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Effects of a Simulated Brazilian Long-Loop Competition on Infrared Thermography and Cortisol Responses in Equine Athletes

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Short Report

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Abstract

Equine athletes require coordinated physiological responses to maintain homeostasis during exercise. This study evaluated infrared thermographic variables and serum cortisol concentrations in 16 trained Criollo horses performing a simulated Brazilian long-loop competition. Thermograms of the lacrimal caruncle, lateral flank, carpal joint, and tarsal joint were obtained before and 30 min after exercise. Serum cortisol was measured before and at 30 min, 2 h, and 24 h post-exercise. Surface temperatures increased significantly in the carpal, tarsal, and lateral flank regions, whereas lacrimal caruncle temperature and serum cortisol concentrations remained unchanged. No significant associations were observed between cortisol and thermographic measurements. Infrared thermography may provide a complementary, non-invasive approach for monitoring responses to exercise in horses.

Introduction

Equine athletes face substantial physiological demands during exercise. Although Criollo horses are recognized for their hardiness and adaptability (Pereira et al., 2024), their physiological responses to traditional disciplines such as long-loop remain poorly characterized. This modality involves intense, intermittent effort and abrupt directional changes that may trigger endocrine and thermoregulatory responses (Compagnoni et al., 2020). Serum cortisol reflects hypothalamic–pituitary–adrenal axis activation during exercise (Ferlazzo et al., 2020; Arfuso et al., 2022), whereas infrared thermography provides a non-invasive means of assessing regional changes in surface temperature (Verdegaal et al., 2024). This study evaluated the effects of a simulated long-loop competition on thermographic parameters and serum cortisol concentrations in Criollo horses.

Methods

2.1 Ethical Aspects

The experimental protocol complied with the National Council for the Control of Animal Experimentation (CONCEA) guidelines and was approved by the Animal Use Ethics Committee of the Universidade Federal da Fronteira Sul (UFFS), Realeza *Campus* (protocol no. 5121280623).

2.2 Experimental Design, Location, and Animals

Sixteen Criollo horses (10 females and 6 males; 395 ± 34 kg; 10 ± 3 years; body condition score, 5 ± 1.30/9) were included. Horses were housed with individual access to the routinely provided diet and water *ad libitum*. Inclusion criteria were clinical health and at least two years of long-loop practice. Health status was assessed by physical examination.

2.3 Simulation of the long-loop competition

At baseline (T0), horses were haltered and assessed for pain and general physical condition, and blood samples were collected for cortisol analysis. They were then saddled and taken to the track to perform 10 lasso attempts using a bovine dummy attached to a motorcycle traveling at an average speed of 25 km/h. The simulation reproduced the movement of a calf during official competitions, with horses completing round trips on a 110-m track. Exercise duration, measured from the first to the last attempt, ranged from 15 to 20 min. The same rider (60 kg) was used for all horses to standardize the exercise stimulus.

2.4 Thermographic Evaluation

Infrared thermography was performed in a shaded area protected from direct sunlight and excessive airflow using a FLIR TG165-X thermal camera (Wilsonville, Oregon, USA), with standardized distance and angle. Images were obtained before exercise (T0) and 30 min afterward (T1) from the lacrimal caruncle, lateral flank, carpal joint, and tarsal joint. These regions were selected to assess thermal responses associated with autonomic regulation, peripheral heat dissipation, and locomotor effort.

2.5 Cortisol Analysis

Blood samples for serum cortisol determination were collected by aseptic jugular venipuncture before exercise (T0) and at 30 min (T1), 2 h (T2), and 24 h (T3) post-exercise into tubes without anticoagulant. Cortisol concentrations were determined using a commercial enzyme immunoassay kit (Cortisol Test System, Monobind Inc., Lake Forest, USA), following the manufacturer's instructions. Absorbance was measured using a Multiskan FC plate reader (Thermo Scientific-Uniscience®), and concentrations were calculated from the standard calibration curve.

2.6 Statistical Analysis

Data normality was assessed using the Shapiro–Wilk test and Q–Q plots. Thermographic variables were compared using paired *t*-tests. Associations between thermographic measurements and cortisol concentrations were evaluated using linear mixed-effects models, with thermographic variables and time as fixed effects and animal as a random effect. Models were fitted using restricted maximum likelihood (REML), with statistical significance set at $p < 0.05$ (95% CI). Analyses were performed in R.

Results

Surface temperature increased significantly from T0 to T1 in the carpal, tarsal, and lateral flank regions, whereas no significant change was observed in the lacrimal region (Fig. 1). The magnitude of thermal variation differed among anatomical regions.

