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Association between Infrared Thermography, Blood Count and Creatine Kinase in the Evaluation of the Welfare of Vaquejada Horses

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Abstract

The Quarter-Horses regularly participate in non-traditional sports that simulate activities on cattle ranches. In the vaquejada races, the horses run at about 30 km/h, reaching maximum heart rates of around 200 bpm, indicating the great physical effort during competitions and with that could impact welfare. To test the hypothesis that vaquejada horses can be evaluated for the quality of welfare through a combination of non-invasive and invasive methods, an experiment was developed that aimed to assess the quality of welfare through thermography and blood biomarkers before and after vaquejada races. Ten vaquejada horses, which were in regular competition, were submitted to the vaquejadas racing test were used. Thermography was performed in 14 regions of interest (head, neck and thorax) in the following phases: pre-race and +1, +4 and +24 hours of the races. Blood samples were collected in pre-race, immediately after and +1, +4 and +24 hours of the races. Results were submitted to ANOVA and Tukey's test, with p set at 5%. Thermography showed that local temperatures before the race and +24 hours after the races were similar ($p > 0.05$), as well as between +1 and +4 hours after the races ($p > 0.05$), which showed higher surface temperatures than the first group (pre-race and +24 hours) ($p < 0.05$). There was also an increase in red blood cells, hemoglobin and hematocrit immediately after the vaquejada races ($p < 0.05$) with a return to normality +1 hour after the races. Plasma proteins rose after the runs and returned to normal values within +1 hour; CK did not change ($p > 0.05$) after the exercises. In conclusion, the vaquejada horses evaluated by infrared thermography and blood analyses maintained the quality of well-being, with the physiological elevation of several parameters after the races and recovery in less than 24 hours after the races. Thermographic images were also able to help in the analysis of the regions of interest that have contact with harnesses, demonstrating that the use of adequate and correct equipment does not compromise animal welfare.

Keywords

Exercise, Equine, CK, Hematology, Equestrian Sports, Well-Being

1. Introduction

Sports with horses are quite widespread, and in most horses, there is a direct relationship with cattle raising, especially in the Americas. Competitions such as roping, paleteada, rodeo, vaquejada and coleada are present in several countries on these continents and involve the use of horses and cattle in the same competition. These ranch types of equestrian competitions are quite different from those commonly known to the urban public, such as races and equestrian events. Due to their importance for job creation and horse agribusiness, these competitions have specific regulations to meet good sports practices and animal welfare. In vaquejada races, the main objective is the good use of horses and cattle involved in official competitions, complying with all official regulations [1] [2] [3], in order to provide the maximum level of welfare to the participating animals.

Different articles have described vaquejada and its impacts on the welfare of horses and cattle, addressing both physiological and behavioral aspects by invasive and noninvasive methods [4] [5] [6] [7]. Some studies have shown that horses exhibit physiological and transient elevation of muscle enzymes, recovering quickly in less than 24 hours [6] [8] [9]. Studies also include epidemiological evaluations, demonstrating the low occurrence of colic syndrome (0.63%) in more than 2000 animals in 12 different events [10]. Additionally, with regard to cattle in vaquejada, studies with this species showed the absence of lesions in the tail and related muscles when they are using the tail protector harness and that the cattle are also adapted to handling the races in the vaquejada events [7] [11] [12].

All of the aforementioned studies used various indicators and signals to evaluate animal welfare [13] in horse and cattle races. However, it is known that animal welfare evaluation methods must be validated, reliable, and viable, in addition to being scientifically established [13], and therefore need to be reviewed regularly and incorporate new evaluation methods, invasive and noninvasive, which can be combined with each other [14]. With these possible combinations, we will be able to more quickly identify the misuse of animals or the inappropriate use of techniques and thus establish new practices to increase animal welfare and the correct use of animals, in addition to promoting the integration between animal, human, and environmental impact [15].

An example of the new processes incorporated is infrared thermography (IRT), which has been shown to be simple and can be used in various conditions, provided that they meet the specifications for use [16]. The IRT has good accuracy in its measurements and is accepted in the scientific community as an effective methodology to assess animal health and welfare [17] [18]. Thus, the combination of thermography with different hematological biomarkers may improve the identification of physiological changes in stress responses and the quality of animal health [19] [20] [21] [22].

Therefore, to test the hypothesis that horses subjected to vaquejada races can be evaluated for quality of welfare through a combination of noninvasive and invasive methods, an experiment was developed to evaluate the quality of welfare for vaquejada horses through noninvasive (thermography) and invasive methods (blood count, plasma proteins, creatine kinase (CK)) before and after physical effort and in the recovery period of vaquejada races. These evaluations may contribute to the evaluation of the animal welfare in this group of athletes and thus meet the current official regulation and establish new concepts to improve the animal welfare for equestrian sports.

2. Materials and Methods

All procedures were approved by the ethics and animal welfare committee of the Ethics Committee on the Use of Animals of the Centro Universitário Cesmac (CEUA/CESMAC) under registration number 01200702819/2016-97.

