

Assessment of Cardiac Stress-Related Reactions in Lactating and Non-Lactating Milking Cows: A Heart Rate Analysis

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Abstract

The manufacturing of dairy products and milk is mostly dependent on dairy farming, with lactating (LACT) cows constituting a major contributor to this industry. Nonetheless, the physiological strain that these cows endure during the milking process is a little-studied facet of dairy farming. This research uses a thorough examination of heart rate (HR) patterns to look into the reactions associated with cardiac stress in milking cows, both lactating (LACT) and non-lactating (N-LACT). To observe cardiovascular effects of palpation per rectum (PPR) in LACT (n = 14) and N-LACT (n = 16) milking cows, we assessed parasympathetic HR variation (HRV) and HR characteristics in the current investigation. HR and HRV were monitored for 60–180 minutes before and after PPR. AUC was determined for LACT and N-LACT cows' HR and HRV specifications to compare heart reactions during PPR. AUC metrics were equal between LACT and N-LACT cows during PPR, whereas HR increased immediately. HR increased in both groups as well as the High Frequency Component (HFC) and Root Mean Square of Successive Differences (RMSSD) decreased While PPR is in progress, indicating a rise in sympathetic and a fall in parasympathetic autonomic nervous system tone. RMSSD increased quickly after PPR, indicating fast parasympathetic activity, which decreased 15 min later. The maximal RMSSD, HF and amplitude values were larger in N-LACT cows compared to LACT cows, indicating that N-LACT cows have a higher short-term cardiac reactivity. However, the examination of AUC parameters showed that the stress reaction was larger and lasted longer in LACT cows. The reaction of cows to PPR was higher significant in parasympathetic HRV than HR. According to our findings, PPR can affect dairy cows' cardiac stress responses, which could affect animal welfare. Studying lactation's effect on cardiac stress reactions can help model bovine stress sensitivity.

Keywords: Cardiac Stress, Milking Cows, Heart Rate (HR), Palpation per Rectum (PPR), High Frequency Component (HFC).

INTRODUCTION

In dairy farming, milking cow wellbeing is critical for achieving maximum productivity and animal welfare. Milking cows, whether they are lactating or not, are subject to a range of physiological and environmental stresses that can have a substantial effect on their heart health. It is essential to comprehend these animals' responses to cardiac stress to put management plans into action and protect their general wellbeing (1, 2).

In milking cows, their circulatory system is essential for maintaining general physiological processes. The proper flow of nutrients and oxygen to essential organs, such as the mammary glands that produce milk, is linked to cardiac function. Heart rhythm abnormalities brought by stress could have a domino impact on an animal's health, affecting things like milk production, reproductive success and general toughness (3).

Lactation is a metabolically taxing procedure that puts extra strain on milking cows' cardiovascular systems. Increased metabolic demands for milk production, in combination with environmental stresses, can result in distinct cardiac responses. Developing stress-reduction strategies to lessen issues associated to stress during

breastfeeding and non-lactating times requires an understanding of how the condition of nursing influences heart dynamics (4, 5).

Numerous stressors, such as poor nutrition, social interactions, temperature variations and management practices, are faced by milking cows. Stress hormones like cortisol are produced as a result of these pressures and they have an impact on the cardiovascular system. Examining the disparities in the effects of these stressors on non-lactating and lactating cows helps to clarify the intricacies of cardiac stress reactions (6, 7).

Accurate assessment of heart stress-related reactivity requires advanced methods. For example, blood biomarker analysis, electrocardiography and heart rate monitoring can provide important information about the cardiac dynamics of milking cows. Additionally, real-time monitoring is made possible by technological advancements that allows for a deeper understanding of the long-term effects of stress on the cardiovascular system (8).

Heart-related stress responses in milking cows have an immediate effect on welfare and productivity in addition to the physiological ramifications. Altered eating along with reproductive patterns and possibly weakened immune systems, stressed cows are more prone to health issues. Understanding these effects is essential for creating comprehensive management plans that prioritize animal welfare and production efficiency (9, 10).

Study (11) examined the hypothalamic-pituitary-adrenal axis in relation to lactation in Holstein-Friesian cattle after artificial insemination (AI), embryo transfer (ET), milking, veterinary along with ultrasound examinations, hoof trimming and natural breeding. Cortisol levels were assessed before, during and after each shock in 24 randomly allocated cattle with control and treatment groups. Hoof trimming (HT) and natural breeding (NB) extended serum cortisol rise. In stress-inducing techniques, controllability and predictability are crucial since Holstein-Friesian cattle react differently to diverse stimuli.

