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Original Research

Description of the Responses of Some Blood Constituents to Rodeo Exercise in Chilean Creole Horses

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ABSTRACT

The aim of this study was to assess the effect of the Chilean rodeo exercise on some blood constituents, classically used to assess welfare, during rest; training with and without steer; and before and after official competitions. During the training season, 13 horses were assessed at their farms of origins, and samples were taken at rest and after training with and without a steer; then during the competition season, 16 horses were assessed at four different times, one before and three after the competition. The blood constituents assessed were packed cell volume (PCV), total proteins, fibrinogen, cortisol, neutrophil:lymphocyte ratio, creatine phosphokinase, aspartate transaminase, glutathione peroxidase, and serum amyloid A. Analysis of variance for repeated measures was applied using the Statistix 8.0 software, and a significance level of $P < .05$ was applied. For horses assessed during exercise with and without a steer, significant differences were observed for PCV, which were significantly lower at rest than after both exercises, and total proteins, which showed a significant increase only after exercise with steer. In the case of official competitions, PCV was significantly higher immediately after rodeo, and the lowest value for cortisol was observed the evening after competition ($P < .05$); among the enzymes assessed, creatine phosphokinase plasma concentrations immediately after rodeo and the evening after competition were significantly higher than concentrations the next morning. The use of physiological indicators did not seem to be sufficient to assess the overall effect of rodeo on the welfare of these horses, and other types of indicators should be considered in future studies.

1. Introduction

In Chile, rodeo is considered a national sport and has been recognized as such by the Sports and Recreation Office [1]. The rodeo represents a sport of traditional character and is followed avidly by the public in Chile. The horse used for rodeo is exclusively the Chilean Creole horse, which is an animal of a muscular type with agile movements. For the horse, the rodeo represents a muscular effort of short duration, but high intensity, and it can be repetitive or nonrepetitive, depending on the number of series in which it participates and its progression through different stages of competition [2]. The main physical effort by the horse (average weight: 392 kg) during the rodeo consists of herding a steer of approximately 320 kg within the "medialuna," which is a circular court with a diameter of 45 m divided into two areas (cancha and apiñadero) where the rodeo takes place, and then pressing it against a padded wall, while transporting on its back the equivalent of 24% of its body weight (rider and saddle, average 92.9 kg) and

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Fig. 1. (**A**) Chilean Creole horse at rest; (**B**) Chilean Creole horse during exercise without a steer; and (**C**) Chilean Creole horse during exercise with the presence of the steer.

moving at a speed of 5-8 m/s on a circular sand surface [2]. Horses competing in rodeos have to move in an unnatural gait called "postura." During this gait, the horse moves sideways crossing its limbs, keeping its body at an angle of 45° to the perimeter of the "cancha" while herding a steer toward the "quincha" (padded part of the wall), point at which the steer is pressed by the horse against it, while in motion (usually at canter).

Despite the general popularity of rodeo, groups of animal protectionists have raised awareness about the possible impact that this sport could have on the welfare of horses and steers that are used. Although Chile has an Animal Protection Law, approved in October of 2009 [3], it has an exclusion for all equestrian sports, on the understanding that they have their own regulations to follow. To include the welfare issue in future regulations, it is important to first obtain scientific evidence of probable causes of poor welfare involved in this sport.

Welfare problems associated with sport horses depend on the activity performed. Injuries and lameness are common in racehorses, whereas for endurance horses most problems are related to the demands of maintaining body temperature and body fluid status [4]. Currently, there is no scientific information about the welfare consequences of rodeo participation for either horses or steers. Although evident injuries do not seem to happen frequently, a certain degree of inflammation and exercise-related stress might occur, thereby affecting the welfare of these horses. Other threats to the welfare of high-performance horses are excessive and/or inadequate training [4]. Training of rodeo horses is usually done once a day and can be divided in two types of exercise: the first phase consists of muscular warming up, with 5 minutes of walk, 3 minutes of trot, and then 2 minutes of canter; the second phase consists of a sequence of exercises with the horse in "postura" and involves 10 minutes of slow canter, 3 minutes of trot, and 2 minutes of walk, and this phase is done inside the medialuna with and without a steer [5].