2.1. Animals and Breeding System

Ten-quarter horses were used (age: 4 - 8 years; weight: ~450 kg; body score: 5 - 6) and housed in a training center for vaquejada races. The experimental horses were fed 20.0 kg of fresh chopped elephant grass (*Pennisetum purpureum* Schum.) divided into three meals, in addition to receiving concentrate supplementation (MaxEquinos Trabalho Mix, CP: 14% (min); EE: 5% (min); FB: 10% (max); BM 10% (max); ADF 15% (max), DuRacho Nutrição Animal), with 6 kg/animal/day, divided into three meals with ~2.0 kg/animal. Mineralized salt and water were available *ad libitum*. Also, the experimental horses were not subjected to changes in their diets to eliminate or reduce acute or chronic stress after the vaquejada challenge, that is, the animals maintained their pre-experimentation diets during whole recovery period.

All animals were housed in individual boxes measuring approximately 15 m^2, ventilated and with ample view of the horses around them. The trot and canter training was performed 3 times a week for 30 to 40 minutes on an official track for the sport, and at least twice a week, they performed 60-minute walks and mounted walks.

2.2. Simulation Test of the Vaquejada Race

The vaquejada race simulation test was performed as previously described in the literature [5] [8] [23], complying with official regulations of the Ministry of Agriculture, the Brazilian Association of Vaquejada, the Brazilian Association of Quarter Horse Breeders and the Brazilian Association of Technical Standards, both for horses and cattle [1] [2] [3] [24].

For vaquejada race simulation test, the horses were warmed for 10 minutes while trotting and cantering. Then, they performed three consecutive runs with cattle on a soft sand track (length 130.0 meters), where the cowboy and his horse gallop to unbalance and knock the cattle (Nelore, ~350 kg) down on a 9.0-meter sand strip, after 90.0 meters run, along with another cowboy and his track horse. Each run lasted 15 to 20 seconds, and then the animals returned to the starting point at a walk until completing three runs. After vaquejada race simulation test, a blood sample was collected, and the animals continued to walk for 15 minutes. Each bull ran only once on the same day and used the official tail protection harness. Both cattle and horses used in this experiment were evaluated 24 hours before, during the test and 24 hours after performing the vaquejada race simulation test using the 5-Domain system [25].

2.3. Collection and Analysis of Blood and Biometric Measurements

Blood samples were collected by jugular puncture in vacuum tubes containing EDTA for complete blood count and subsequent collection of plasma, which was stored in a freezer at −20˚C for further analysis (plasma protein and creatine kinase). These samples were collected in the following phases: pre-race with the animals fasting, immediately after exercise, and after 1, 4 and 24 hours of exercise. Samples after 24 hours were collected with the horses fasting.

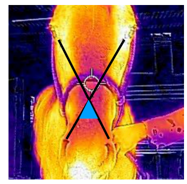
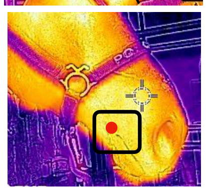
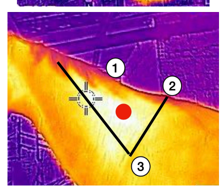
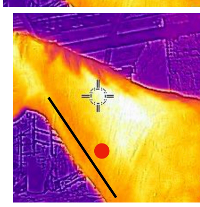
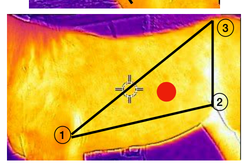
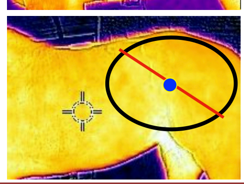
Complete blood counts were performed in semiautomatic equipment (Roche® Poch 100iv), and plasma protein determination was obtained by refractometry. The analyses to determine the CK concentrations were performed in semiautomatic equipment (Doles® D-250) using commercial kits (Doles®) following the manufacturer's recommendations. The weight of the horses was determined using equine tape, and the body score was determined using the model by Henneke *et al.* (1983) [26].

2.4. Thermographies and Environmental Conditions

An infrared thermograph (E4 Wifi, FLIR Systems AB, Sweden) was used to perform the thermography in the following periods: pre-race with the animals fasting for food and 1, 4 and 24 hours after the three vaquejada races. These thermographic examinations were performed in a covered environment, without drafts and with the animals dry, at an average distance of 150 cm from the target. After the races, the animals were sent to their respective boxes with water and fresh forage, and they were not bathed to allow the natural drying of the body to avoid compromising the thermographic images.

The body regions evaluated were the ocular caruncle, the corner of the mouth, face/bridge of nose, jaw/masseter muscle region, neck/trapezius muscle region, neck/brachiocephalicus muscle region, barrel/costal arches and flank (Table 1). All regions of interest were photographed on the left and right sides, with the exception of the ocular caruncle and face/bridge of nose.

Table 1. Region of interest in horses to obtain thermographic images.

