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Muscle Stress and Thermal Discomfort of Equines in a Brazilian Rodeo-Style Sport (Vaquejada)

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Abstract

This study aimed to evaluate the effect of the exercise performed under the semi-arid weather on horses submitted to a Rodeo-style Sport (Vaquejada) through hemogasometric, clinical, and thermographic variables. To conduct this study, a Vaquejada simulation test (VST) was conducted in agreement with the rules of the Brazilian Association of Vaquejada. Physiological, thermographic, hemogasometry, and biochemistry data were collected before and after a single race. After it, significant changes were observed for thermographic, physiological, hemogasometric, and biochemical variables. The general surface temperature (ST) and those by body region differed ($P < 0.01$) between pre- and post-exercise conditions. The RR, HR, CK, Lactate, K^+, and AG increased ($P < 0.01$) after the exercise. A negative correlation between the maximum lactate concentration and the reduction ($P < 0.01$) of pH, HCO_3-, TCO_2, and EB was also observed after the exercise. No significant changes were observed in PO_2, PCO_2, SO_2, and other serum electrolytes after the exercise. The thermographic profile evidences the environment of thermal, critical, and dangerous discomfort to which the animals are exposed. Thus, we believe horses used in this activity should not be submitted to consecutive and immediate exercises so that pathological complications from repetitive exertion are avoided.

Keywords

Blood gas analysis; thermal comfort; physical performance

1. Introduction

The Vaquejada, a manifestation of the culture in the Brazilian Northeast, emerged in the mid-twentieth century from a simulation of cattle management performed by a cowboy (local rural worker) and his horse. This competition consistently reduces gas variables, such as EB, HCO_3-, and TCO_2 after high-intensity exercises [1], which is equivalent to high-goal polo [2] and high-speed treadmill activities [3]. In addition, physical-motor injuries, such as tendinitis and tenosynovitis associated with biochemical and hematological imbalances due to intense exercise, have already been described [4–7].

In addition to the intense physical activity, the high environmental temperatures of the event sites can negatively influence the welfare and performance of animals [8]. Therefore, meteorological variables and bioclimatological indexes, such as the temperature-humidity index (THI), comfort index (CI), and degree of comfort of the animal, combined with infrared thermography (IT); a noninvasive and precise method that detects minimal variations in surface temperature; have been used in the monitoring of animal welfare **[9–11]**. In this context, the analysis of the thermograms integrated with the changes in the physiological responses of the animals allows for a better understanding of thermoregulatory mechanisms because of the increase in surface temperature and the action of the environment on the thermal condition of animals **[12]**.

When considering the significant impact that climatic variables may have on sport horses and their physiological responses, we measured the impact of the environment and physical exercise on the physiological responses of vaquejada equines through gasometric, thermographic, and clinical biochemical analyzes before and after the intense physical activity of the event.

2. Material and Methods

2.1. Study Site

The experimental phase was a cross-sectional study, conducted in São Mamede, a municipality in the semi-arid region of northeastern Brazil, with a climate characterized as hot and dry with an average annual rainfall of 780.8 mm and average temperature ranging from 21°C (minimum) to 33°C (maximum), according to data from the National Institute of Meteorology (INMET). The study site was a vaquejada park (Lat: -6.925144°; Long: -37.098774°) with a track measuring approximately 160 m in length, with variations in width, covered in fine sand with a thickness of about 50 cm.

2.2. Animals, Management, and the Vaquejada Simulation Test

Twelve Quarter Horses were divided into six pairs, composed of the pull and helper horses, comprising five males and seven females, with an average age of 3.6±1.2 years and a weight of 305.6±49.8 kg, active in the vaquejada events, with training three times a week in fine sand for 60 minutes through walking, trotting, galloping, and racing activities. All animals belonged to private breeders in São Mamede. Their diet consisted of chopped elephant grass (*Pennisetum purpureum* Schum.) or Tifton (*Cynodon dactylon*) divided into two (5 AM and 5 PM) daily portions and commercial concentrate[1] (1.0 kg/100 kg body weight, with 12% crude protein) provided fractionally. An inorganic mineral salt mixture was not offered and water was always available. The criterion for the selection of healthy horses was based on clinical and laboratory aspects by consulting the histories of the animals on the veterinarian's domain of the properties. The clinical aspects of the physical examination involved measurement of rectal temperature, heart and respiratory rates, skin turgor, capillary refill time, evaluation of lymph nodes, inspection of mucous membranes, cardiac and pulmonary auscultation, abdominal palpation, and inspection of the external genitalia and laboratory (blood count), whose variables analyzed were red blood cell count (He), hemoglobin concentration (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (CHCM), total and differential leukocyte count and platelet count. In this study, animals with a recent history of pathologies, therapeutic treatment, and with reports of recent participation (two weeks) in sports competitions were removed from the experimental group. Furthermore, all selected animals were evaluated for hematological parameters (complete blood count) one week before the simulation experiment and immediately before the race. As there were no significant differences between the pre- and post-race moments, they were not described. The animals were brought to the site of the vaquejada simulation test (VST) by their halters, from nearby properties (400 meters). The time interval between going to the test site and data collection was two hours. The official vaquejada park is the same place used for training the selected animals. The VST was conducted between 9 AM and 1 PM.