The physiological adaptation of Chilean rodeo horses has been studied in the past by Pérez et al. [2] and García et al. [5]. The first study [2] assessed changes in blood variables during official rodeo competitions in terms of exercise physiology, but indirect factors that could affect these variables such as transport to the venue, unknown conspecifics, and housing at the place of the rodeo were not considered. The second study [5] assessed the adaptation to the rodeo exercise during a period of 45 days through blood variables; however, neither study provided basal normal ranges for many of the blood variables commonly used in both exercise physiology and welfare studies for Chilean Creole horses. Although physiological data by itself might not explain the welfare state of an individual, according to Broom [6], many of the signs of poor welfare are related to physiological responses after a particular challenge, resulting in changes in heart rate, adrenal activity, or reduction in the immune response among others, with some of these physiological changes being indicative of a prepathological state [7]. A complication is that most owners are reluctant to allow scientists to sample their horses, especially if a welfare issue is involved; this makes it difficult to obtain samples from large groups of horses, particularly if samples are taken during official rodeo competitions. However, we were successful in obtaining permission from a range of owners for the present study. Our aim was to evaluate the effect of the rodeo exercise on some blood constituents, classically used as welfare indicators, during different situations commonly faced by Chilean Creole horses: (1) rest, (2) training with and without steer, and (3) before and after competition.

2. Materials and Methods

Twenty-nine Chilean Creole horses were included in the study, 13 were assessed at their farms of origin and 19 during official Chilean rodeo competitions. The criteria for inclusion in both experiments were that the horses were clinically sound at the moment of sampling, currently participating in Chilean rodeo competitions, and consent could be obtained from owners to withdraw a blood sample. These criteria were used so as to obtain samples from horses under the real conditions in which they participate in this sport. Provision of water was given to all horses after exercise, as normal husbandry practice used by each owner.

2.1. Horses Assessed during Exercise with and without a Steer

The study was conducted during the summer of the 2010-2011 training season of Chilean Creole horses for rodeo under the approval of the Bioethical Committee for

Use of Animals in Experimentation of the Universidad Austral de Chile. Thirteen Chilean Creole horses were included in the study—five mares, six stallions, and two geldings—aged between 4 and 10 years of age, belonging to two breeding centers located in the XIV Region. Owner consent was obtained to take blood samples. All horses were clinically sound before sampling.

2.1.1. Collection of Blood Samples

Samples were obtained through jugular puncture of horses at their farms of origin between 9 and 10 AM on three different days during three situations that normally occur during the training of these horses for the rodeo season. In each sampling, 10 mL of blood was withdrawn from each horse. Sampling was done on three consecutive days (Tuesday, Wednesday, and Thursday) in a counter-balanced order for exercise with and without the presence of a steer. Samples at rest were always taken on a Tuesday morning because Monday is a day of complete rest for these horses, thereby allowing horses to have more than 24 hours of rest before sampling.

1. Sample at rest (R): This sample was taken with the horse outside its own box between 8 and 9 AM the morning after its resting day, so no exercise had been performed by the horse during the past 24 hours (Fig. 1A).
2. Sample after exercise without presence of a steer (NS): This sample was taken during the morning (between 8 and 9 AM) outside the medialuna, between 5 and 10 minutes after the horse had finished its normal training session without the presence of a steer. This exercise consisted of approximately 5 minutes of walk, 5 minutes of trot, and 10 minutes of canter in "postura" (Fig. 1B).
3. Sample after exercise with presence of a steer (WS): This sample was taken during the morning (between 8 and 9 AM) outside the medialuna, between 5 and 10 minutes after the horse had finished its normal training session with a steer. This exercise consisted of approximately 5 minutes of walk, 5 minutes of trot, and 10 minutes of canter in "postura" while following a steer (Fig. 1C).

Riders were asked to conduct both exercises (NS and WS) at the same intensity.

2.2. Horses Assessed during Official Rodeo Competitions

The study was conducted during three official rodeo competitions between November and December (spring time) corresponding to the 2009-2010 rodeo season. Sixteen Chilean Creole horses—four mares, three stallions, and nine geldings—aged between 5 and 15 years, belonging to eight different owners, entered the study. Owner consent was obtained to take blood samples. All horses were transported to the place of the rodeo event over distances ranging between 100 and 200 km. During the weekend, horses were kept at the site of the event, being in visual contact with other horses the whole time, even during blood samplings. All horses were clinically sound before the start of the rodeo event, and none of the horses sampled were injured during the exercise.