Region	Description	Location
Ocular Caruncle	Region at the inner angle of the eye	
Face/Bridge of Nose	Region on the anterior surface of the head, between the forehead and the nostrils	
Jaw/Masseter Muscle Region	Region on the lateral surfaces of the head, with the masseter muscle	
The Corner of the Mouth	The point where the lips meet at the corner of the mouth	
Neck/Trapezius Muscle Region	Muscle near the upper border of the neck, near the base (1-upper edge of neck; 2-withers; 3-midpoint of base of neck).	
Neck/Brachiocephalicus Muscle Region	Muscle near the lower border of the neck, and dorsal to the jugular groove.	
Barrel/Costal Arches	Region referring to the costal arches (1-elbow; 2-stifle; 3-point of hip)	
Flank	Midpoint between the point of hip and the stifle	

The emissivity for the readings was set at 0.95 ε, and the procedures adopted for performing the thermographs were in accordance with those described in the literature [7] [27] [28]. The thermography images were processed by a software (FLIR Tools, FLIR Systems AB, Sweden) to adjust the results for the temperature and relative humidity of the environment at the time of images' collection [7] [28].

The ambient temperature and relative humidity were measured using a thermohygrometer (Thermo-Hygrometer #7666.02.0.00, Incoterm Indústria de Termómetros LTDA, Brazil) with a temperature resolution of 0.1˚C and accuracy of +1˚C to 0˚C. at 50˚C, the relative humidity had a resolution of 1% (precision ±5%), according to the manufacturer's instructions. The thermal stress index (TSI) was calculated according to the formulas described in the literature [29] [30] (**Table 1**), which is the sum of the ambient temperature in Fahrenheit and the relative humidity. The following formula for converting Celsius to Fahrenheit was used: ˚F = (5/6 × ˚C) + 32, and the following formula was used to calculate the temperature and humidity index (THI): 0.8 × T (˚C) + RH × (T − 14.4) + 46.4, with moisture expressed numerically (75% should be 0.75) [31] [32] (**Table 2**).

2.5. Statistical Analyses

The results were subjected to one-way ANOVA and Tukey's test to identify possible differences between periods. In all cases, the significance level was set at 5%, and the results are expressed as the means ± mean standard error. The statistical analysis software SigmaPlot 13.0 (Systat Software Inc., San Jose, CA, USA) was used in all analyses.

Table 2. Thermal stress index (TSI) and temperature and humidity index (THI) risk rating.

Model	Index	Risk Rating	Effects on the Metabolism of Horses
TSI	1	<120/130	Normal cooling of the horse's body, through evaporation, respiration and sweating, unless the horse is obese or very hairy
	2	>140	Responsible for heat loss is sweat, which can be aggravated by obesity or very hairy
	3	>150	Evaporative heat loss is compromised, especially if the relative humidity is above 50%, but sweat is still important for heat loss
	4	>180	The natural dissipation of heat does not occur, and subsequent increases in body temperature may occur. There is great danger for the occurrence of thermal stress
THI	Light	72 - 79	Mild risk of thermal stress and responsible for heat loss is sweat. In horses with a lot of hair or obese, a greater impact may occur
	Moderate	80 - 89	Moderate risk of heat stress
	Severe	>90	Severe risk of heat stress

Notes: TSI: thermal stress index, adapted from Cymbaluk and Christison (1990) [30] and Loving and Johnston (1995) [29]; **THI:** temperature/relative humidity index, adapted from NOAA (1976) [31] and Vitali *et al.* (2009) [32].

3. Results

Room temperature and relative humidity varied between 22˚C and 29˚C and 67% and 85%, respectively, under the conditions of the vaquejada simulation test. With these variations, the classification of the thermal stress index (TSI) and the air temperature/humidity index (THI) was at level 3 and mild, respectively, in the different evaluation phases of these parameters (Table 3). The highest TSI was observed in the +1 hour phase (160.2), while the THI was observed in the +4 hour phase (79.5) after the races.

Surface temperatures in the regions of interest were higher 1 and 4 hours after the races compared to those obtained before the race and after 24 hours ($p < 0.05$). It was also observed that the temperatures before the test and +24 hours after the tests were similar ($p > 0.05$), as well as between +1 and +4 hours after the tests ($p > 0.05$) (Table 3).

Table 3. Result of ambient temperature (˚C), relative humidity (%), thermal stress index, temperature/humidity index and thermographic image temperature (˚C) in the region of interest for pre- and post-race vaquejada horses (n = 10).

