second	65	10.4	1.24	8.9	1.03
Third and more	69	10.8	1.16	9.0	0.99
Farm		P< 0.001 61.71%		P< 0.001 60.62%	
Farm A	20	6.5[a]	2.73	7.9[ab]	2.28
Farm B	27	24.3[c]	2.39	22.0[c]	2.06
Farm C	51	4.0[a]	1.41	2.0[a]	1.23
Farm D	33	6.6[a]	2.07	4.4[a]	1.77
Farm E	49	11.5[b]	1.92	7.6[b]	1.60
Daily milky yield, kg		P= 0.011 31.40% 0.1546*		P= 0.003 32.48% 0.0928*	
Error		4.70%		3.45%	
Overall mean	180	10.6	2.10	8.8	1.79

Abbreviations, as before.

Each curve in *Figure 7* shows that driving causes a significant increase in heart rate for all cows monitored irrespective of the farm types, - farms A, B, C, D and E. The greatest deviation in HR from the baseline was recorded for cows on Farm B that uses a 2×2×12-stall parallel milking system. This pattern of uppermost heart rate deviation on farm B is continued throughout the milking process. This graph supports our hypothesis in that the lowest heart rate deviations are to be found for those on small pasture based farm A and large scale operations using herringbone milking system (C and D). Contrary to this, the rotary milking system (farm E) resulted in elevated HR which remained above the average with the last stage of milking being an exception. Waiting after milking has the lowest impact on heart rate deviation from the baseline.

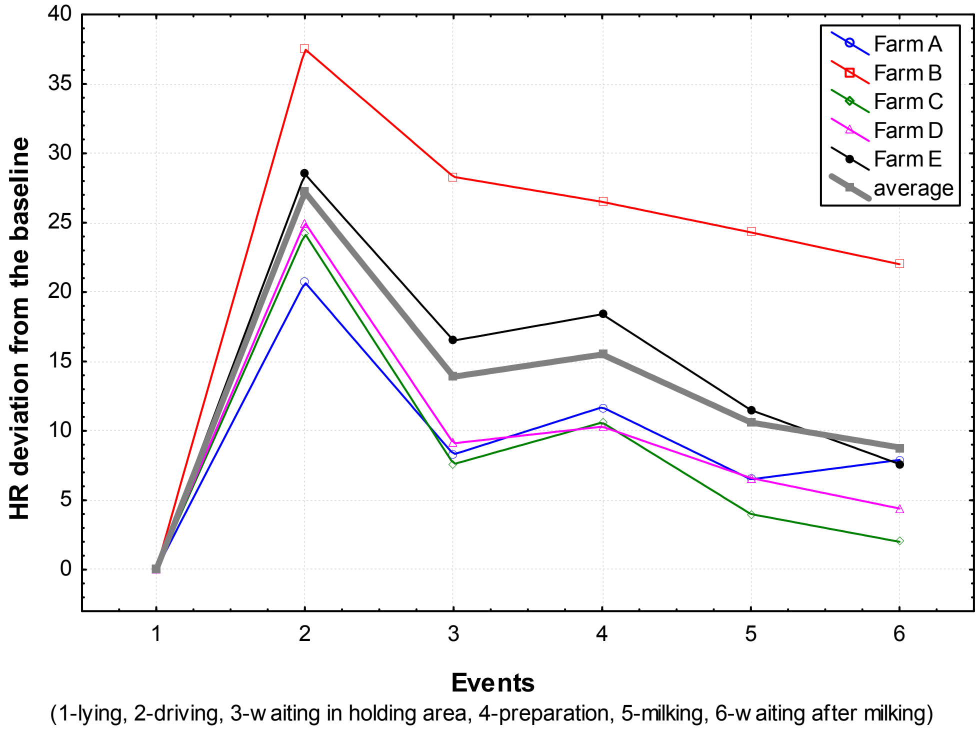

Figure 7: HR deviation from the baseline at different stage of milking according to the farms

The data presented in *Figure 8* shows similar patterns in HR as in *Figure 7*. It supports the conclusion that the HR level in milking reflects the baseline to a certain extent and the values move together. The lowest heart rate values were observed on farm A at lying and throughout the milking process. A single exception was farm B where total heart rate level greatly exceed initial predictions and contradicts this original hypothesis for this study.