2.2.1. Collection and Analysis of Physiological Data

Horses were restrained with a halter, and blood samples were taken from each one by jugular puncture at the following sampling times:

1. Sample collected before rodeo (T-Pre Rodeo): This sample was taken between 8 and 9 AM, in the morning before the rodeo competition. All horses had arrived either the night before or early that morning; all of them were at the venue for more than 1 hour when the sample was taken;
2. Sample collected after rodeo (T-Post Rodeo). This sample was taken 5-10 minutes after the horse finished the first series of the competition;
3. Sample collected the evening after rodeo (T-Post Eve): This sample was taken between 8 and 9 PM the same evening after the competition;
4. Sample collected a day after rodeo (T-Next Day): This sample was taken the morning after the rodeo between 8 and 9 AM.

2.3. Handling of Blood Samples

Blood samples were separated in two tubes: one without additives for the measurement of serum cortisol (μg/dL) and serum amyloid A (SAA) (mmol/L) concentration (only for horses at official rodeo competitions); the second tube contained ethylenediaminetetraacetic acid and the sample was used for the measurement of plasma creatine phosphokinase (CK) and aspartate transaminase (AST/serum glutamic oxaloacetic transaminase) activity (U/L), packed cell volume (PCV) (%), neutrophil:lymphocyte ratio (N:L), and glutathione peroxidase (GSH-Px).

Samples were processed at the Clinical Pathology Laboratory of the Veterinary Faculty of the Universidad Austral de Chile, with the exception of cortisol, which was analyzed at the Animal Physiology and Endocrinology Laboratory of the Veterinary Faculty at Universidad de Concepción. The following kits and equipment were used for each variable:

1. CK: 2015 Human instrument and a Metrolab 2300 autoanalyzer, Wiener Lab, Argentina.
2. AST: 12021 Human instrument and a Metrolab 2300 autoanalyzer, Wiener Lab.
3. GSH-Px: RS 506 Randox instrument and a Metrolab 2300 autoanalyzer, Wiener Lab, UK.
4. For manual complete blood count, the Haraeus Biofuge Haemo centrifuge (Kendro Lab. Germany); and for automatic complete blood count, a Sysmex KX 21 N hematology analyzer (USA).
5. Cortisol: Radioimmunoassay (Belgium) with the commercial kit Diasource validated for animals was used with a level of detectability of 1 μg/dL and a coefficient of variation within assay of under 5%.
6. SAA: Enzyme-linked immunoabsorbent assay kit TP-802 Phase SAA from Tridelta Development Ltd (Ireland).

Reference values used as normal ranges in this study were as follows:

1. Cortisol: 0.9-5.6 μg/dL [8];
2. PCV: 35%-47% [9];

3. Total proteins: 68-84 g/L [9];
4. Fibrinogen: 2-4 g/L [10];
5. N:L ratio: 0.8-2.8 [11];
6. CK: 113-333 U/L [9];
7. AST: 120-480 U/L [9];
8. GSH-Px: 130-270 U/g Hb [9];
9. SAA: 500-20,000 ng/mL [12].

2.4. Statistical Analysis

For the statistical analyses, Statistix 8.0 software (Analytical Software in Tallahasee, Florida) was used. Data obtained from blood variables were checked for normal distribution and homogeneity of variance using the Kolmogorov–Smirnov test and the Levene test, respectively. When these requirements were not fulfilled, data were transformed by logarithmic transformation before analysis using analysis of variance for repeated measures and Tukey post hoc tests. Pearson correlations were examined between all pairs of variables. A P value of $P < .05$ was considered significant.

3. Results

3.1. Horses Assessed during Exercise with and without Steer

We were able to observe differences in the changes of blood variables in Chilean Creole horses when they were doing the same exercise with and without the presence of a steer. Mean values during the sampling at rest (R) were observed within normal ranges for all the variables assessed with the exception of fibrinogen and enzyme CK (Tables 1 and 2). Although when looking at individual values, none of the variables included all 13 horses within the normal range.

Changes within variables between samplings occurred for all variables assessed, but these changes were significant only for the PCV and for total proteins. The PCV was significantly higher after both exercises, NS and WS, than at rest, whereas the total protein values were significantly higher after WS than at R and NS (Table 1).

Cortisol serum concentrations were not significantly different between samplings, but there was a tendency for an increase in mean values observed between R, NS, and WS, respectively. The N:L ratio was not significantly different between the three samplings, and only one horse had an increased N:L ratio after the NS exercise (Table 1). None of the enzymes analyzed showed significant differences between samplings; it is important to highlight that the CK average level was above normal range in the sample at R. In the case of GHS-Px, a slight decrease was observed after exercise with NS and WS, with only one horse presenting levels below the normal range at R and after WS (Table 2).