Surface temperature increased significantly in the carpal (mean difference = 2.23°C; $t = 2.76$; $p = 0.0146$; 95% CI: 0.51–3.95), tarsal (1.96°C; $t = 2.69$; $p = 0.0167$; 95% CI: 0.41–3.52), and lateral flank regions (1.41°C; $t = 2.96$; $p = 0.0097$; 95% CI: 0.39–2.42). No significant change occurred in the lacrimal region (0.64°C; $t = 1.28$; $p = 0.2193$; 95% CI: −0.42 to 1.70). Serum cortisol concentrations did not differ

significantly across time points ($F(3,45) = 1.46$; $p = 0.238$), despite nonsignificant decreases at T1 ($\Delta = -1.459$; $p = 0.088$) and T2 ($\Delta = -1.571$; $p = 0.067$) and a smaller decrease at T3 ($\Delta = -1.004$; $p = 0.237$) relative to baseline. Substantial inter-individual variability was observed, with animal-level variance comparable to residual variance (Fig. 2).

In linear mixed-effects models adjusted for animal and time, no thermographic variable was significantly associated with serum cortisol concentrations (carpal: $\beta = 0.299$; tarsal: $\beta = 0.436$; lateral flank: $\beta = -0.338$; lacrimal: $\beta = -0.129$; all $p > 0.05$; Fig. 3). Effect estimates were small and inconsistent in direction. Time point T1 showed a nonsignificant trend toward lower cortisol concentrations ($\beta = -2.422$; $p = 0.066$).

Discussion

The significant temperature increases in the carpal, tarsal, and lateral flank regions suggest region-specific thermal responses to exercise. Surface temperature may increase with exercise intensity and duration and remain elevated during recovery (Janczarek et al., 2021). Thermal changes in regions associated with locomotion have also been reported in different exercise protocols, potentially reflecting muscular activity and biomechanical demand (Redaelli et al., 2019; Sikorska et al., 2025).

In contrast, lacrimal caruncle temperature did not change significantly. Although the periocular region is sensitive to autonomic activity, ocular temperature responses vary with exercise, transport, athletic performance, experience, and individual characteristics (Soroko et al., 2021; De Mira et al., 2021; Mira et al., 2021; Aragona et al., 2024). Environmental conditions, breed, sex, season, and measurement procedures may further contribute to variability (Jansson et al., 2021). Thus, the absence of a significant change may reflect individual variability or an insufficient stimulus to elicit a detectable periocular response.

Serum cortisol concentrations also remained unchanged across sampling times, differing from findings in horses subjected to racing, training, and other exercise protocols (Arfuso et al., 2022; Cravana et al., 2025). Cortisol responses may vary with exercise intensity, conditioning, previous experience, and individual characteristics, while novel training stimuli can elicit physical and emotional responses (Holtby et al., 2023; Cravana et al., 2025). Accordingly, the simulated protocol may not have elicited a detectable endocrine response, potentially because of its intensity or the horses' familiarity with the activity.

No significant associations were found between thermographic variables and cortisol concentrations. Although previous studies have reported relationships between temperature and cortisol, these associations may be weak or dependent on the anatomical region and sporting context (Redaelli et al., 2019; Soroko et al., 2021; Mira et al., 2021; Aragona et al., 2024). Temperature increases without significant cortisol changes have also been reported in exercising horses (Filho et al., 2024). These

findings suggest that regional thermal and endocrine responses may follow distinct physiological pathways.

Conclusion

Infrared thermography detected significant post-exercise temperature increases in the carpal, tarsal, and lateral flank regions, whereas lacrimal temperature and serum cortisol concentrations remained unchanged. The absence of significant associations between thermographic and cortisol measurements suggests that these variables may reflect distinct physiological responses to exercise. Further studies involving larger populations, different exercise protocols, and additional physiological biomarkers are needed to clarify the relationship between thermal and endocrine responses.

Declarations

Author Contribution

Priscila Correa Frison: Project administration, conceptualization, investigation, methodology, writing – original draft, writing – review and editing. Bruna Alves Ottobeli: Conceptualization, investigation, methodology, writing – original draft, writing – review and editing. Mayara Freire da Silva: Conceptualization, investigation. Rafael Luan Perin: Formal analysis. Amália Vitória Zimpel: Investigation, data curation. Marina Marangoni: Investigation, data curation. Ana Letícia Rodrigues Marques: Investigation, data curation. Tatiana Champion: Investigation, data curation. Dalila Moter Benvegnú: Supervision, writing – review and editing. Maiara Garcia Blagitz: Supervision, project administration, writing – review and editing. All authors read and approved the final version of the manuscript.