Parameters	Experimental Periods			
	Pre-race	+1 Hour after the 3 Races	+4 Hours after the 3 Races	+24 Hours after the 3 Races
Ambient Temperature, ˚C (˚F)	22.8 (73.0)	24.0 (75.2)	29.0 (84.2)	24.1 (75.4)
Relative Humidity, %	76.8	85.0	67.6	76.9
TSI	149.8	160.2	151.8	152.3
THI	71.11	73.76	79.50	73.20
Face/Bridge of Nose	30.45 ± 0.53 **B**	36.51 ± 0.47 **A**	36.65 ± 0.30 **A**	30.79 ± 0.87 **B**
Ocular Caruncle	34.24 ± 0.36 **B**	36.98 ± 0.26 **A**	37.48 ± 0.20 **A**	34.74 ± 0.48 **B**
The Corner of the Mouth L	34.50 ± 0.34 **B**	37.15 ± 0.20 **A**	37.50 ± 0.28 **A**	35.34 ± 0.46 **B**
The Corner of the Mouth R	34.68 ± 0.30 **B**	37.06 ± 0.30 **A**	37.59 ± 0.22 **A**	35.42 ± 0.45 **B**
Jaw/Masseter Muscle Region L	33.29 ± 0.54 **B**	36.48 ± 0.34 **A**	37.59 ± 0.32 **A**	34.44 ± 0.57 **B**
Jaw/Masseter Muscle Region R	32.71 ± 0.50 **B**	36.00 ± 0,45 **A**	37.16 ± 0.34 **A**	34.25 ± 0.58 **B**
Neck/Trapezius Muscle Region L	34.39 ± 0.46 **B**	36.94 ± 0.47 **A**	37.63 ± 0.41 **A**	34.62 ± 0.82 **B**
Neck/Trapezius Muscle Region R	34.16 ± 0.40 **B**	36.42 ± 0.56 **A**	37.00 ± 0.32 **A**	34.54 ± 0.50 **B**
Neck/Brachiocephalicus Muscle Region L	33.70 ± 0.26 **B**	37.05 ± 0.34 **A**	37.32 ± 0.32 **A**	33.51 ± 0.33 **B**
Neck/Brachiocephalicus Muscle Region R	33.22 ± 0.36 **B**	37.20 ± 0.38 **A**	36.80 ± 0.28 **A**	33.74 ± 0.38 **B**
Barrel/Costal Arches L	33.17 ± 0.46 **B**	36.41 ± 0.43 **A**	37.63 ± 0.26 **A**	33.23 ± 0.60 **B**
Barrel/Costal Arches R	33.05 ± 0.29 **B**	36.49 ± 0.45 **A**	36.04 ± 0.30 **A**	33.48 ± 0.60 **B**
Flank L	33.62 ± 0.36 **B**	36.97 ± 0,46 **A**	38.01 ± 0.41 **A**	33.68 ± 0.42 **B**
Flank R	33.42 ± 0.30 **B**	36.56 ± 0.37 **A**	36.78 ± 0.30 **A**	33.86 ± 0.53 **B**

Notes: different capital letters on the same line indicate that $p < 0.05$ by Tukey's test; **TSI:** heat stress index; **THI:** air humidity temperature index; **L:** left side; **R:** right side.

The results of the hematological analyzes indicated an increase in the concentrations of total plasma proteins, erythrocytes, hemoglobin and hematocrit immediately after vaquejada ($p < 0.05$), but without an increase in the other parameters analyzed ($p > 0.05$) (**Table 4**). In the biochemical analyses, there was an increase in plasma proteins ($p < 0.05$) immediately after the races. Even so, these also returned to normal values within 60 minutes, remaining so up to 24 hours after the races. There was no change in CK concentrations ($p > 0.05$).

4. Discussion

The current study combined analyses of thermographic images, in 14 regions of interest, and blood analyses to assess the effect of harnesses used in riding, as well as the impact of physical exertion during vaquejada. The thermographic images showed a similarity pattern of elevation after 1 and 4 hours of the runs and recovery in 24 hours of surface temperatures in the regions of interest. These regions of interest are influenced by environmental conditions, physical effort, harnesses, and the recovery model, but without compromising animal health and welfare, as they recovered without compromising health. In the blood evaluations, similar patterns of variation were also found to those observed in other studies [5] [8] [23]. Thus, it was observed that the effects on the stress system and on general metabolism were transient when the animals participated in races following the current rules [1] [2] [3] [24] without impairment horses' health and well-being in the vaquejada. Thus, when these evaluations are combined, they can promote the creation of new systems for evaluating animal welfare during competitions and the appropriate use of equipment used by horses and riders.

Table 4. Results of hematological and biochemical tests of vaquejada horses (n = 10), pre- and post-race.

Parameters	Experimental Periods				
	Pre-race	Immediately after the 3 Races	+1 Hour after the 3 Races	+4 Hours after the 3 Races	+24 Hours after the 3 Races
Plasma Proteins, mg/dL	6.56 ± 0.06 **AB**	6.90 ± 0.09 **A**	6.42 ± 0.09 **B**	6.66 ± 0.14 **AB**	6.74 ± 0.10 **AB**
Creatinekinase, UI/L	192.45 ± 22.71	217.03 ± 29.63	218.95 ± 26.13	214.80 ± 24.44	235.30 ± 30.30
Red Blood Cells, $\times 10^6/\mu L$	8.04 ± 0.43 **B**	11.46 ± 0.45 **A**	7.67 ± 0.18 **B**	7.96 ± 0.42 **B**	7.80 ± 0.34 **B**
Hemoglobin, g/dL	12.50 ± 0.70 **B**	17.91 ± 0.75 **A**	11.94 ± 0.34 **B**	21.37 ± 0.68 **B**	12.12 ± 0.65 **B**
Hematocrit, %	35.11 ± 1.82 **B**	50.82 ± 1.99 **A**	33.54 ± 0.80 **B**	34.68 ± 2.00 **B**	34.01 ± 1.69 **B**
MCHC, g/dL	35.56 ± 0.30	35.19 ± 0.34	35.56 ± 0.30	35.70 ± 0.31	35.59 ± 0.29
MCV, fL	43.72 ± 0.46	44.38 ± 0.49	43.76 ± 0.52	43.51 ± 0.53	43.52 ± 0.54
RDW-CV, %	20.62 ± 0.21	20.71 ± 0.20	20.67 ± 0.23	20.69 ± 0.20	20.36 ± 0.21
RDW-SD, fL	35.80 ± 0.40	36.01 ± 0.50	35.97 ± 0.46	35.64 ± 0.41	35.54 ± 0.41
Platelets, $10^3/\mu L$	116.90 ± 10.59	136.80 ± 18.40	126.90 ± 13.3	137.50 ± 14.26	126.40 ± 15.54
White blood Cells, $10^3/\mu L$	7.86 ± 0.45	9.56 ± 0.54	8.35 ± 0.71	8.40 ± 0.56	7.99 ± 0.60
Lymphocytes, $10^3/\mu L$	2.71 ± 0.24	3.72 ± 0.24	2.72 ± 0.21	2.59 ± 0.24	2.76 ± 0.24
Other White Blood Cells, $10^3/\mu L$	5.15 ± 0.37	5.84 ± 0.40	5.47 ± 0.44	5.81 ± 0.46	5.23 ± 0.48

