THE EFFECT OF AIR TEMPERATURE ON THERMOGRAPHIC IMAGE OF THE INNER CANTHUS AND HEART RATE VARIABILITY PARAMETERS IN WARMBLOOD MARES AND GELDINGS

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Summary

This study was based on the premise that the thermographic image of the medial canthus and heart rate variability (HRV) parameters in horses are influenced by thermoregulation, which in turn is affected by ambient temperatures. It was hypothesized that an air temperature of 0°C or lower would result in a higher medial canthus temperature and reduced parasympathetic nervous system activity compared to above-freezing air temperature. Significant changes might indicate stress. The aim of this study was to evaluate the medial canthus temperature and HRV parameters in mares and geldings under varying air temperatures. The study included 12 adult warmblood mares and geldings (mean age: 11.42 ± 2.71 years). The experiment was conducted over three selected days with air temperatures of 5.0°C, 0.0°C, and –5.0°C, respectively. Medial canthus temperature was measured using a FLIR E53 thermal imaging camera and ThermaCAM Researcher Pro 2.8 SR-2M software. HRV parameters were determined using Polar RS800CX heart rate monitors together with Polar Pro Trainer 5 and Kubios HRV software. Measurements were taken at rest, immediately after the animals returned from the paddock, and 30 minutes later. The results showed that keeping horses for several hours at an air temperature of 0°C or lower caused a significant increase in medial canthus temperature and a change in HRV parameters compared to above-freezing temperatures. However, these changes were not indicative of distress that could compromise the horses' well-being; rather, they suggested the activation of thermoregulatory mechanisms. Although changes were more frequently observed in geldings than in mares, a definitive effect of sex could not be established. The results suggest that it is unnecessary to limit paddock time for horses at temperatures below 0°C, which supports the beneficial behaviors associated with free movement.

Keywords: horse, paddocks, air temperature, thermography, HRV

Introduction

Stress is a non-specific response of an organism to various stimuli, known as stressors (Van Reenen et al., 2005). It is a natural physiological and behavioral reaction that enables adaptation to environmental conditions and, ultimately, survival. However, prolonged exposure to stress adversely affects multiple body systems. In horses, chronic stress can impair welfare, contributing to disorders such as gastric ulcers and reproductive disorders (Niemczycka and Walczak, 2019).

Stress-inducing factors in horses are highly diverse. Common stressors include training, transport, and environmental changes (Budzyńska, 2014). According to Zeitler-Feicht (2014), ambient temperature should also be considered a causal factora, as it influences rest, movement, thermoregulation, circadian rhythm, and behavior. Since the complete elimination of stress in horse management is impossible, it is essential to identify and minimize avoidable stressors.

The physiological stress response involves two main pathways: the sympathetic–adrenal axis and the hypothalamic–pituitary–adrenal (HPA) axis (Van Reenen et al., 2005). The sympathetic–adrenal axis is activated immediately, leading to an increase in heart rate and respiration, elevated blood glucose levels, and a reduction in digestive activity. The HPA axis responds more slowly, stimulating the release of glucocorticoids, primarily cortisol. Together, these mechanisms can trigger undesirable behavioral changes in horses (Budzyńska, 2014). Current approaches to stress assessment range from subjective behavioral observations to more precise physiological indicators, such as cortisol concentration in saliva, blood, or feces (Kołodziejczyk and Cywińska, 2019). Other sensitive markers include heart rate variability (HRV) and respiration rate (Visser et al., 2002).

A relatively recent method for evaluating stress intensity is infrared thermography of the medial canthus (inner corner) of the eye (Dai et al., 2015). This technique detects temperature fluctuations—reflecting autonomic responses to stressors—without causing additional stress during measurement (Valera et al., 2012). The medial canthus is richly innervated, making it a suitable site for monitoring blood flow changes linked to autonomic activity (Soroko et al., 2016). Resulting temperature shifts provide a non-invasive indicator of stress (Valera et al., 2012). The method offers key advantages, such as avoiding the need for biological sampling and providing immediate results. Its main limitation, however, is high sensitivity to minor external factors, which may compromise accuracy (Piccini et al., 2021).