Study (12) examined the function of autonomic nerve, as measured by blood pressure and heart rate variability (HRV) parameters in Friesian-Holstein cattle among clinically healthy (CH) puerperal cows and those experiencing postpartum fever (PF). Holter-type electrocardiograms and power spectral analysis were used to measure HRV. Compared to CH cows, PF cows had faster heart rates, lower HRV parameters as well as altered blood parameters, indicating sympathetic nerve function and inflammation.

Study (13) analysed oxytocin's (OXT) neurophysiologic modulation, effects on mother behaviour, bonding and breastfeeding in domestic animals after parturition. OXT and hormones including Prolactin, estradiol, cortisol, relaxin, connexin and prostaglandins are the hormones that were examined in mammalian birth, maternal behaviour, imprinting, social cognition and affiliative behaviour. OXT was important for parturition, maternal behaviour, bonding and breastfeeding in domestic animals.

Study (14) evaluated the consequences of a high-concentrate (HC) on the diet oxidative stress in goat livers and the related molecular pathways. Twelve goats were received a nutrition of low-concentrate (LC) or a high-concentrate (HC) diet plan for five weeks and analyses of antioxidant parameters, gene expression and protein levels revealed that HC-fed goat's livers had decreased NRF2-dependent antioxidant responses and increased glucocorticoid receptor (GR) nuclear translocation.

Study (15) demonstrated that because of changed insulin, glucose metabolism and skeletal muscular activity, contemporary dairy calves under heat stress have a large negative energy balance (NEBAL). To assist, the physiological responses and general welfare of heat-stressed cows, mitigation options include evaluating and placing cooling systems into place, treating dietary deficits and investigating metabolic and antioxidant supplements.

Study (16) investigated whether prolactin, cortisol and stress behaviour were connected in dogs that are sheltered. Prolactin and cortisol were measured in samples of blood using a kit for hormone-specific ELISA. The results demonstrated a slight negative connection between the amounts of prolactin and cortisol. The two hormones weren't connected; their levels appear to be in line with the stress score, either changed by expressing fear gripped in the collection room.

Study (17) explored the metabolic adaptability of high-yielding dairy cows to heat stress. Various physiological indicators, including as water and feed intake, vital signs, milk output and blood samples, were recorded and evaluated to determine the metabolic effects of heat stress on the cows. The results indicate the need of understanding the physiological stage of dairy cows when creating future dietary solutions to reduce health and performance deficiencies caused by ambient heat.

Study (18) examined how inflammation, pre-partial diet as well as overfeeding affect dairy cow lactation and offered New Zealand farmers practical advice. In three trials, hepatic and adipose tissue gene expression, transcriptomes and metabolic pathways were studied. Transitional results were studied from various treatments and dietary methods. Avoid early postpartum pharmaceutical therapies and concentrate on adequate prepartal nutrition for dairy cows, emphasizing body condition score.

Study (19) investigated to find out how a compound of traditional Chinese herbal medicine (TCHMC) affected the dairy cows ability to produce throughout their peripartum period. Eighteen pregnant Holstein dairy cows who are not nursing, 1 to 2 parity and comparable body conditions were divided into three groups at random (n = 6) and from 14 to 9 days prepartum, they were fed a baseline diet containing 200 (T-200 group), 300 (T-300 group) and 0 (CON group) g TCHMC daily. The results showed that the TCHMC treatments shortened the time it took for a calf to reach its first service, first visible estrus and days during pregnancy.

Study (20) was to compare actively cooled (CL) and non-cooled (HT) dry cows to identify temperature-humidity index (THI) thresholds for heat strain indicators. At a THI of 77, dry cows without active cooling exhibited higher rectal temperature and respiration rate, emphasizing the significance of monitoring and adopting treatments to minimize heat-stress-related difficulties throughout the dry phase and after lactation.

The purpose of this research was to compare and examine the heart rate variability and other cardiac stress-related reactions in lactating and non-lactating milking cows during peristalsis per rectum (PPR). This analysis aimed to shed light on the physiological and management-related variations that might affect the sensitivity of cows to stress in dairy farming environments.