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Figures

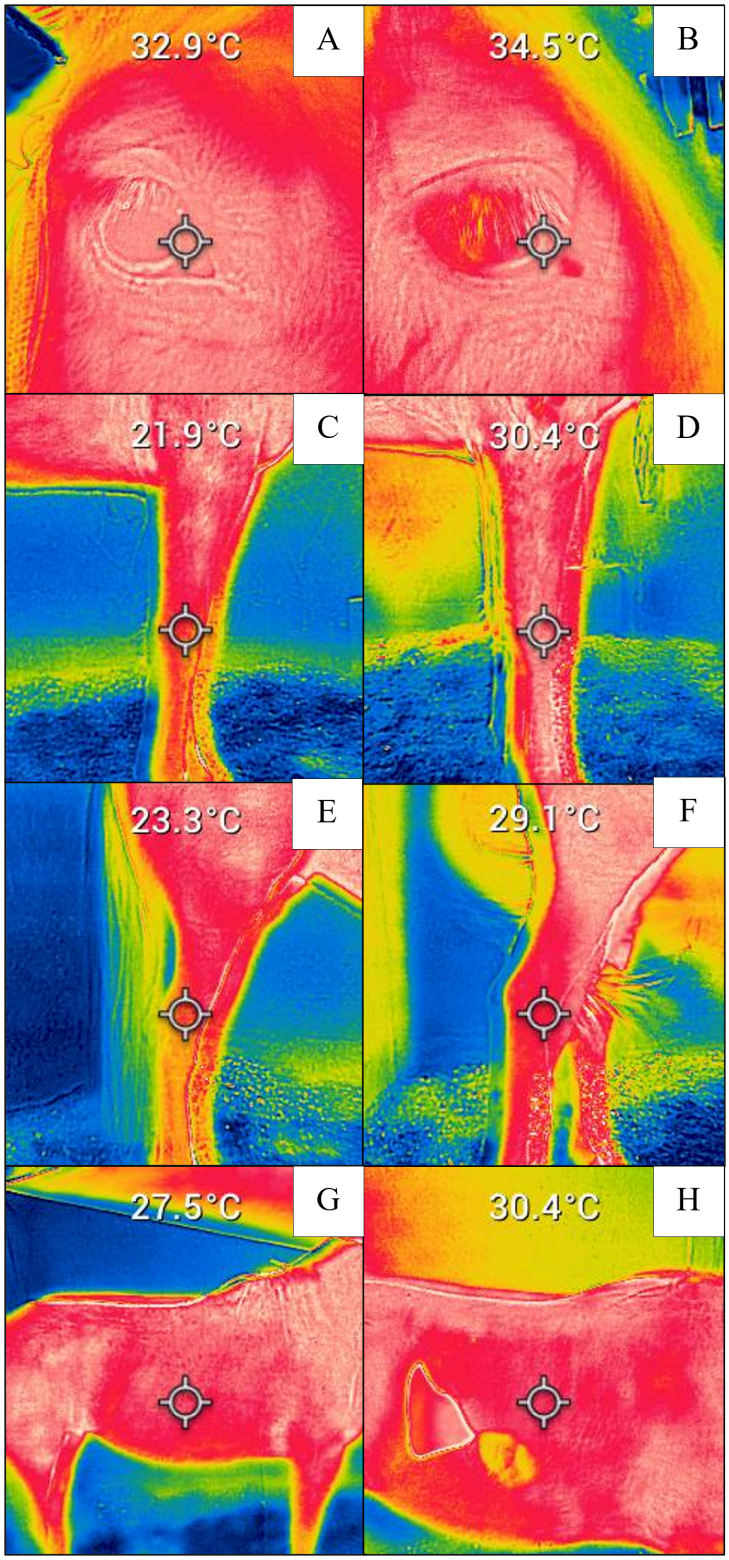


Figure 1

Thermographic images of 16 Criollo horses before (left) and 30 min after (right) simulated long-loop exercise. (A–B) Lacrimal caruncle; (C–D) carpal joint; (E–F) tarsal joint; and (G–H) lateral flank. Values indicate mean surface temperature (°C). Increased thermal intensity was observed in the carpal, tarsal, and lateral flank regions ($p < 0.05$).