The VST consisted of the execution of a single race without successive repetitions as occurs in the official events (a cycle of three races), as regulated by the Brazilian Association of Vaquejada **[13]**. The pull horse/cowboy and helper horse/cowboy ran on a 160-m long soft sand track after a bovine specimen (bull). The pull horse/cowboy pulls the bull down after 100 m of running and the other helper horse/cowboy helps the first one to keep the bull running in a straight line. The pull horse gallops at approximately 8.0 m/s, which is completed with a tug until the bull lies on the track, while the helper horse gallops at the same speed to the place where it is laid **[14,15]**. Each bull was used only once, and the horses were ridden by their usual riders.

2.3. Thermographic Data

The surface temperature (ST) of each animal was measured with an infrared thermographic camera (Fluke Ti 25®) while the animals were immobile, with no restriction, and with little manipulation to avoid possible stress. Images were taken from the right lateral plane, the first in resting conditions in the shade (pre-exercise) and the second after the vaquejada exercise, under direct sunlight (post-exercise). Later, the thermograms were analyzed by the Smartview software version 3.1, for obtaining the general average temperature and that for each body region, that is, of the thigh, rump, buttock tip, neck, and head, to calculate the representative surface temperature of the animals, considering an emissivity of 0.98 (**Figure 1**).

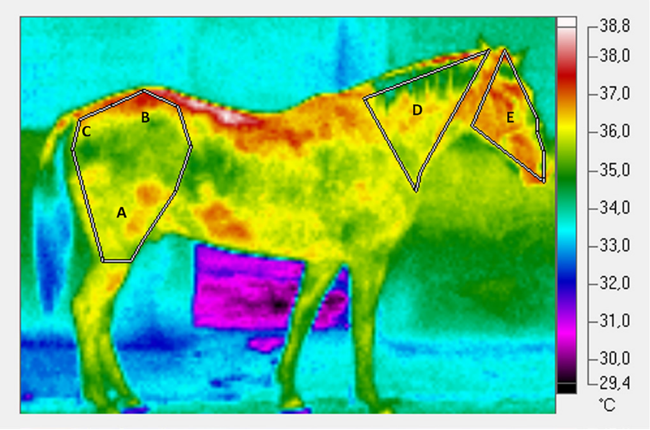

Figure 1: Equine infrared thermography. Images were obtained from the right lateral plane of the following body regions: thigh **(A)**, rump **(B)**, buttock tip **(C)**, neck **(D)** head **(E)**, analyzed using Smartview version 4.1 (Fluke® Ti25 thermographic camera).

The environmental data included air temperature (AT), relative humidity (RH), and maximum (Tmax) and minimum (Tmin) temperatures, obtained from the meteorological station of the National Institute of Meteorology (INMET), in Patos, the closest city (24 km) with a climate similar to that of the study site. From these climatic variables, two climatic condition indices were calculated. The Temperature-Humidity Index (THI) was calculated from the equation developed by **[16]** for dairy cows: THI = AT + 0.36Td + 41.5, where: AT = air temperature (°C) and Td = dew point temperature (°C). THI values up to 70 indicate a nonstress environment, between 71 and 78 indicate a critical one, between 79 and 83 a dangerous one, and above 83, an emergency condition **[16,17]**. To characterize whether the thermal environment during the collection period was stressful or not for equines, we used the Comfort Index (CI), employed by **[9]**, with the following formula: CI = Air Temperature (°F) + Relative Humidity (%).

2.4. Sample Handling and Analysis

Physiological data and blood samples were collected immediately before and after the exercise. The heart and respiratory rates were obtained with the aid of a stethoscope. Blood samples were collected anaerobically by puncturing the external jugular vein after physical containment of the animal and local disinfection. A 5.0 mL collection was made in a tube with sodium fluoride (BD Vacutainer®)[3] to perform the glucose and lactate dosage in plasma and another 5.0 mL collection was made in a tube with lithium heparin to perform hemogasometry and serum and electrolytic biochemistry. Blood gas analysis was performed immediately after collection by direct aspiration in a piece of portable equipment. The remaining blood was stored and transported refrigerated to the Laboratory of Veterinary Clinical Pathology of the University Veterinary Hospital (LPCV/HUV) of the Federal University of Campina Grande (UFCG) where they were processed. The plasma was obtained by centrifugation at 600 g for 10 minutes and analyzed.