MATERIALS AND METHODS

This research uses heart rate analysis to explore cardiac stress-related responses in lactating and non-lactating milking cows. The investigation intends to identify any physiological variations via heart rate pattern comparisons, offering important new information about the health of lactating dairy cows.

Animals and Shelter

A total of 30 multiparous [14 lactating (LACT) and 16 non-lactating (N-LACT)] clinically healthy Holstein-Friesian cows were chosen from large-scale herd (4,000 lactating cows). Table (1) illustrate the covariates value of Age, Parity and BCS were comparable across the LACT and N-LACT groups. Cows were kept in sand-filled free stall barns. Self-locking headlocks were installed throughout the whole length of the feeding area. Cows locked themselves in place after being milked when they stuck their heads in the stanchions to feed. On the farm, frequent inspections and treatments were performed while the animals were bound and standing after eating.

Table (1). Mean value (Source: Author)

Covariates	LACT	N-LACT
Age	4.8 ± 2.3	4.5 ± 1.1 year
Parity	4.2 ± 1.9	3.6 ± 1.7
BCS	4.1 ± 1.6	4.3 ± 1.8

This kind of feeding and restraint system has been in use for many years. The animals were given a total mixed feed once per day at 700 h and had unrestricted access to water. Lactating cows were milked three times per day in a 78-stall rotary milking parlour. Non-lactating animals were pregnant and had at least 10 days after drying off and 20 days before calving. Lactating animals were not pregnant and had experienced recent PPR exposure.

HRV Analysis

Interbeat Intervals (IBI) from 60 minutes before the assessment to 180 minutes following was employed in the analysis. A custom filter was used to remove any artifacts. Interbeat intervals that differed by greater than 40% from the preceding IBI were classified as artifacts. For analysis, an average error rate of 7% was acceptable. In addition, a visual assessment of the restored data was undertaken to remove any remaining artifacts. Due to artifacts, only one 5-min time frame from the baseline measurements and four 5-min time windows from the post-PPR recordings were used. IBI data between 160 and 180 minutes after PPR were not obtained for 1 cow due to equipment failure.

Before using Fast Fourier transformation (FFT) for analysis, segments of 512 IBI were assessed for IBI time series interpolation. HRV was analyzed during the following periods: (1) 60 minutes before PPR (Pre-PPR); (2) 5 minutes after PPR and (3) 180 minutes after PPR (Post-PPR). The mean results in the 20 minutes before the assessment were utilized as the baseline. The time domain measurements examined were mean HR and RMSSD. FFT was used to compute (high-frequency component) HF, a frequency domain parameter reported in normalized units. Recommendations were met by limiting the spectral components (low frequency = 0.07-0.30 Hz and high frequency = 0.30-0.68 Hz). Earlier reports on dairy cattle for HRV study utilized this frequency band width for the HF power.

Statistical Analysis

A generalized linear model technique with penalized quasi-likelihood was used to assess heart rate, RMSSD and HF. The Kolmogorov-Smirnov test was used to visually assess the models residuals for variance distribution and homogeneity. Because the data were not normally distributed, the HR, RMSSD and HF parameters (as dependent variables) were transformed before analysis. As fixed variables, covariates (parity, condition and age) were introduced to the model. The Bonferroni correction was employed for intra-group comparisons of HR and HRV data. A ($P < 0.05$) value was considered significant.

Changes in HR and HRV characteristics were computed in terms of area under the curve (AUC) to reduce the number of statistical comparisons between collectives throughout the Post-PPR phase. The AUC represents the magnitude and changes in the response over time and simplifies statistical analysis using multivariate data transformation into univariate space when the quantity of successive measurements is large and it is necessary to compile the information. The parameters studied were the baseline and maximum values of HR, RMSSD and HF; the amplitude of the HR, RMSSD and HF response and long-term measurements of cardiac responses to the PPR (AUC response and time to return to baseline). Using a technique, the area under the response curve AUC_{RESP} was calculated for the time it took to return to baseline as shown in Equation (1).

$$AUC_{RESP} = \sum \left[\frac{C_n + C_{n+1}}{2 \times h - baseline} \right] \quad (1)$$

where C is the cardiac parameter (HR, RMSSD, or HF) at a specific moment in time, h is the difference between the two C values in hours, where baseline is the mean value during the last 20 minutes of collected HR and HRV data prior to PPR. The AUC 60 minutes before PPR for the LACT and N-LACT groups AUC_{PRE} was computed. Because there was no response beyond baseline levels of either HR or HRV parameters to measure, the area was estimated simply as follows in Equation (2):

$$AUC_{PRE} = \sum \left[\frac{C_n + C_{n+1}}{2 \times h} \right] \quad (2)$$

Absolute values for the AUC_{PRE} and AUC_{POST} parameters are given AUC parameters were compared across groups using the nonparametric Friedman test since numerous parameters showed significant non-normality. A significant threshold of ($P < 0.06$) was established.