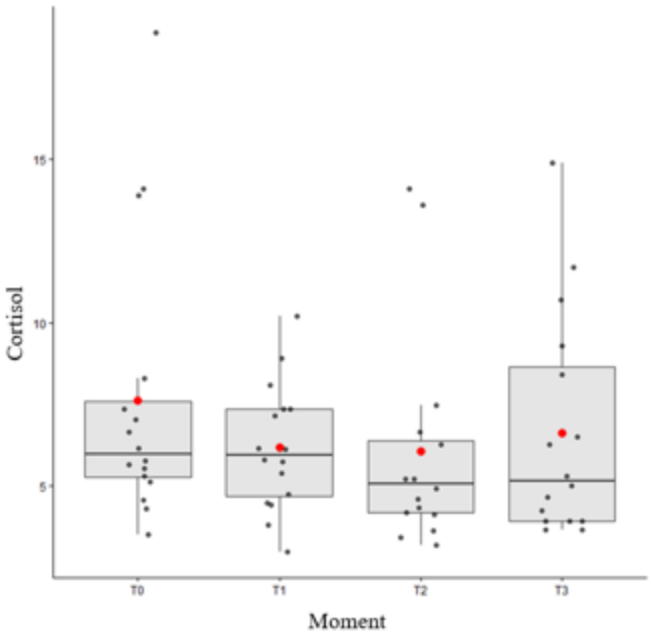


Figure 2

Serum cortisol concentrations before (T0) and 30 min after (T1) simulated long-loop exercise in 16 Criollo horses. Black dots represent individual values, red dots indicate means, horizontal lines within boxes indicate medians, boxes represent the interquartile range (25th–75th percentiles), and whiskers show data spread.

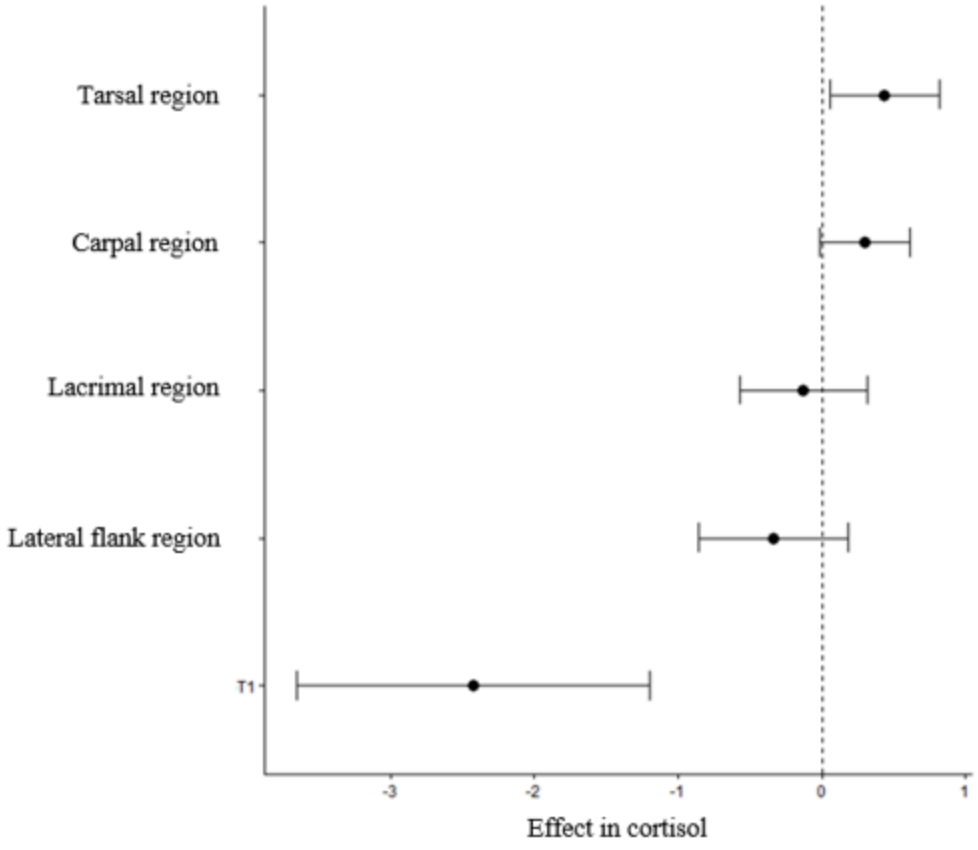


Figure 3

Associations between thermographic variables and serum cortisol concentrations estimated using mixed-effects models in 16 Criollo horses after simulated long-loop exercise. Black points indicate effect estimates (β), horizontal bars represent confidence intervals, and the dashed vertical line denotes the null effect (β = 0). Confidence intervals crossing zero indicate nonsignificant associations.