Gasometric analysis was performed on-site in heparinized whole venous blood, using a portable hemogasometer[4] (AGS-22, Drake®). The hydrogen ion concentration (pH), partial pressure of carbon dioxide (PCO_2), and partial pressure of oxygen (PO_2) were determined by specific electrodes. The concentrations of bicarbonate (HCO_3-), excess base (EB), total carbon dioxide (TCO_2), and oxygen saturation (%) were calculated indirectly through the equipment software. The biochemical analysis was performed using a Cobas C111 automated biochemical apparatus[5] (Roche®) using spectrophotometry in enzymatic or colorimetric kinetic assays when using the commercial biochemical kits from the manufacturer (Roche Diagnostics, Mannheim, Germany) to measure Aspartate Aminotransferase (AST), Creatine Kinase (CK), Lactate Dehydrogenase (LDH), Glucose, Total Calcium (tCa), and Lactate. The electrolytic analysis was performed in an automated analyzer for electrolytes[6] (Max Ion - Med max®) by the direction selectivity method for Sodium (Na), Potassium (K), Chlorine (Cl), and Ionized Calcium (iCa). The anion gap was calculated using the values of sodium, chlorine, and bicarbonate.

2.5. Statistical Analysis

The statistics evaluated the effect of the main factors, sport modality (pull and helper), experimental period (pre- and post-exercise), and the possible interaction between them, also considering the possibility of nonparametric effects for the nonsignificant variables in the ANOVA F-test. The thermographic, physiological, biochemical, and hemogasometric parameters were interpreted by analyzing parametric variance using the F-test and comparing the means by the Tukey test at 5%, and by the paired t-test at 5% for data that did not present parametric organization. Analyses were performed using the Graph Pad Prism software for Windows[7](GraphPad Software version 5.01).

3. Results

At the time of the experiment, the average ambient temperature (AT) was 32.5°C, the maximum temperature (Tmax) was 37.0°C, the minimum temperature (Tmin) was 29.0°C and the relative humidity was 45%. The comfort index (CI) was 135.5 and the temperature-humidity index (THI) was 74, which reached 79 when obtained from the maximum temperature. The physiological, hemogasometric, and biochemical variables investigated were significantly affected only by the exercise factor, with a tendency to rise. The RR and HR increased ($P < 0.01$) after exercise **(Table 1)**. The surface temperature (ST), generally and by regions, differed ($P < 0.01$) between pre- and post-exercise conditions **(Table 1)**, except for the T in the head region (H) which did not differ significantly between the moments.

The significant elevation rates were 111.0% and 1,062% for CK and Lactate, respectively **(Table 2)**.

Table 1: Respiratory rate (RR), heart rate (HR), general surface temperature (ST), and ST by body regions: thigh (RT), buttock tip (BT), rump (R), and head (H) of the equine pre- and post-exercise.

Physiological responses	Pre-exercise	Post-exercise	*P*	Reference
RR (bpm)**	31.0^b	39.7^a	0.0001	8-16[#]
HR (bpm)**	49.0^b	106.2^a	0.0073	30-40[#]
Surface temperatures (°C)				
sT**	37.7^b	40.3^a	0.00008	na
sT (RT)**	37.5^b	40^a	0.00004	na
sT (BT)**	37.9^b	40.5^a	0.00012	na
sT (R)**	38.3^b	43.7^a	0.00003	na
sT (H)ns	37.6	38.3	0.08888	na

Means followed by the same letters do not differ by the Tukey test with 5% probability; (**) significance at $P \leq 0.01$; (ns) not significant; bpm - beats per minute or breaths per minute; (na) not available; (#)**[18]**.

Table 2: Serum enzymes and metabolites of the equine pre- and post-exercise.

	Pre-exercise	Post-exercise	*P*	Reference$^{\#}$
CK$_{(\text{U/L})}^{**}$	283.2±160.9^{b}	599.0±340.5^{a}	0.0056	108.0-380.0
AST$_{(\text{U/L})}^{\text{ns}}$	271.1±112.0	286.3±118.3	0.7544	220.0-600.0
LDH$_{(\text{U/L})}^{\text{ns}}$	378.5±95.2	446.4±112.3	0.1307	160.0-410.0
Lactate$_{(\text{mmol/L})}^{**}$	0.83±0.45^{b}	9.69±5.32^{a}	0.0001	1.11-1.80
Glucose$_{(\text{mmol/L})}^{\text{ns}}$	5.78±0.84	6.08±0.88	0.4087	4.20-6.40

Means followed by equal letters do not differ from the Tukey test with 5% probability; (**) significance with $P \leq 0.01$; (ns) not significant; (#) [18].