RESULTS

The HR changes in the LACT and N-LACT cows throughout the trial followed a similar trend Figure (1). HR did not alter in either group over the Pre-PPR period. HR during PPR ($P < 0.001$, in both groups), declined in the first five minutes after PPR and stabilized at baseline levels. HR amplitude did not vary across groups, despite baseline and maximal HR in the LACT group are greater ($P < 0.001$, in both instances) than in the N-LACT group Table (2). Lactation had no influence on the Pre-PPR or Post-PPR periods, according to the AUC study. Following the restoration to baseline, HR in the two groups under study remained comparatively consistent, with an average for LACT cows that was marginally below physiological level and an average that was marginally above it Figure (1).

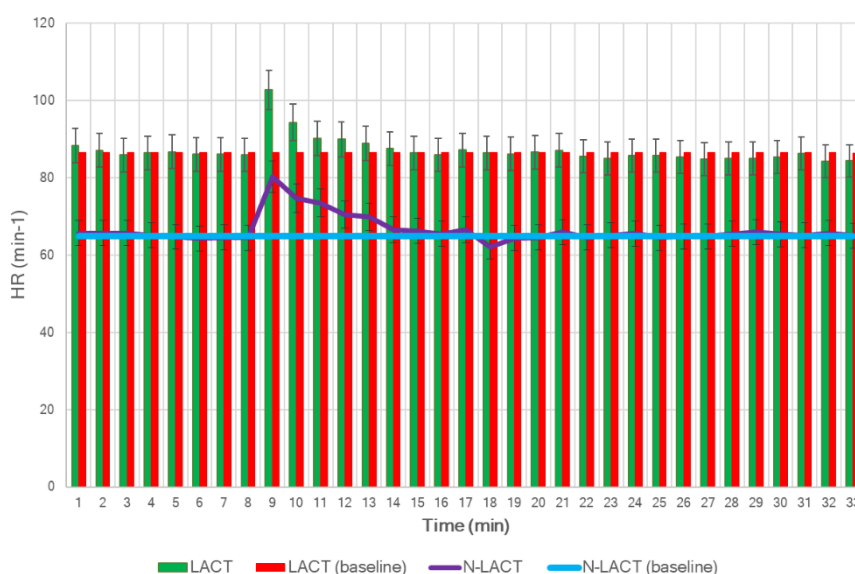


Figure (1). Variations in the heart rate (HR; beats per minute) (Source: Author)

Table (2). Mean value of LACT and N-LACT $HR_{response}$ parameter (Source: Author)

$HR_{response}$ parameter	LACT		N-LACT	
	Mean	Standard deviation	Mean	Standard deviation
AUC_{PRE} (beats)	14.9	15.8	27.2	18.5
Amplitude of response (beats/min)	16.5	3.6	16.2	4.6
AUC_{PRE} (beats)	183.1	86.6	278.8	112.1
Baseline values (beats/min)	87.3	6.5	65.9	5.6***
Maximum values (beats/min)	103.7	6.8	82.0	5.7***
Time to return to (baseline min)	30.8	22.1	38.5	13.2

Similar to HR, neither group's root mean square of consecutive differences changed over the Pre-PPR period Figure (2). The LACT group had a lower baseline RMSDD than the N-LACT group ($P < 0.001$); Table (3) shows that the AUC_{PRE} values of both groups were similar. RMSDD dropped in N-LACT and LACT cows during PPR ($P < 0.001$ and $P < 0.01$, respectively), which was consistent with the abrupt drop in PNS

activity. In both groups, the RMSSD during the first five minutes of Post-PPR surpassed the baseline ($P < 0.001$). In the LACT and N-LACT groups, the average growth rate was 24.4 and 41.7%, respectively. When comparing the LACT group to the N-LACT group, the RMSSD maximum and amplitude were less. RMSSD Required a longer time and AUC_{PRE} was higher in LACT cows than in other cows.