We hypothesized that the thermographic profile of the medial canthus and heart rate parameters in horses are influenced by thermoregulation, which depends on ambient air temperature. Specifically, we expected that temperatures at or below 0 °C would lead to a greater increase in medial canthus temperature and reduced parasympathetic nervous system (PNS) activity compared with above-freezing conditions. Pronounced changes were anticipated to indicate stress. The aim of this study was therefore to evaluate medial canthus temperature and HRV parameters in mares and geldings under varying ambient air temperatures.

Materials and methods

The study was conducted on 12 adult warmblood horses (mean age: 11.42 ± 2.71 years), including five mares and seven geldings, representing the Małopolski and Polish Sport Horse breeds. All horses were housed in the same box-stall stable and fed traditional rations three times daily. They were used for recreational riding for 1–2 hours per day, six days a week, and had daily access to outdoor paddocks for 4–6 hours. At the time of the study, all horses were clinically healthy.

The experiment was carried out on three separate days, selected based on specific atmospheric conditions, primarily air temperature. On each study day, the horses were turned out to a rectangular, unshaded sand-and-earth paddock (30 × 70 m) between 9:00 AM and 3:00 PM. A hay rack was placed at the center of the paddock. Atmospheric conditions on the study days are presented in Table 1.

Stress levels were evaluated using thermographic imaging of the medial canthus (inner corner) of the eye, captured with a FLIR E53 thermal imaging camera (Bartolomé et al., 2013). The left eye of each horse was scanned at a 90° angle from a distance of 1 m (Soroko, 2016). Using Therma Cam Researcher Pro 2.8 SR-2m software (FLIR Systems AB, FLIR Systems, Inc., Sweden), the maximum temperature was recorded within an oval region of interest that included the eyeball and a 1-cm margin around the outer eyelid.

Table 1. Weather conditions on the following days of the study

Day of experience	I day	II day	III day
Air temperature (°C)	5.0	0.0	-5.0
Relative air humidity (%)	76	73	72
Atmospheric pressure (hPa)	1002	999	1009
Wind speed (m/s)	0.3	0.4	0.5
Cloudy	total	total	total

Heart rate variability (HRV), an indicator of autonomic regulation and emotional excitability, was recorded using Polar ELECTRO OY RS800CX monitors with H2 transmitters, to which the horses had been previously habituated. The transmitter was fastened with an elastic strap to the left side of the chest at heart level, and the heart rate monitor was secured close to the electrode. Data were transmitted to a computer via an IrDA USB 2.0 peripheral adapter and analyzed using Polar Pro Trainer 5 and Kubios HRV software. Low-power filters were applied to remove artifacts. The following HRV parameters were analyzed:

— RR interval [ms] – time between successive R waves of the QRS complex,
— rMSSD [ms] – root mean square of successive differences,
— HF [ms^2] – high-frequency spectral power,
— LF [ms^2] – low-frequency spectral power,
— LF/HF [%] – ratio of low-frequency to high-frequency spectral power (Laborde et al., 2017).

Measurements were taken three times per study day: 15 minutes before turnout, and 5 and 30 minutes after the horses were returned to the stable.

Statistical analyses were performed using SAS 9.4. The normality of distribution was tested with the Kolmogorov–Smirnov test ($\alpha = 0.05$). Descriptive statistics (mean and standard deviation) were calculated. The effect of fixed factors on the studied traits was evaluated using multifactorial analysis of variance (GLM model). Differences between means were assessed with Tukey's test. Correlations between traits across all horses were determined using Pearson's correlation coefficient ($P \leq 0.05$).

Results

Thermographic assessment of the eye canthus

Medial canthus temperatures in mares and geldings differed significantly across study days (Table 2). Before turnout, the highest values were observed at an ambient temperature of –5 °C. Five minutes after the horses were returned to the stable, the temperature was significantly higher compared with pre-turnout measurements on both the second and third study days. After 30 minutes, the elevated temperature persisted only in geldings. In mares, no significant differences were noted compared with pre-turnout values.