In the hemogasometric study, pH, HCO_3-, TCO_2, K^+, and AG were significantly affected **(Table 3)**. A slight acidification of the blood after physical activity was observed, followed by a 39% reduction in bicarbonate concentration. The venous oxygen saturation was reduced by 39% and AG presented an increase of 85%. A negative correlation ($P < 0.001$) between the maximum lactate concentration and the pH value, HCO_3-, EB, and AG after exercise was observed. A significant elevation ($P < 0.05$) for the electrolyte K^+ was verified, however, physiologically, it remained within the reference range as well as the other electrolytes after exercise.

4. Discussion

The CI obtained in the experimental period classifies the environment as of thermal discomfort according to [9], since the limit value that would demonstrate nonactivation of the animal thermoregulatory system is 130. The THI values between 74-79 demonstrate an environment that can vary from critical to dangerous for the animals [17]. The physiological parameters (RR and HR) at rest exceeded the reference range of 8 to 16 breaths min^{-1} and 30 to 40 beats min^{-1}, respectively, according to [18]. These data are related to the THI value obtained, because when animals are inserted in an unfavorable thermal environment, there is the activation of the thermoregulatory center, with increased peripheral sweating and vasodilation, followed by increased HR and subsequent elevation of RR [19]. The ST, in general, and in different body regions studied, was higher due both to the influence of the thermal environment and the thermal energy generated by the muscle contraction required in high-speed running. The heat production, even being an expected physiological event, in most cases is greater than the dissipation capacity of the body, causing it to experience increased temperature [20]. The heat produced by exercise in a horse can increase its body temperature between 3.0 to 5.0 °C. If effective heat dissipation does not occur, this temperature may rise to a risk of thermal shock or integrated physiological disturbances [21]. Unfortunately, thermographic data collections in a second post-race moment were not performed. Thus, the effective heat dissipation and the return to the physiological state of thermal comfort have not been evaluated.

Associated with the environmental thermal stress detected, intense exercise promoted a proportional increase in the respiratory rate in response to high blood flow velocity in the pulmonary artery to the increase in output and heart rate as observed by [22]. Thus, there was a reduced time of erythrocytes in alveolar capillaries, which decreased the absorption time of O_2. Consequently, the association of reduced PO_2 values with intense exercise caused a change in the production of aerobic muscle energy of myocytes by an anaerobic metabolic pathway with massive lactate production [23]. Glucose, an important source of energy for muscle activity, however, did not undergo significant serum changes since, in intense exercise, much of the energy is generated from muscle glycogen hydrolysis through anaerobic glycolysis.

Despite the nonsignificant reduction of PO_2 and the saturation of O_2 in venous blood after only one event, the lactate level approaches the values of equines in longer activities, such as cross-country races [24] and high-speed treadmill [3]. Combined with changes in O_2 pressure, hypercapnia can occur with values above 50 mmHg [25]. However, this was not observed in our study since the activity was extremely short, with less than a minute between the start and rest in the post-exercise period.

The transient metabolic acidosis profile is well-known in sports medicine for equines [26], including with vaquejada animals at milder temperatures [1]. Transient acidosis in sports horses, which have hypoxemia because of high-intensity exercise, is correlated with post-exercise lactate levels. Lactate levels and HCO_3- concentration are inversely proportional because bicarbonate is consumed in the processing of Lactic Acid buffering [27]. According to [28], the increase in AG levels and reduction of EB and TCO_2 is justified by the reduction of bicarbonate levels consumed in the buffering of the excess lactic acid produced. After just one race, we observed a significant reduction in HCO_3- associated with reduced pH levels, so that, given a greater effort, as in consecutive races, a possible picture of marked metabolic acidosis may arise. Although consecutive runs are infrequent, it is common during unofficial vaquejadas, despite restrictions issued by the Brazilian Association of Vaquejada.

Table 3: Blood gas and serum electrolytes of the equine pre- and post-exercise.