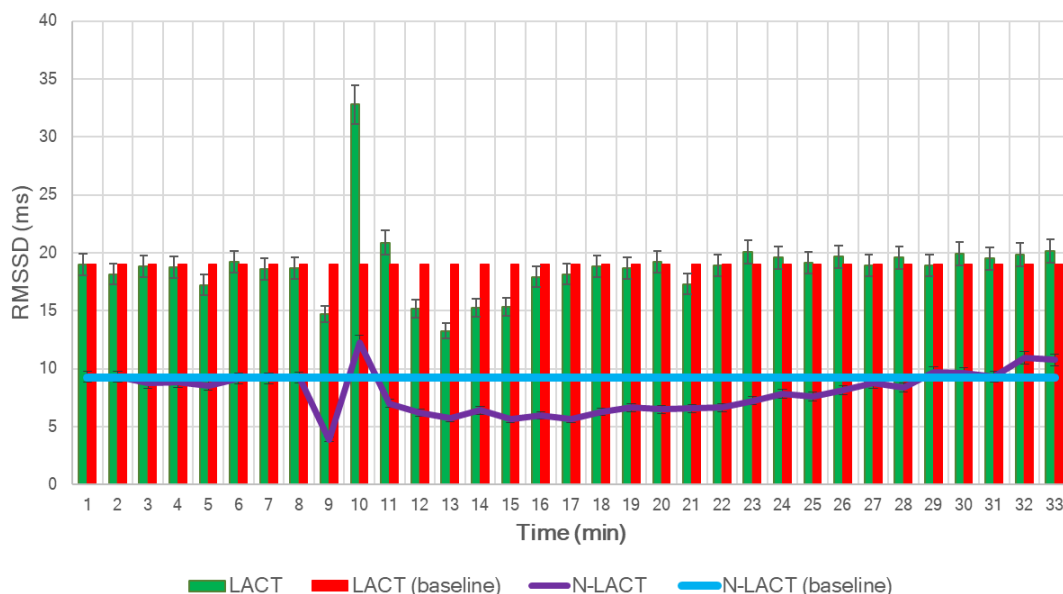


Figure (2). The successive difference root mean square (RMSSD) (Source: Author)

Table (3). Mean value of LACT and N-LACT $RMSSD_{response}$ parameter (Source: Author)

RMSSD _{response} parameter	LACT		N-LACT	
	Mean	Standard deviation	Mean	Standard deviation
AUC_{PRE} (ms × min)	17.9	25.6	22.4	47.6
Amplitude of response (ms)	5.6	3.8	15.8	13.2**
AUC_{PRE} (ms × min)	195.4	93.8	17.9	207.3*
Baseline values (ms)	9.8	1.2	19.9	3.3***
Maximum values (ms)	34.8	14.8***	12.8	5.5
Time to return to baseline (ms)	88.4	18.9	43.6	14.4***

Baseline HF in LACT compared to N-LACT cows was reduced ($P < 0.001$), in accordance with RMSSD. Both groups had a decrease in HF during PPR ($P < 0.001$) Figure (3). In LACT cows, the average rate of decrease was 28.5%, but in N-LACT cows, it was 38.1%. The groups HF area under the curve parameters varied as well. In HF, N-LACT cows exhibited a bigger AUC_{PRE} ($P < 0.001$) and longer duration to return to baseline ($P < 0.001$), whereas LACT cows displayed higher amplitude ($P < 0.05$) and a higher maximum in the 5 minutes after PPR ($P < 0.001$), which is shown in Table (4)