Note: different capital letters on the same line indicate that $p < 0.05$ by Tukey's test.

5. Environmental Conditions and Thermography

The vaquejada simulation tests were performed in the early morning, as the animals would perform 3 races in a row, thus the horses would have a short recovery interval (<2 minutes), walk at a pace, and on horseback, between races. This system has already been described in the literature and accurately reflects regular vaquejada competitions without harming the health and well-being of equine athletes [5] [8] [23]. In the present experiment, the horses ran after blood collections in the pre-race and warm-up periods, when average temperatures were close to 22.8˚C and relative humidity was 76.8%. Thus, the thermal stress and temperature/humidity indices would be classified around 3 and mild, respectively [29] [31] [32], similar to what occurred in evaluations +1, +4 and +24 hours after races periods.

The Infrared thermography (IRT) was performed in 14 regions of interest with some relation to the driving harness and aids. The bridge of the nose (place of action noseband), The corner of the mouth (place of action of the bridle), and the jaw (muscular region of the impact of the jaw under pressure) are related to the steering harness and are used to evaluate the action of the rider in these structures and the stress level in the region [33] [34]. The neck regions (reflecting the movement of the animal on the competition track), barrel/costal arches, and flank (reflecting the action of the spurs) [34] are more associated with the rider's mount, which is widely used for evaluations of well-being during competitions. Thus, the performance of thermographs in the 14 regions of interest analyzed various aspects of the use of horses in sports, in this case with emphasis on the vaquejada horse.

The IRT indicated that the horses recovered less than 24 hours after vaquejada simulation tests, as there were no differences between the temperatures in the regions of interest in the pre-race and +24 hours. However, in phases +1 and +4 hours after the races, the surface temperatures were higher, reflecting the impact of the physical effort of the vaquejada simulation tests, the temperature and relative humidity of the air in the environment, and the recovery model. The indicators of environmental stress, TSI and THI, were higher precisely at +1 and +4 hours after the races, impacting heat loss. It is also believed that the decision not to bathe the animals after the races, which does not occur in the vaquejadas races, also influenced the results of surface temperatures, but this methodology also avoided possible interference with local thermal changes in the regions of interest such as inflammation, excessive cooling or post-exercise injuries. The methodology adopted in the recovery allowed the achievement of accurate and unimpaired results of the thermographic images. Finally, when the results of the thermography in the regions of interest are combined, they indicate that there were no negative impacts of the driving and mounted harnesses used (nosebands, bridles, and saddles) and of the aids (spurs) on the vaquejada horses during physical exertion.

Horses are bred and trained under different environmental conditions around the world, and thermo-neutrality is not well-defined for horses [30]. The experimental animals had been housed and trained in the training center for more than 4 months, in addition to competing regularly in different locations in the region. They also had a body score compatible with the sport, between 5 and 6, were not obese, and had short hair, and no difficulty in losing heat in the test environment was observed, even without having been bathed after exercise.

Under the current conditions of the experiment, the horses depended on sweating to facilitate heat loss [29] [30], which made it impossible to collect thermographic images immediately after the races in the regions of interest. The sweat resulting from physical exertion evaporated naturally with the horses in their boxes, and 1 hour after the races, they were dry and ready to obtain the thermographic images, demonstrating that they were well adapted to the environmental conditions in this region. This procedure precisely identified the effects of physical exercise on heat generation, stress hormones, and post-exercise thermographic images in the regions of interest surveyed.

Different studies have used the information from electro-myography and thermography to evaluate muscle movement during exercises and the possible impact of the use of driving harnesses (bites and halters) on structures that have local contact during mounts [33] [34] [35] [36], which facilitates the understanding of the impact of physical effort and the characteristics of the rider during riding. The temperatures in the head region, at five points (bridge of the nose, the corner of the mouth, and the jaw), showed similar patterns of variation, following the same pattern of elevation and recovery observed in the ocular caruncle, but with different values because those structures are more affected by the environmental conditions than that evaluated in the ocular caruncle. Loss of rise/fall patterns in surface temperature immediately after exercise may indicate increased local stress, either from inflammation (hyperthermia) or impaired blood supply (hypothermia) [35]. This assessment can easily be done by thermography, provided the appropriate techniques are used [16] [27] [28], and should be used both for assessing health and well-being in equine athletes in different sports.