3.2. Horses Assessed during Official Rodeo Competitions

During the T-Pre Rodeo, all variables were within normal ranges, with the exception of fibrinogen, N:L ratio, and CK that were all elevated (Tables 3 and 4).

Cortisol, PCV, and CK were the only variables that showed significant differences between sampling times, with all three presenting their highest level in the sample taken immediately after participating in the rodeo (T-Post Rodeo). The cortisol level was significantly higher at T-Post Rodeo in relation to T-Post Eve and T-Next Day; the PCV was significantly higher at T-Post Rodeo when compared with all other sampling times (Table 3), whereas CK was significantly lower at T-Next Day when compared with all other sampling times (Table 4). Most horses presented values within the species normal range for all variables, except for the enzymes CK and AST, for which more than half of the horses already had high values at T-Pre Rodeo (Table 4).

Two horses also had elevated values of SAA, and nine had elevated fibrinogen at the sample taken before competing in the rodeo (T-Pre Rodeo) (Table 3).

No significant correlations were found between any of the blood parameters assessed.

Table 1
Blood variables assessed including their normal range and mean values; standard deviation (SD); minimum (min) and maximum (max) values; and number of horses below, within, and above normal range observed during three situation (R, rest; NS, rodeo exercise without steer; WS, rodeo exercise with presence of steer)

Blood Variables	R	NS	WS
PCV (35%-47%)			
Mean	42[a]	51.3[b]	54[b]
SD	7.4	5.1	6.8
Min and max values	32-58	44-60	42-65
Below	2	0	0
Normal	9	4	3
Above	2	9	10
Total proteins (68-84 g/L)			
Mean	70.2[a]	71.5[a]	77.3[b]
SD	4.9	3.7	4.7
Min and max values	62-76	62-76	70-87
Below	4	1	0
Normal	9	12	12
Above	0	0	1
Fibrinogen (2-4 g/L)			
Mean	4.8	4.7	5.5
SD	1.4	1.8	0.8
Min and max values	3.0-7.0	3.0-7.0	4.0-7.0
Below	0	0	0
Normal	4	6	1
Above	9	7	12
Cortisol (0.9-5.6 μg/dL)			
Mean	7.4	8.5	9.0
SD	1.8	1.7	2.6
Min and max values	4.3-9.9	5.8-11	4.3-12.9
Below	0	0	0
Normal	2	0	2
Above	11	13	11
N:L ratio (0.8-2.8)			
Mean	1.3	1.3	1.1
SD	0.6	0.6	0.4
Min and max values	0.67-2.33	0.48-2.83	0.49-1.94
Below	2	3	2
Normal	11	9	11
Above	0	1	0

[a,b] Variables with different superscripts within the same row are significantly different at $P < 0.05$.

4. Discussion

4.1. Horses Assessed during Exercise with and without Steer

When eliminating most of the stimuli present at the rodeo competition by sampling horses in their farms of

Table 2
Enzymes assessed including their normal range and mean values; standard deviation (SD); minimum (min) and maximum (max) values; and number of horses below, within, and above normal range observed during three situation (R, rest; NS, rodeo exercise without steer; WS, rodeo exercise with presence of steer)

Blood Variables	R	NS	WS
CK (113-333 U/g Hb)			
Mean	516.4	756.5	372.1
SD	837.4	1669.9	313.4
Min and max values	122-3206	149-6292	139-1257
Below	0	0	0
Normal	10	9	8
Above	3	4	5
AST (120-480 U/g Hb)			
Mean	303.9	293.8	294.3
SD	78	38.8	35.4
Min and max values	202-513	207-341	221-350
Below	0	0	0
Normal	12	13	13
Above	1	0	0
GSH-Px (130-270 U/g Hb)			
Mean	262.2	236.5	234.2
SD	75.2	60.5	65.7
Min and max values	104-367	130-339	125-342
Below	1	0	1
Normal	7	8	9
Above	6	5	3

Table 3
Blood variables assessed including their normal range and mean values; standard deviation (SD); minimum (min) and maximum (max)values; and number of horses below, within, and above normal range observed during four different times at an official rodeo competition.