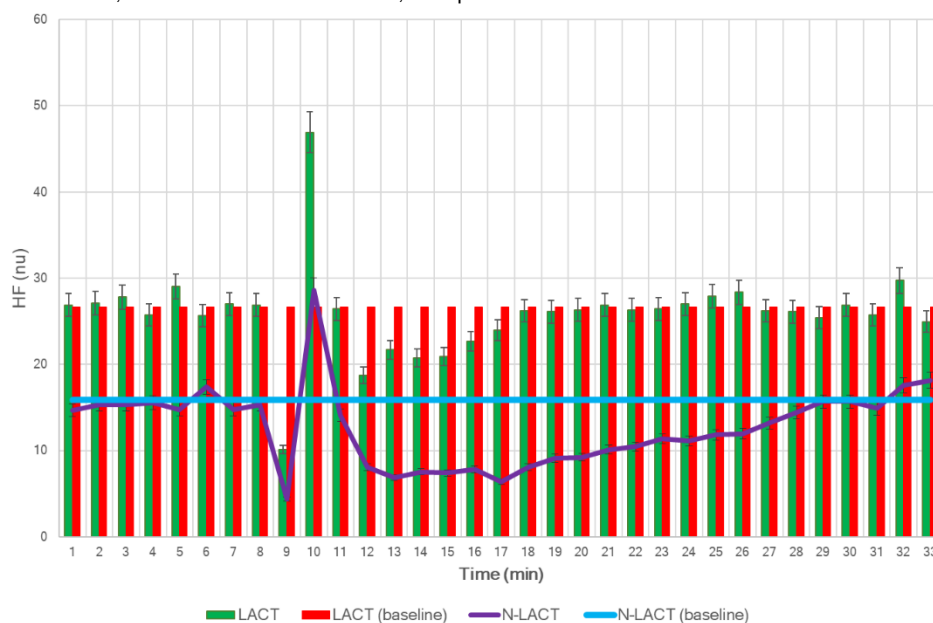


Figure (3). The HRV high frequency component in both lactating and non-lactating cows (Source: Author)

Table (4). Mean value of LACT and N-LACT HF_{response} parameter (Source: Author)

HF _{response} parameter	LACT		N-LACT	
	Mean	Standard deviation	Mean	Standard deviation
AUC _{PRE} (nu × min)	45.3	42.8	21.8	39.4
Amplitude of response (nu)	13.9	6.5	20.6	4.6*
AUC _{PRE} (nu × min)	485.2	260.4	86.9	191.7***
Baseline values (nu ³)	16.3	4.3	25.8	5.5***
Maximum values (nu)	29.7	6.5	46.1	6.6***
Time to return to baseline (min)	103.8	7.8	55.2	18.6***

DISCUSSION

When stillness or surroundings that the animal associates with unpleasant past experiences can cause minor stress reactions, baseline recordings can be distorted even in the absence of manipulation (21). Our study's cows were monitored during their routine feeding sessions rather than being housed in isolation. Notwithstanding their confinement and the presence of researchers, the animals were silent throughout and after PPR. We can conclude that the observed cardiac responses are a result of the stress induced by the PPR process. A 15 beats per minute rise in heart rate was noted on average (22) when they were examining the short-term stress reactions after castration in bull calves without the use of local anaesthetic. HR began to decline two minutes after the surgery ended, according to those researchers. The decline in our research was more gradual, owing to the differing degree or form of pain. During PPR, RMSSD and HF fell in both groups, indicating a quick decrease in PNS activity, which was consistent with (23) polyvagal theory that painful stimuli elicit a decrease in vagal tone.

The AUC approach, which has been widely used in endocrine-logical investigations on several species, was used to describe the size and duration of cow's cardiac responses. In previous studies, HR and HRV (i.e., RMSSD) were reported as AUC in horses traveling by road (24) and the technique was shown to be helpful in differentiating between the stress levels of horses throughout various travel times. Lactation had no influence on

the size and length of the HR response, according to our study's sections below the HR curve and time to get back to the initial state values, which did not represent the variations in stress-responsiveness between N-LACT and LACT animals.

In HF values, these variations were more apparent. It is worthwhile to look into whether these lactation-associated distinctions are connected to physiology or management. The greater physiological load associated with breastfeeding can be a reason for the extended drop in PNS tone found in LACT cows throughout the Post-PPR period; however, a previous investigation (25) did not find any cardiac differences between LACT and NLACT cows. It is significant to remember that the HRV parameters at rest were evaluated in the study (26). The PNS indexes of HRV in our study characterized variations in the short or long-term stress response to PPR.

As a result, table (2), (3) and (4) illustrate measures of cardiac function and heart rate (HR) response, namely the high frequency component (HF) of HR variability (HRV) determined as the area under the curve (AUC) in non-lactating (n = 16) and lactating (n = 14) cows before, during and after palpation per rectum (PPR).