6. Blood Analysis

The present experiment showed that after three races in a row, vaquejada horses show a rapid recovery in hematological parameters and plasma proteins, with no variation in CK concentration, similar to other studies with race and vaquejada horses [5] [6] [8] [37]. In the biochemical analyses, there was an increase in plasma proteins, followed by an increase in the number of red blood cells (He) and hematocrit (HT) immediately after the races, reflecting the movement of fluids during physical effort; 1 hour after the action of the stressor, the concentrations of red blood cells returned to the values observed pre-race and 24 hours after.

However, despite the significant increase in Ht (42%), the increase in plasma proteins was slight (5%), suggesting a reduction in fluid loss immediately after vaquejada races, even with intense sweat production to help with thermoregulation after racing, which is very typical of short-duration, high-intensity sports. One hour after the exercises, the concentrations of plasma proteins and He, in addition to Ht, were already similar to the values found in experimental animals pre-race/fasting and 24 hours after the races ($p > 0.05$), indicating a quick recovery after physical exertion. These variations observed in vaquejada horses are similar to those described by other authors for this sport, in addition to also occurring in other equestrian sports of high intensity and short duration [5] [6] [8]. The current experimental animals recovered in the stalls with water and forage ad libitum, in addition to mineralized salt, which may have contributed to these results.

Creatine kinase (CK) concentrations after exercise are an indicator of the health of the muscular system in athletic horses. Animals in good health and well-conditioned may show a slight increase in CK concentration after physical exertion due to changes in plasma volume, but with the recovery of pre-test values in a few hours [6] [8] [9]. For CK evaluation, the blood of the animals must be collected between 4 and 6 hours after the exercise [38], and in the present experiment, the CK concentrations remained below the limit that indicates muscular injury (<400/500UI) [8] [39] after 1, 4 and 24 hours after the races, demonstrating that the horses used in the current experiment did not develop muscle tissue injuries that would compromise their health and well-being.

Finally, carrying out simulation tests in equestrian sports when carried out under real conditions of animal use is always a challenge for researchers as it presents several limitations such as the number of animals able to be used and the impact of local meteorological conditions. In the current experiment, the biggest limitation was the number of animals used, which were 10 animals. They should be in competition conditions, and receive very similar diets as well as training practices, factors that reduce the inclusion of animals even in places with many animals. Another point that can be observed and could reduce some deficiencies in the current experiment would be the determination of the concentration of stress hormones, such as Cortisol, and also the heart rate during exercise for a better understanding of the degree of physical effort to which the animals were submitted. However, as there are other publications on the subject, we opted to carry out a greater number of thermography images in the areas of interest, as this was the focus of the current study.

7. Conclusion

The animals subjected to the vaquejada simulation test, under current conditions and following the official rules, when evaluated through the combination of infrared thermography and blood analyses, maintained their health and quality of welfare. In the tests, there was a physiological elevation of the surface temperature and of some blood biomarkers after the races, but with the recovery of these parameters in less than 24 hours. Thermographic images were also able to assist in the analysis of the regions of interest, which have contact with harnesses, demonstrating that the correct use of this equipment does not compromise the animal welfare of the vaquejada horses.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] GMAPA (2017) Ministério da Agricultura e Pecuária. Portaria MAPA No. 1.781, 14/08/2017. Nota técnica 08/2017/MAPA-CTBEA, Regulamento Geral da Vaquejada.

[2] Associação Brasileira de Vaquejada (ABVAQ) (2020) Manual Bem-Estar Animal. https://drive.google.com/file/d/1bM1qpXZ6eredn5G1xSZR3qLhQYIn5Fn0/view

[3] Associação Brasileira de Criadores de Cavalo Quarto de Milha (ABQM) (2020) Manual de boas práticas para bovinos participantes de atividades esportivas equestres. https://abqm.com.br/app/webroot/documentos/manualdeboaspraticasembovinos-site.pdf

[4] Pimentel, M.M.L., Camara, F.V., Dantas, R.A., Freitas, Y.B.N., Dias, R.V.C. and Souza, M.V. (2011) Biometria de equinos de vaquejada no Rio Grande do Norte, Brasil. *Acta Veterinaria Brasilica*, **5**, 376-379.

[5] Santiago, T.A., Manso, H.E.C.C.C., Abreu, J.M.G., Melo, S.K.M. and Filho, M.H.C. (2014) Blood Biomarkers of the Horse after Field *Vaquejada* Test. *Comparative Clinical Pathology*, **23**, 769-774. https://doi.org/10.1007/s00580-013-1683-y

[6] Souza, T.M.S., Rêgo, G.M., Nunes, G.S., Paraguaio, P.E. and Machado, L.P. (2014) Elevação transitória da atividade sérica das enzimas musculares em equinos após exercício de vaquejada. *Ciência Veterinária nos Trópicos*, **17**, 56-57.