The farm, which have adopted the rotary milking system, has the second highest total heart rate level during milking.

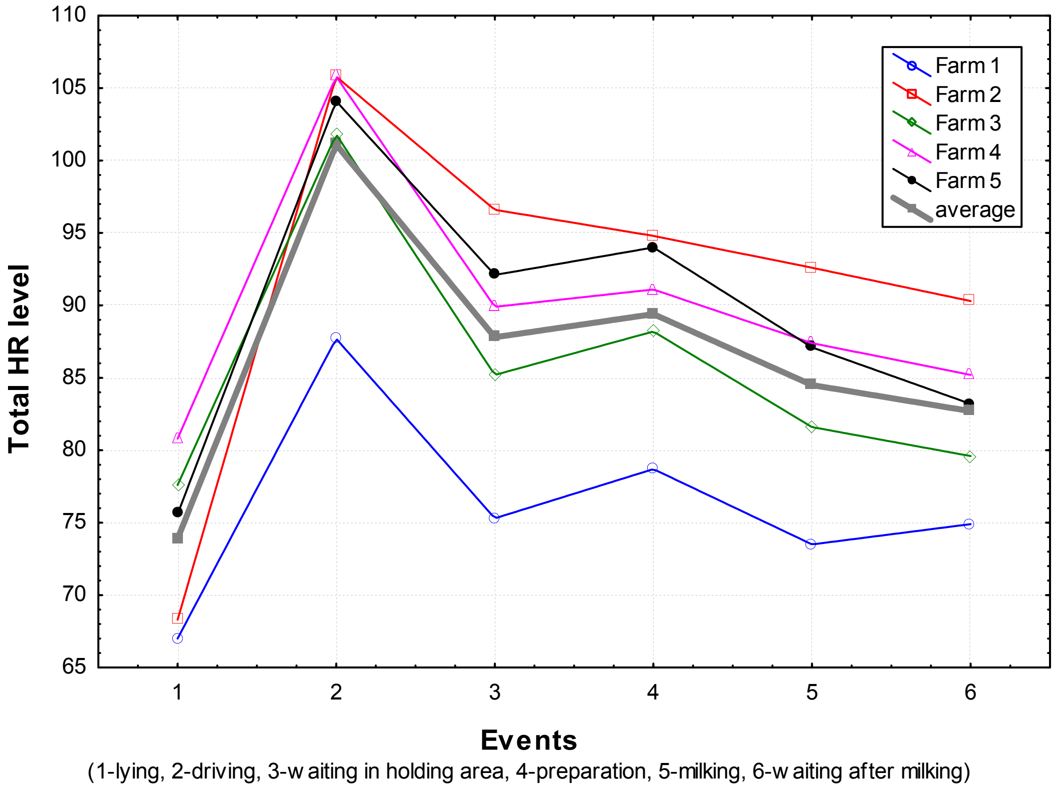

Figure 8: Total HR level in lying and at different stage of milking according to the farms

Chapter 5. Conclusion and Recommendations

This study investigated heart rate as a measure of stress in dairy cows milked in five different working farms in Hungary. The facilities, which included herringbone, parallel and rotary milking systems, and size of each farm location varied. Heart rate was recorded at different stages of milking process.

The initial hypothesis for this study expected lower heart rates would be recorded for dairy cattle on farms with smaller cow groups, milking parlours and a rotary milking system compared to any other farms equipped with larger sized milking parlours.

The Polar Equine T56H mobile device used in this study was reliable. Initial attachment of the electrode belt to each cow was a non-invasive and quick procedure that caused minimal stress. It was recognised that false readings might arise from differences in the size of each cow in relation to the proximity of the electrode sensors. Ultrasound transmission gel was used to optimise the sensitivity of the electrodes to optimise readings.