CONCLUSION

During palpation per rectum (PPR), this study provides significant new data on the cardiac stress responses of lactating (LACT) and non-lactating (N-LACT) milking cows. N-LACT cows showed greater short-term cardiac responsiveness, despite the fact that both groups experienced sudden increases in heart rate (HR) and changes in parasympathetic heart rate variation (HRV). Remarkably, in spite of these variations, lactating cows showed a greater and longer stress response during PPR. In order to improve animal welfare policies in the dairy sector, a more detailed knowledge of these physiological dynamics is necessary. The research emphasizes the possible influence of PPR on the cardiac stress responses of dairy cows. Modeling the stress sensitivity of cows can benefit from more research on the impact of lactation on cardiac stress responses.

REFERENCES

- [1] Soumya, N. P., Banerjee, R., Banerjee, M., Mondal, S., Babu, R. L., Hoque, M., ... & Agarwal, P. K. (2022). Climate change impact on livestock production. In *Emerging Issues in Climate Smart Livestock Production* (pp. 109-148). Academic Press. DOI: 10.1016/B978-0-12-822265-2.00010-7
- [2] Mee, J. F., & Boyle, L. A. (2020). Assessing whether dairy cow welfare is “better” in pasture-based than in confinement-based management systems. *New Zealand Veterinary Journal*, 68(3), 168-177. DOI:10.1080/00480169.2020.1721034
- [3] Bienboire-Frosini, C., Muns, R., Marcet-Rius, M., Gazzano, A., Villanueva-García, D., Martínez-Burnes, J., ... & Mota-Rojas, D. (2023). Vitality in Newborn Farm Animals: Adverse Factors, Physiological Responses, Pharmacological Therapies, and Physical Methods to Increase Neonate Vigor. *Animals*, 13(9), 1542. DOI: 10.3390/ani13091542
- [4] Surai, P. F., Kochish, I. I., Fisinin, V. I., & Juniper, D. T. (2019). Revisiting oxidative stress and the use of organic selenium in dairy cow nutrition. *Animals*, 9(7), 462. DOI: 10.3390/ani9070462
- [5] Islam, M. A., Lomax, S., Doughty, A., Islam, M. R., Jay, O., Thomson, P., & Clark, C. (2021). Automated monitoring of cattle heat stress and its mitigation. *Frontiers in Animal Science*, 2, 60. DOI: 10.3389/fanim.2021.737213
- [6] Berget, B., Vas, J., Pedersen, G., Uvnäs-Moberg, K., & Newberry, R. C. (2023). Oxytocin levels and self-reported anxiety during interactions between humans and cows. *Frontiers in Psychology*, 14. DOI: 10.3389/fpsyg.2023.1252463
- [7] EFSA Panel on Animal Health and Welfare (AHAW), Nielsen, S. S., Alvarez, J., Bicout, D. J., Calistri, P., Canali, E., ... & Herskin, M. (2022). Welfare of cattle during transport. *EFSA Journal*, 20(9), e07442. DOI: 10.2903/j.efsa.2022.7442
- [8] Napolitano, F., Arney, D., Mota-Rojas, D., & De Rosa, G. (2020). Reproductive technologies and animal welfare. In *Reproductive technologies in animals* (pp. 275-286). Academic Press. DOI: 10.1016/B978-0-12-817107-3.00017-5
- [9] Shaji, S. (2021). New approaches to the impact of heat stress on production in dairy cattle. DOI: 10.25814/rxjx-3g23