[7] Cruz, R.K.S., Vilela, C.F., de Freitas, D.B., Paixão, C.C., da Silva, C.J.F.L., Trindade, K.L.G., da Silva, J.G., da Costa Cordeiro Manso, H.E.C. and Filho, H.C.M. (2021) Effort and Recovery in Nellore Oxen during Vaquejada Assessed with Ocular and Tail Infrared Thermography Superficial Temperature. *Open Journal of Veterinary Medicine*, **11**, 258-271. https://doi.org/10.4236/ojvm.2021.117017

[8] Hunka, M.M., Souza, L.A., Almeida, T.T.S., Nery, P.C.R., Manso, H.E.C.C.C. and Filho, H.C.M. (2018) Metabolic and Physiological Changes during and after Vaquejada Exercise in Horses. *Medicina Veterinária* (*UFRPE*), **12**, 254-262. https://doi.org/10.26605/medvet-v12n4-2454

[9] Santos, A.L., Ferreira, D.S.A., Neto, J.R., Bessa A.F.O., Filho, M.C., Costa, M.L.L. and Moreira, G.R. (2019) Recuperação de cavalos em teste de simulação de vaquejada. *Sigmae*, **8**, 266-273.

[10] Dias, R.V.C., Bevilacqua, D., Filho, J.D.R., Ribeiro Junior, J.I. and Souza, M.V. (2013) Avaliação física e laboratorial da síndrome cólica de equinos em parque de vaquejada. *Veterinária e Zootecnia*, **2**, 658-672.

[11] Cruz, R.K.S., Ribeiro, M.L.A., Macedo, J.S., Ferreira, B.A., Silva, M.A., Neves, D.A., Manso, H.E.C.C.C. and Filho, H.C.M. (2021) Clinical and Radiographic Evaluation of Cattle Tail before and after the Vaquejada Race. *Open Journal of Veterinary Medicine*, **11**, 165-176. https://doi.org/10.4236/ojvm.2021.116012

[12] Cruz, R.K.S., Ribeiro, M.L.A., Sarmento, E.C.B., Oliveira, C.F., Alencar, H.A.G., Silva, J.G., Manso, H.E.C.C.C. and Filho, H.C.M. (2021) Manejo nas corridas de vaquejada e na vacinação modificam as concentrações da creatinaquinase e do Cortisol em bovinos. *Medicina Veterinaria* (*UFRPE*), **15**, 196-203. https://doi.org/10.26605/medvet-v15n3-3578

[13] Lesimple, C. (2020) Indicators of Horse Welfare: State-of-the-Art. *Animals*, **10**, Article 294. https://doi.org/10.3390/ani10020294

[14] Bozzo, G., Dimuccio, M.M., Casalino, G., Ceci, E., D'Amico, F., Petrontino, A., Bonerba, E., Camarda, A. and Circella, E. (2022) Preliminary Evidence Regarding the Detection of Cortisol and IL-6 to Assess Animal Welfare in Various Rabbit Housing Systems. *Agriculture*, **12**, Article 1622. https://doi.org/10.3390/agriculture12101622

[15] Tarazona, A.M., Ceballos, M.C. and Broom, D.M. (2020) Human Relationships with Domestic and Other Animals: One Health, One Welfare, One Biology. *Animals*, **10**, Article 43. https://doi.org/10.3390/ani10010043

[16] Yanmaz, L.E., Kumus, Z. and Dogan, E. (2007) Instrumentation of Thermography and Its Applications in Horses. *Journal of Animal and Veterinary Advances*, **6**, 858-862.

[17] Martins, J.N. and Silva, S.R. (2022) Use of Infrared Thermography to Assess Body Temperature as a Physiological Stress Indicator in Horses during Ridden and Lunging Sessions. *Animals*, **12**, Article 3255. https://doi.org/10.3390/ani12233255

[18] Young, P., Russo, I., Gill, P., Muir, J., Henry, R., Davidson, Z. and Costa, R.J.S. (2023) Reliability of Pathophysiological Markers Reflective of Exercise-Induced Gastrointestinal Syndrome (EIGS) in Response to 2-h High-Intensity Interval Exercise: a Comprehensive Methodological Efficacy Exploration. *Frontiers in Physiology*, **14**, Article 1063335. https://doi.org/10.3389/fphys.2023.1063335

[19] Alberghina, D., Pasquale, A., Piccione, G., Vitale, F. and Panzera, M. (2015) Gene Expression Profile of Cytokines in Leukocytes from Stereotypic Horses. *Journal of Veterinary Behavior*, **10**, 556-560. https://doi.org/10.1016/j.jveb.2015.08.007

[20] Pawluski. J., Jego, P., Henry, S., Bruchet, A., Palme, R., Coste, C. and Hausberger, M. (2017) Low Plasma Cortisol and Fecal Cortisol Metabolite Measures as Indicators of Compromised Welfare in Domestic Horses (*Equus caballus*). *PLOS ONE*, **12**, e0182257. https://doi.org/10.1371/journal.pone.0182257

[21] Witkowska-Piłaszewicz, O., Baska, P., Czopowicz, M., Zmigrodzka, M., Szarska, E., Szczepaniak, J., Nowak, Z., Winnicka, A. and Cywinska, A. (2019) Anti-Inflammatory State in Arabian Horses Introduced to the Endurance Training. *Animals*, **9**, Article 616. https://doi.org/10.3390/ani9090616

[22] Slusher, A.L. and Acevedo, E.O. (2023) Stress Induced Proinfammatory Adaptations: Plausible Mechanisms for the Link between Stress and Cardiovascular Disease. *Frontiers in Physiology*, **14**, Article 1124121. https://doi.org/10.3389/fphys.2023.1124121

[23] Hunka, M.M., Lima, L.C.F.L., Souza, L.A., Silva, C.J.F.L., Silva, E.R.R., Manso, H.E.C.C.C. and Filho, H.C.M. (2018) Heart Rate and Velocity in Vaquejada Horses during Field Tests. *Comparative Exercise Physiology*, **13**, 25-30. https://doi.org/10.3920/CEP160027

[24] Associação Brasileira de Normas Técnicas (ABNT) (2020) Equipamentos utilizados na prática de atividades esportivas equestres da raça de cavalos Quarto de Milha.