It was found that heart rate in cows was lowest on farm A (small cow group, small sized Herringbone milking system, and grazing possibility) and the highest in another lower-scale farm B (small cow group, Parallel milking system), which is contrary to our initial hypothesis. From the results, milking with rotary milking systems (farm E) appear to induce a great stress in cows. Compared with the herringbone parlour (farm C), operators of the parallel milking system (farm D) have difficulty attaching the milking units due to the angle of cow standing position. This added complexity may prolong the milking process, impacting on HR levels and stress in cows.

The results strongly suggest that the level of stress is similar for cows being milked in both large-sized farms working with large milking parlours and in a smaller-scale farm with a pasture-based system. The statistically higher heart rates of cows milked in the rotary milking parlour in the holding pen and during the milking process might be due to the high stocking density of animals in the holding area resulting in a prolonged effect on heart rate measured during milking. The high heart rate changes on farm B should be further evaluated involving more factors of the milking technology, human handling or other features that can be presumably related to the stress state of the cows during the milking process. The results of this study and their relevance to animal welfare might conclude that stress in dairy cattle can be minimised by adopting a pasture based Herringbone milking system for all stages of the milking process.

The data recorded from cows being milked at the 2x2x12 stall parallel system on farm D should be further evaluated for any possible variations in methodology. If the methods applied on this farm are consistent with those adopted on all the other farms involved in this study, then concerns about the efficacy of using a 2x2x12 stall parallel milking system, in which base HR is raised at each stage of the milking process, might be indicated. However, cows on this farm were milked more frequently (three times a day) than on other farms (with an exception of farm E, also milked three times a day but in a rotary milking system).

Cows that are milked in both farms using the parallel and rotary milking systems have equally high total heart rate level during milking. This might suggest that this system should not be the system of choice from an animal welfare perspective.

In particular it would be valuable to carry out further studies to identify why the waiting period after cows have finished the milk let down is associated with an increase in HR on farm A compared to all other farms.

This study was based on data gathered from five different farms. It is unclear if the geographical and climatic location of data sampling might be significant and which might be an area for further examination and study. The results dismiss any impact on any variation in season.

Chapter 6. Acknowledgements

I would like to express my sincere gratitude to my supervisors, Dr. András Gáspardy and Dr. Levente Kovács from the University of Veterinary Medicine, Budapest for their guidance, expertise and continuous support during my thesis work. I would also like to thank APHA, Veterinary Laboratories Agency, UK for allowing open access to their archives and assisting with my research. Finally, I am grateful for all the prior research that has been done on this topic, which has allowed me to put together this thesis.

Chapter 7. Summary/ összefoglalás

The Heart Rate as indicator in Relation to Stress in Dairy Cows

In this field study, we investigated heart rate as acute measure of stress in dairy cows milked in five different working farms. Our aim was to evaluate cow's stress responses during the entire milking procedure. We hypothesised lower heart rates of cows in farms working with smaller milking parlours (A and B) and in a farm where a rotary milking system was in operation (E) compared to any other farms either equipped with larger or smaller-sized milking parlours.

Heart rate was recorded using a Polar Equine T56H mobile recording system and analysed during undisturbed lying posture of the cows and during five stages of the evening milking procedure (driving, in the holding area, udder preparation, milking and waiting after milking). Raw data was analysed by single trait general linear models that included season, parity and farm as fixed effects, and milk yield on the day of the investigation as covariate.

During lying lower heart rates were found in lower-scale farms than in larger-scale farms ($P < 0.05$ for all comparisons). During driving the increase of heart rate was the lowest in a pasture-based system (lower-scale farm A, LSM ± SE = 20.7 ± 3.3) and the highest in lower-scale farm B with mattress-equipped cubicle system (LSM ± SE = 37.5 ± 2.9). Heart rate of cows in larger-scale farms showed similar increments during driving up to the milking parlour ($P > 0.05$ for all comparisons). On farms A, C and D similar heart rate changes were found in the holding pen, during udder preparation, milking or waiting. ($P < 0.05$ for all comparisons during all phases). Partly controversially to the hypothesis, also farm B - beside farm E - heart rate changes were significantly higher in the holding pen or during the phases of milking compared.