- [10] Silva, T. H. D. (2021). Alternative strategies and new insights into immunology, health, and performance of dairy cattle (Doctoral dissertation, Universidade de São Paulo). DOI: 10.11606/T.74.2021.tde-03052021-141531
- [11] Koenneker, K., Schulze, M., Pieper, L., Jung, M., Schmicke, M., & Beyer, F. (2023). Comparative assessment of the stress response of cattle to common dairy management practices. *Animals*, 13(13), 2115. DOI: 10.3390/ani13132115
- [12] Aoki, T., Itoh, M., Chiba, A., Kuwahara, M., Nogami, H., Ishizaki, H., & Yayou, K. I. (2020). Heart rate variability in dairy cows with postpartum fever during night phase. *PLoS One*, 15(11), e0242856. DOI: 10.1371/journal.pone.0242856
- [13] Mota-Rojas, D., Marcet-Rius, M., Domínguez-Oliva, A., Martínez-Burnes, J., Lezama-García, K., Hernández-Ávalos, I., ... & Bienboire-Frosini, C. (2023). The Role of Oxytocin in Domestic Animal's Maternal Care: Parturition, Bonding, and Lactation. *Animals*, 13(7), 1207. DOI: 10.3390/ani13071207
- [14] Wang, Y., Salem, A. Z., Tan, Z., Kang, J., & Wang, Z. (2021). Activation of glucocorticoid receptors is associated with the suppression of antioxidant responses in the liver of goats fed a high-concentrate diet. *Italian Journal of Animal Science*, 20(1), 195-204. DOI: 10.1080/1828051X.2021.1873706
- [15] Sammad, A., Wang, Y. J., Umer, S., Lirong, H., Khan, I., Khan, A., ... & Wang, Y. (2020). Nutritional physiology and biochemistry of dairy cattle under the influence of heat stress: Consequences and opportunities. *Animals*, 10(5), 793. DOI: 10.3390/ani10050793
- [16] Gutiérrez, J., Gazzano, A., Pirrone, F., Sighieri, C., & Mariti, C. (2019). Investigating the role of prolactin as a potential biomarker of stress in castrated male domestic dogs. *Animals*, 9(9), 676. DOI: 10.3390/ani9090676
- [17] Vailati Riboni, M. P. E. (2019). Management during the dry period and its effect on hepatic and adipose tissue molecular biomarkers of metabolism and health in grazing dairy cows. DOI: 10.1186/s12864-016-3191-3
- [18] Rosa, E. O. A. (2019). The effect of Swine's semiochemicals on pigs' behavior, physiology and production (Doctoral dissertation, dissertation May 2019. (Year: 2019)).
- [19] Ran, M., Cha, C., Xu, Y., Zhang, H., Yang, Z., Li, Z., & Wang, S. (2022). Traditional Chinese herbal medicine complex supplementation improves reproductive performance, serum biochemical parameters, and anti-oxidative capacity in periparturient dairy cows. *Animal Biotechnology*, 33(4), 647-656. DOI: 10.1080/10495398.2020.1819823
- [20] Ouellet, V., Toledo, I. M., Dado-Senn, B., Dahl, G. E., & Laporta, J. (2021). Critical temperature-humidity index thresholds for dry cows in a subtropical climate. *Frontiers in Animal Science*, 2, 706636. DOI: 10.3389/fanim.2021.706636
- [21] Jaskowski, J. M., Kaczmarowski, M., Kulus, J., Jaskowski, B. M., Herudzinska, M., & Gehrke, M. (2019). Rectal palpation for pregnancy in cows: A relic or an alternative to modern diagnostic methods. *Med. Weter*, 75(5), 259-264. DOI: dx.doi.org/10.21521/mw.6156
- [22] dos Reis, B. R., Tedeschi, L. O., Netto, A. S., Silva, S. L., Lancaster, P. A., & Silva, L. (2021). Grazing beef cows identified as efficient using a nutrition model partition more energy to lactation. *Animal Production Science*, 62(1), 40-54. DOI: 10.1071/AN20558
- [23] Hernández-Avalos, I., Mota-Rojas, D., Mendoza-Flores, J. E., Casas-Alvarado, A., Flores-Padilla, K., Miranda-Cortes, A. E., ... & Mora-Medina, P. (2021). Nociceptive pain and anxiety in equines: Physiological and behavioral alterations. *Veterinary World*, 14(11), 2984. DOI: 10.14202%2Fvetworld.2021.2984-2995
- [24] Alterisio, M. C., Micieli, F., Valle, G. D., Chiavaccini, L., Vesce, G., Ciaramella, P., & Guccione, J. (2023). Cardiovascular changes, laboratory findings and pain scores in calves undergoing ultrasonography-guided bilateral rectus sheath block before herniorrhaphy: a prospective randomized clinical trial. *BMC Veterinary Research*, 19(1), 191. DOI: 10.1186/s12917-023-03754-6
- [25] Jaskowski, J. M., Sobolewski, J. A. R. O. S. Ł. A. W., Wiczorkiewicz, M. A. R. I. A., Gehrke, M. A. R. E. K., & Herudzinska, M. (2020). Modern techniques of teaching bovine rectal palpation: Opportunities, benefits and disadvantages of new educational devices. *Med Weter*, 76, 5-10. DOI: dx.doi.org/10.21521/mw.6324

- [26] Wu, L., Shi, P., Yu, H., & Liu, Y. (2020). An optimization study of the ultra-short period for HRV analysis at rest and post-exercise. *Journal of Electrocardiology*, 63, 57-63. DOI: 10.1016/j.jelectrocard.2020.10.002