Emotional excitability

The mean RR interval was generally lowest on the third study day (Table 3). The highest values were recorded on the first day, with the exception of geldings measured 5 minutes after return from turnout, where the greatest values occurred at 0 °C. Significant sex-related differences appeared only 30 minutes after return, when mares showed significantly higher values.

The rMSSD parameter was lowest on the third study day and highest on the first (Table 4). Significant sex-related differences were observed at 0 °C and –5 °C prior to turnout, at 5 °C and –5 °C immediately after return, and at 5 °C thirty minutes after return. Except for the last measurement, geldings consistently showed higher values than mares.

According to the results presented in Table 5, HF values in mares differed significantly across study days, with the lowest values recorded on the third day. In geldings, significant differences were noted only after turnout, with the lowest values also occurring on the third day. Significant sex-related differences were observed 15 minutes before turnout on the third day, 5 minutes after return on the second and third days, and 30 minutes after return on all three days. In each case, geldings displayed higher values than mares.

LF values before turnout differed significantly between study days (Table 6). In mares, the lowest values were recorded on the first and second days, whereas in geldings this occurred only on the second day. After turnout, the lowest LF values were observed on the first day and the highest on the third day in both groups. The values obtained on the second day in mares were intermediate. Significant sex-related differences were found only 5 minutes after return, when mares showed higher values than geldings, and before turnout on the first day, when the opposite pattern was observed.

In mares, significantly higher LF/HF values were recorded on the third study day (Table 7). The lowest values were observed on the first day, with intermediate values (except 15 minutes before turnout) on the second day. In geldings, the highest results were also noted on the third day, while the lowest occurred on the second day before turnout and on the first day after return. In all cases, significant sex-related differences were noted. With the exception of the pre-turnout measurement on the first day, geldings showed lower values than mares.

For the RR interval, a single significant correlation with eye canthus temperature was noted (Table 8). This negative correlation occurred 5 minutes after return on the third day. The rMSSD parameter showed a negative correlation with canthus temperature 5 minutes after return on the second day. HF values correlated negatively 5 and 30 minutes after return on the third day. No significant correlations were observed for LF and LF/HF.

Table 2. Temperature (°C) from the corner of the horse's eye on subsequent days of the study

Measurement time	Sex	I day		II day		III day	
		Mean	SD	Mean	SD	Mean	SD
15 minutes before being released into the paddock	Mares	35.56ax	1.55	35.87ax	2.02	44.56bx	11.34
	Geldings	37.45ay	1.98	36.66ax	2.05	43.11bx	9.67
5 minutes after returning from the paddock	Mares	32.45ax	1.56	43.21bx	4.45	45.23bx	7.87
	Geldings	33.56ax	3.34	45.56bx	4.03	44.77bx	10.09
30 minutes after returning from the paddock	Mares	34.34ax	2.13	36.42ax	2.89	36.87ax	3.33
	Geldings	35.71ax	2.66	40.12by	2.71	41.54by	2.78

Means marked with different letters a, b, c (in rows), x, y (in columns) are significantly different at P≤0.05.

Table 3. RR parameter (ms) of horses on subsequent days of the study

Measurement time	Sex	I day		II day		III day	
		Mean	SD	Mean	SD	Mean	SD
15 minutes before being released into the paddock	Mares	456.23ax	125.63	418.34ax	111.34	321.11bx	100.34
	Geldings	523.12ax	121.45	432.19bx	111.54	289.43cx	121.45
5 minutes after returning from the paddock	Mares	543.23ax	119.45	600.34ax	201.23	323.45bx	115.56
	Geldings	576.45ax	155.11	634.45bx	118.63	453.34ax	172.84
30 minutes after returning from the paddock	Mares	654.34ax	211.46	565.32acx	128.48	456.34bcx	112.43
	Geldings	546.56ay	200.34	453.56acy	226.67	358.73bcy	118.45

Means marked with different letters a, b, c (in rows), x, y (in columns) are significantly different at P≤0.05.