Blood Variables	T-Pre Rodeo	T-Post Rodeo	T-Post Eve	T-Next Day
PCV (35%-47%)				
Mean	35.9[a]	42.4[b]	35.5[a]	34.8[a]
SD	5.1	5.7	3.2	4.2
Min and max values	31-48	35-54	30-44	31-44
Below	8	0	6	9
Normal	7	13	10	7
Above	1	3	0	0
Total proteins (68-84 g/L)				
Mean	73	75	73	73
SD	4.2	4.0	3.5	3.2
Min and max values	66-80	66-80	68-80	69-80
Below	1	1	0	0
Normal	15	15	16	16
Above	0	0	0	0
Fibrinogen (2-4 g/L)				
Mean	5	5	4	5
SD	1.5	1.4	1.5	0.9
Min and max values	2.0-7.0	3.0-7.0	2.0-8.0	3.0-6.0
Below	0	0	0	0
Normal	7	8	8	6
Above	9	8	8	10
SAA (500-20,000 ng/mL)				
Mean	8.603	10.165	10.541	13.669
SD	13.433	17.063	18.77	21.544
Min and max values	66-52267	118-55911	15-63200	44-71911
Below	5	4	5	4
Normal	9	9	9	9
Above	2	3	2	3
Cortisol (0.9-5.6 μg/dL)				
Mean	6.5[ab]	7.9[b]	3.9[c]	5.4[a]
SD	13.4	4.3	3.9	2.9
Min and max values	0-11.18	0-15.89	0-17.82	0-10.54
Below normal range	2	2	2	2
In normal range	4	1	12	6
Above normal range	10	13	2	8
N:L ratio (0.8-2.8)				
Mean	3.3	3.1	2.4	2.4
SD	2.8	2.1	0.8	1.2
Min and max values	0.9-9.9	0.6-7.6	0.9-3.7	0.8-5.1
Below	0	1	0	0
Normal	11	9	12	13
Above	5	6	4	3

[a,b,c] Variables with different superscripts within the same row are significantly different at $P < 0.05$.

origin, we observed differences in the changes of blood variables when horses did the same exercise with and without the presence of a steer, suggesting that steer itself is a challenging stimulus, separate from exercise, during rodeo competition. The study by García et al. [5] on the assessment of traditional training of Chilean Creole horses is probably the study that is most similar to the first section of the present study, although in their sample at rest, it was not specified for how long before the sampling had the horses not performed any type of exercise, and their postexercise sample was taken after exercise without the presence of a steer only.

The significant increase of the PCV found during the NS and WS exercises (Table 1) can be explained by the spleen contraction and subsequent release of erythrocytes during exercise, an increase that normally returns to baseline values 2 hours after exercise or excitement [13]. The slight increase of cortisol observed between NS and WS, although not significant, could be the result of the excitement produced by the presence of the steer, which is known to increase cortisol levels in horses [14]. García et al. [5] described significant increases in the PCV of Chilean Creole horses after rodeo exercise training in relation to resting values, although their PCV values were lower than the ones reported in the present study at both rest (35.4%) and after the exercise (46.3%).

The significant increase in total proteins during WS exercise was similar to the 80 g/L after exercise reported by García et al. [5], although higher than the 71.5 g/L (Table 1) reported in this study after NS exercise. Increase in total proteins can be attributed to the normal intercompartmental fluid shifts, with greater increases during maximal exercise [13]; this could explain the significant increase during WS exercise (Table 1). It is expected that after a short-duration exercise such as the one performed during rodeo, this increase should return to baseline after 30 minutes. Although the increase was significant during WS exercise, only one horse presented an increase over the normal level, leading us to assume that dehydration was not a problem for these horses.

García et al. [5] reported a significant increase in cortisol release when comparing resting values with those after exercise without a steer. In the present study, the horses assessed presented an R value of 7.4 μg/dL, which was higher than both resting value (3.6 μg/dL) and after exercise value (6 μg/dL) reported by García et al. [5], with no significant changes between samplings and above normal ranges [8]. The tendency for both the average values and number of individuals to increase above normal values when comparing R, NS, and WS, respectively, observed in Table 1, could be the result of cortisol release owing to physical exercise after NS; then after WS, the added effect of excitement due to the steer's presence is another

Table 4
Enzymes assessed including their normal range and mean values; standard deviation (SD); minimum (min) and maximum (max) values; and number of horses below, within, and above normal range observed during four different times at an official rodeo competition