Our results suggest that milking is not more stressful for cows in large-sized farms working with large milking parlours than in a smaller-scale farm with a pasture-based system. The statistically higher heart rates of cows milked in the rotary milking parlour in the holding pen and during the milking process might be explained with the high stocking density of animals in the holding area that had a prolonged effect on heart rate measured during milking. The high heart rate changes on farm B should be further evaluated involving more factors of the milking technology, human handling or other features that can be presumably related to the stress state of the cows during the milking process.

A szívritmus mint a tejhasznú tehén stressz jelzője

Ebben a termelés helyszínén megvalósított vizsgálatban a szívritmust mint a tejhasznú tehén stressz állapotának azonnali mutatóját értékeltük öt üzemben. Célul a tehenek stresszre, jelen feldolgozásba a fejés alkalmával fellépő stresszre adott válaszának elemzését tűztük ki. Feltételeztük, hogy kevesebb férőhelyes fejőállásban (egyúttal kisebb üzem- és csoportméret mellett; A és B üzemek) kisebb fokú kedvezőtlen hatás éri a teheneket, mint hatalmas karusszelben (E) vagy akár más közepes méretű állásokban (C és D).

A szívritmust hordozható Polar Equine T56H eszközzel az alábbi alkalmakkor vettük fel: nyugalomban töltött fekvés és az esti fejés öt részre osztott szakaszai (felhajtás, elő-várakozó, tőgy előkészítés, fejés és fejést követő várakozás). Az alapadatokat általános lineáris egyenlettel dolgoztuk fel az idény, az ellésszám és az üzem fix hatásainak és a napi tejtermelés mint együttes változó figyelembe vételével.

A fekvési szívritmus vonatkozásában alacsonyabb értékeket kaptunk a kisebb üzemekben, mint a nagyobbakban (üzemhatás, $P < 0,05$). A felhajtás során a legkisebb szívritmus emelkedés a legelőn tartott állatok esetében volt tapasztalható (A-1 üzem, $LSM \pm SE = 20,7 \pm 3,3$), a legnagyobb pedig a matrac padozatú, egyedi fekvőhelyes istállóban tartottak esetében (B üzem, $LSM \pm SE = 37,5 \pm 2,9$). A nagyüzemi állományok tehenei egymáshoz hasonló szívritmus változást mutattak a fejőházba történő felvezetés során (üzemhatás, $P > 0.05$). Az A, C és D üzemek tehenei hasonló értékekkel voltak jellemezhetők mind az elő-várakozóban, mind pedig a fejésre való előkészítés, maga a fejés, valamint a fejést követő várakozás idején (üzemhatás valamennyi helyzetben, $P < 0.05$). Feltételezésünkkel részben ellentétben nem csak az E, hanem a B üzem teheneire is nagyfokú szívritmus emelkedést állapítottunk meg a fejés különböző szakaszaiban.

Eredményeink rámutatnak arra, hogy a fejés nagyüzemi körülmények között lényegesen nagyobb stresszt jelent a teheneknek, mint legelővel bíró kisebb üzemekben. Fejőkarusszel használatakor az elő-várakozóban igazoltan megemelkedett és a fejés során is fennmaradó magasabb szívritmus a nagyobb csoportmérettel magyarázható. A B üzem szívritmusának értelmezése olyan újabb, pl. technológiai vagy emberi tényezők bevonását teszi szükségessé, amelyek segítségével az itt tapasztalt változást jobban megérthetjük.

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Chapter 9. Table of Abbreviations

PMS	Parallel milking system
HMS	Herringbone milking system
RMS	Rotary milking system
a, b, c	Different letters mean significant (P< 0.05) differences (Tukey's post-hoc test)
*	Regression coefficient
VC	Variance components
LSM	Least squares means
SE	Standard Error

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I hereby confirm that I am familiar with the content of the thesis entitled

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