	Pre-exercise	Post-exercise	*P*	Reference$^{\#}$
pH**	7.44±0.08^{a}	7.21±0.07^{b}	0.0001	7.32-7.46
pCO$_{2\,(mmHg)}^{ns}$	39.33±3.58	41.17±3.75	0.2341	38.0-46.0
pO$_{2\,(mmHg)}^{ns}$	58.42±16.83	57.33±16.51	0.8751	35.0-45.0
Sat O$_{2}^{ns}$	86.83±18.11	74.75±15.59	0.0937	80.0-90.0
HCO$_{3}$- $_{(mmol/L)}^{**}$	27.75±4.48^{a}	16.75±2.71^{b}	0.0001	23.0-32.0
BE $_{(mmol/L)}^{**}$	4.58±0.70^{a}	-11.5 ±-1.76^{b}	0.0001	0.0-6.0
TCO$_{2\,(mmol/L)}^{**}$	28.92±4.55^{a}	17.5±2.75^{b}	0.0001	22.0-31.0
K^{+}$_{(mmol/L)}^{*}$	4.02±0.18^{b}	4.18±0.19^{a}	0.0497	3.0-5.0
Na^{+}$_{(mmol/L)}^{ns}$	129.22±1.84	129.74±1.55	0.5113	132.0-146.0
Cl^{-}$_{(mmol/L)}^{ns}$	108.04±4.63	107.99±4.32	0.9797	98.0-110.0
iCa^{+2}$_{(mmol/L)}^{ns}$	1.40±0.07	1.37±0.06	0.3397	1.40-1.70
tCa^{+2}$_{(mmol/L)}$ns	2.80±0.13	2.74±0.18	0.3602	2.80-3.44
AG $_{(mEq/L)}^{**}$	-6.36±-0.88^{b}	5.58±0.77^{a}	0.0001	10.0-25.0

Means followed by equal letters do not differ from the Tukey test with 5% probability; (*) significance with $P \leq 0.05$; (**) significance with $P \leq 0.01$; (ns) not significant; (#)**[18]**.

According to **[29]**, depending on the time of duration and the type of physical exertion, an acute inflammatory process of physiological character is produced, altering the cellular permeability of myocytes. This change in permeability of the muscle enzymes CK, AST, and LDH, transfers them from the intracellular space to the extracellular one, causing an increase in serum level. The CK values in the pre-test were similar to those from **[14]**. Sodré *et al.* **[15]** found, for vaquejada horses, even with a single run, that the results in the post-test were higher than those observed in a post-test with successive runs. This significant increase in CK may be related to an intense and continuous muscular injury **[30]**, or the lack of physical conditioning of some animals that made up the experimental groups and that, consequently, increased the CK values in the post-race.

The elevation of K^{+} levels in maximal exertion activities may occur because of the transition of cations from the intracellular medium to the extracellular one, by the action of α-adrenergic receptors and some catecholamines present in red blood cells, liver, and muscle tissues **[31]**. Regarding the harmful effects on animals exposed to vaquejadas, it is pertinent to report that there will be a significant reduction 30 minutes after the completion of the activity, which will occur due to the same catecholamines that will act on β-adrenergic receptors, thereby correcting the hyperkalemia **[32]**.

5. Conclusions

For the vaquejadas performed in the Brazilian Northeast, thermal discomfort characterized by a critical to dangerous environment for animals was observed in the thermographic analysis. The association of this thermal stress with high-intensity physical exertion caused transient metabolic acidosis with a marked base deficit and acute energetic and muscle tissue stress. Because of this, we believe that consecutive races in vaquejadas should be discouraged because they may involve muscle damage and acid-base imbalance, which can represent the genesis of contusions, strains, and myopathies by exertion in sports horses.

Authors' Contributions

Thiago A. Gurjão, Gabriella S.B. Souto, Cledson C. Oliveira and Maycon R. Da Silva contributed to study design and execution, data analysis and interpretation, and preparation of the manuscript. Bonifácio B. de Sousa contributed to study execution. Antônio F.M. Vaz contributed to study design, data analysis and interpretation and preparation of the manuscript. All authors read and approved the final manuscript.

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Data Availability Statement

The data supporting the findings of this study are available within the article.

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Manufacturers

- [1]Rações Supra Alisul Alimentos S. A, São Leopoldo, RS, Brazil.
- [2]Fluke Corporation, Everett, Washington, USA.
- [3]BD Vacutainer, Franklin Lakes, NJ, USA.
- [4]Drake, São José do Rio Preto, SP, Brazil.
- [5]Fritz Hoffmann-La Roche, Basel, Switzerland.
- [6]Medmax, Barueri, SP, Brazil.
- [7]GraphPad Software, San Diego, CA, USA.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Ethical Approval

The research project was approved by the Research Ethics Committee (REC) of the Federal University of Campina Grande with protocol number 306/2015. All procedures for handling animals related to this study were conducted in accordance with national or institutional guidelines for the care and use of animals.

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