Table 4. RMSSD parameter (ms) of horses on subsequent days of the study

Measurement time	Sex	I day		II day		III day	
		Mean	SD	Mean	SD	Mean	SD
15 minutes before being released into the paddock	Mares	67.34ax	15.56	56.45bx	13.15	43.23cx	12.45
	Geldings	62.34ax	11.44	66.45ay	13.61	55.55by	14.34
5 minutes after returning from the paddock	Mares	72.34ax	12.82	65.34bx	11.64	45.37cx	10.05
	Geldings	67.45ay	13.73	65.43ax	12.07	51.53by	9.12
30 minutes after returning from the paddock	Mares	77.37ax	12.35	65.45bx	13.56	55.34cx	10.34
	Geldings	69.94ay	13.11	67.34ax	12.66	56.43bx	11.13

Means marked with different letters a, b, c (in rows), x, y (in columns) are significantly different at P≤0.05.

Table 5. HF parameter (ms^2) of horses on subsequent days of the study

Measurement time	Sex	I day		II day		III day	
		Mean	SD	Mean	SD	Mean	SD
15 minutes before being released into the paddock	Mares	123.56ax	45.74	113.45ax	44.02	89.34bx	22.22
	Geldings	127.45ax	52.11	122.58ax	37.41	119.38ay	19.83
5 minutes after returning from the paddock	Mares	145.67ax	58.71	104.23bx	39.34	78.12cx	28.45
	Geldings	155.23ax	39.43	146.34ay	33.09	123.45by	23.31
30 minutes after returning from the paddock	Mares	127.38ax	30.17	100.03bx	26.34	66.11cx	18.81
	Geldings	142.54ay	51.28	133.18ay	31.43	100.66by	23.95

Means marked with different letters a, b, c (in rows), x, y (in columns) are significantly different at P≤0.05.

Table 6. LF parameter (ms^2) of horses on subsequent days of the study

Measurement time	Sex	I day		II day		III day	
		Mean	SD	Mean	SD	Mean	SD
15 minutes before being released into the paddock	Mares	987.34ax	177.56	1073.23ax	200.14	1265.23cx	199.34
	Geldings	1234.45ay	162.34	1000.22bx	166.28	1199.24ax	177.38
5 minutes after returning from the paddock	Mares	789.27ax	78.89	902.55bx	100.02	1029.28cx	109.27
	Geldings	652.18ay	99.28	721.29ay	96.36	899.29by	111.04
30 minutes after returning from the paddock	Mares	678.39ax	102.22	739.49bx	102.28	999.27cx	117.15
	Geldings	627.28ax	98.23	712.18ax	98.49	1003.23bx	89.67

Means marked with different letters a, b, c (in rows), x, y (in columns) are significantly different at P≤0.05.

Table 7. LF/HF ratio (%) of horses on subsequent days of the study

Measurement time	Sex	I day		II day		III day	
		Mean	SD	Mean	SD	Mean	SD
15 minutes before being released into the paddock	Mares	799.08ax	115.42	745.99ax	125.89	1416.20bx	112.74
	Geldings	968.58ay	105.68	815.97bx	101.12	1004.56ay	104.52
5 minutes after returning from the paddock	Mares	541.82ax	79.47	865.92bx	84.69	1317.56cx	94.57
	Geldings	420.14ay	76.96	492.89ay	81.45	728.46by	89.99
30 minutes after returning from the paddock	Mares	532.57ax	95.67	739.27bx	68.47	1511.53cx	76.53
	Geldings	440.07ay	86.85	534.75by	79.33	996.65cy	58.72

Means marked with different letters a, b, c (in rows), x, y (in columns) are significantly different at P≤0.05.