Parte 5: Modalidade-Vaquejada. ABNT/DE Publicações.

[25] Mellor, D.J. (2017) Operational Details of the Five Domains Model and Its Key Applications to the Assessment and Management of Animal Welfare. *Animals*, **7**, Article 60. https://doi.org/10.3390/ani7080060

[26] Henneke, D.R., Potter, G.D., Kreider, J.L. and Yeates, B.F. (1983) Relationship between Condition Score, Physical Measurements and Body Fat Percentage in Mares. *Equine Veterinary Journal*, **15**, 371-372. https://doi.org/10.1111/j.2042-3306.1983.tb01826.x

[27] Bartolomé, E., Perdomo-González, D.I., Sánchez-Guerrero, M.J. and Valera, M. (2021) Genetic Parameters of Effort and Recovery in Sport Horses Assessed with Infrared Thermography. *Animals*, **11**, Article 832. https://doi.org/10.3390/ani11030832

[28] Idris, M., Uddin, J., Sullivan, M., McNeill, D.M. and Phillips, C.J.C. (2021) Non-Invasive Physiological Indicators of Heat Stress in Cattle. *Animals*, **11**, Article 71. https://doi.org/10.3390/ani11010071

[29] Loving, N.S and Johnston, A.M. (1995) Veterinary Manual for the Performance Horse. Blackwell Science Ltd, Hoboken, NJ.

[30] Cymbaluk, N.F. and Christison, G.I. (1990) Environmental Effects on Thermoregulation and Nutrition of Horses. *Veterinary Clinics of North America*: *Equine Practice*, **6**, 255-372. https://doi.org/10.1016/S0749-0739(17)30546-1

[31] National Oceanic Atmospheric Administration (NOAA) (1976) Livestock Hot Weather Stress. Regional Operations Manual Letter C-31-76. US Department of Commerce, National Oceanic and Atmospheric Administration, National Weather Service Central Region.

[32] Vitali, A., Segnalini, M., Bertocchi, L., Bernabucci, U., Nardone, A. and Lacetera, N. (2009) Seasonal Pattern of Mortality and Relationships between Mortality and Temperature-Humidity Index in Dairy Cows. *Journal of Dairy Science*, **92**, 3781-3790. https://doi.org/10.3168/jds.2009-2127

[33] Sainsbury, P. and Gibson, J.G. (1954) Symptoms of Anxiety and Tension and the Accompanying Physiological Changes in the Muscular System. *Journal of Neurology*, *Neurosurgery and Psychiatry*, **17**, 216-224. https://doi.org/10.1136/jnnp.17.3.216

[34] Rankins, E.M., Manso Filho, H.C., Malinowski, K. and McKeever, K.H. (2022) Muscular Tension as an Indicator of Acute Stress in Horses. *Physiological Reports*, **10**, e15220. https://doi.org/10.14814/phy2.15220

[35] McGreevy, P., Warren-Smith, A. and Guisard, Y. (2012) The Effect of Double Bridles and Jaw-Clamping Crank Nosebands on Temperature of Eyes and Facial Skin of Horses. *Journal of Veterinary Behavior*, **7**, 142-148. https://doi.org/10.1016/j.jveb.2011.08.001

[36] Mott, R.O., Hawthorne, S.J., and McBride, S.D. (2020) Blink Rate as a Measure of Stress and Attention in the Domestic Horse (*Equus caballus*). *Scientific Reports*, **10**, Article No. 21409. https://doi.org/10.1038/s41598-020-78386-z

[37] Cappelli, K., Amadori, M., Mecocci, S., Miglio, A., Antognoni, M.T. and Razzuoli, E. (2020) Immune Response in Young thoroughbred Racehorses under Training. *Animals*, **10**, Article 1809. https://doi.org/10.3390/ani10101809

[38] Ribeiro, W.P., Valberg, S.J., Pagan, J.D. and Gustavsson, B.E. (2004) The Effect of Varying Dietary Starch and Fat Content on Serum Creatine Kinase Activity and Substrate Availability in Equine Polysaccharide Storage Myopathy. *Journal of Veterinary Intern Medicine*, **18**, 887-894. https://doi.org/10.1111/j.1939-1676.2004.tb02637.x

[39] Valberg, S., Häggendal, J. and Lindholm, A. (1993) Blood Chemistry and Skeletal

Muscle Metabolic Responses to Exercise in Horses with Recurrent Exertional Rhabdomyolysis. *Equine Veterinary Journal*, **25**, 17-22.
https://doi.org/10.1111/j.2042-3306.1993.tb02894.x