Table 8. Correlations between medial canthus temperatue and heart rate variability parameters in horses on subsequent days of the study

Measurement time	I day	II day	III day
RR parameter			
15 minutes before being released into the paddock	-0,321	0,086	-0,205
5 minutes after returning from the paddock	-0,046	-0,226	-0,533*
30 minutes after returning from the paddock	-0,432	-0,357	-0,229
rMSSD parameter			
15 minutes before being released into the paddock	0,067	0,097	-0,334
5 minutes after returning from the paddock	-0,227	-0,556*	-0,408
30 minutes after returning from the paddock	-0,267	-0,333	-0,151
HF parameter			
15 minutes before being released into the paddock	-0,285	-0,199	0,098
5 minutes after returning from the paddock	-0,451	-0,499	-0,602*
30 minutes after returning from the paddock	-0,221	-0,456	-0,559*
LF parameter			
15 minutes before being released into the paddock	-0,168	0,267	0,353
5 minutes after returning from the paddock	0,224	0,325	-0,078
30 minutes after returning from the paddock	0,308	0,303	0,222
LF/HF parameter			
15 minutes before being released into the paddock	-0,123	0,098	0,228
5 minutes after returning from the paddock	-0,226	-0,112	-0,399
30 minutes after returning from the paddock	0,056	-0,166	-0,289

*Significant correlation at $P \leq 0.05$

Discussion

The results confirmed that medial canthus temperature in horses is influenced by ambient temperature. Significant changes were already evident before turnout, suggesting that air temperature may affect horses even while stabled. Caretakers should therefore pay particular attention to horses during such periods, as Camargo et al. (2018) reported that dangerous behaviors toward humans may occur in these conditions.

The effect of air temperature became even more pronounced when horses spent several hours outdoors. The lowest canthus temperatures were recorded at positive air temperatures, while the highest were observed at sub-zero conditions, indicating that changes begin to occur around 0 °C. This may be explained by the fact that warmblood horses originate from warm and dry climates (Olczak and Klocek, 2003). Temperatures around freezing likely trigger thermoregulatory mechanisms leading to somatic changes (Mejdell et al., 2020). However, only extreme changes may lead to mood deterioration and increased stress (Cymbaluk and Christison, 1990). Such drastic effects were not observed in the present study. Therefore, there is no indication that blanketing horses is necessary at the analyzed temperature ranges in winter. Hammer and Gunkelman (2020) and Mejdell et al. (2019) reported different conclusions, which may be attributable to methodological differences.

The findings regarding changes in canthus temperature were only partially confirmed by HRV analysis, which reflects autonomic regulation and emotional excitability (Soroko et al., 2016). Interestingly, similar RR responses to decreasing air temperature were observed in both sexes. This is notable, as mares are often considered more temperamental and sensitive to environmental conditions (Crowell-Davis, 2007). Significant differences were most commonly observed 30 minutes after return from turnout. This suggests that geldings required a longer recovery time to return to resting levels compared with mares, consistent with observations by Lesimple et al. (2020).

Conversely, opposite patterns were observed for rMSSD and HF, parameters reflecting parasympathetic activity of the autonomic nervous system (Kreuzer, 2019). Although both parameters showed stronger decreases at 0 °C and below, these changes were less pronounced in geldings than mares. In contrast, LF and LF/HF values increased under the same conditions.

At this stage, the results do not allow for a clear conclusion regarding sex-related differences in physiological responses to air temperature. Considering the large standard deviations observed, individual variation likely plays an important role.
No consistent relationships were found between canthus temperature and HRV parameters, as correlations were limited to a few cases. Soroko et al. (2016) also reported that maximum canthus temperature was not correlated with established stress indicators, such as salivary cortisol or heart rate. Thus, the present findings can be regarded as only partially consistent, possibly due to differences in the analyzed parameters.

Conclusion

Keeping horses outdoors for several hours at air temperatures of 0°C or below resulted in a significant increase in canthus temperature and changes in HRV parameters compared with positive air temperatures. However, these changes did not reach levels indicative of stress with negative welfare implications; instead, they most likely reflected activation of thermoregulatory mechanisms. Although more pronounced changes were observed in geldings than mares, no consistent sex-related effect could be confirmed. The results suggest that restricting turnout time during sub-zero temperatures is unnecessary. Allowing horses free movement under such conditions may promote desirable behaviors and support welfare.

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