Blood Variables	T-Pre Rodeo	T-Post Rodeo	T-Post Eve	T-Next Day
CK (113-333 U/g Hb)				
Mean	518.1[ab]	546.9[a]	484.3[a]	350.3[b]
SD	491	543	272	149
Min and max values	145-2162	150-2448	154-1249	155-657
Below	0	0	0	0
Normal	8	6	5	8
Above	8	10	11	8
AST (120-480 U/g Hb)				
Mean	448.8	402.7	474.1	444.6
SD	363	325	400	313
Min and max values	113-1367	107-1246	105-1518	86-1364
Below	0	0	0	0
Normal	3	6	4	2
Above	13	10	12	14
GSH-Px (130-270 U/g Hb)				
Mean	168.8	159.6	156.8	172.9
SD	71.7	54.8	53.3	89.8
Min and max values	67-324	36-286	67-241	54-445
Below	4	4	4	6
Normal	12	12	12	10
Above	0	0	0	0

[a,b] Variables with different superscripts within the same row are significantly different at $P < 0.05$.

possibility that could indicate that the steer acts as a stressor stimuli during the rodeo. The four horses that presented resting values over normal range could have anticipated exercise when the head collar was placed in the box to take the blood sample.

The N:L ratio did not present significant differences between the three samplings (Table 1); an increase in the N:L ratio is expected to occur because of cortisol released after a stress response and could be a more accurate indicator of stress than just the cortisol levels [15]. In the present study, only one horse presented an increased N:L ratio after the NS exercise (Table 1); these results are in accordance with the cortisol levels noted, suggesting that the release of cortisol observed is the result of a normal stress response with no negative effect on the horses.

Enzymes were also assessed to determine possible muscular damage as a result of the rodeo exercise. AST levels were within normal range in all samplings and also slightly higher than the 196 IU/L at rest and 224 IU/L after exercise reported by García et al. [5]. CK plasma concentrations were above normal range already in the R sample. At R sampling, horses had been in rest for more than 24 hours; we could assume that the three horses that had higher concentrations of CK in blood than normal could have had bruising or had performed more strenuous exercise during the days previous to sampling. When analyzing the other variables associated with these horses, all three had elevated fibrinogen level (6-7 g/L), which normally takes between 24 and 72 hours to reach peak values after induction of inflammation [10], and one had low levels of GSH-Px, which could indicate a selenium deficiency and a higher risk of muscular damage [16]. CK is usually within the muscular cell, and the concentration in blood is elevated when the cell membrane permeability is increased or suffers damage. Elevated levels can normally be found after exercise; abnormal elevations can occur after intense exercise, bruising, or transport [17]. This is why the European Commission [18] has established that this enzyme can be a useful welfare indicator when used in conjunction with other variables. Increases in blood levels of both enzymes are normally found after exercise [17], and similar increases were reported by Marlin et al. [19] for endurance horses after exercise. The increases reported by García et al. [5] for AST and CK were not significant in either case, although the concentration of CK at rest and after exercise (63 IU/L and 70 IU/L, respectively) reported by these authors is lower than those found in the present study at both rest and after exercise samples.

In the case of GHS-Px, a slight decrease was observed after exercise with NS and WS, with only one horse presenting levels below the normal range at R and after WS. Marlin et al. [19] reported a significant reduction in GSH-Px levels in endurance horses. but only after prolonged exercise. All horses included in this study received oral selenium and vitamin E supplementation; thus this it was expected that all horses were going to present normal values at rest despite the fact that the area in which they were located is selenium deficient because of the volcanic soil present. Proper selenium levels avoid risk of muscular disease [16], and adequate levels of GSH-Px are also important as a defense mechanism toward reactive oxygen species, which have been linked to some of the deleterious effects of exercise [20,21]. Neither García et al. [5] nor Pérez et al. [2] assessed this enzyme; we considered it was important because of the high selenium deficiency prevalent in the area of study [22].

4.2. Horses Assessed during Official Rodeo Competitions

Rodeo exercise during official competitions resulted in physiological changes in the blood parameters assessed. The fact that animals had been transported to the venue, housed in a novel environment, and exposed to the presence of unknown conspecifics makes it difficult to assess how much these changes are the result of the exercise itself.

The sampling times of this part of our study are comparable with the study of Pérez et al. [2] where they assessed the cardiovascular and biochemical changes in Chilean Creole horses before and after rodeo competition.

The significant increase of the PCV found in T-Post Rodeo (Table 3) can be explained by the spleen contraction and subsequent release of erythrocytes during exercise, an increase that returns to baseline values 2 hours after exercise or excitement [13]. Decreases in PCV were already observed by T-Post Eve (Table 3).

Mean cortisol values fluctuated above normal range for the species (0.9-5.6 μg/dL) during T-Pre Rodeo and T-Post Rodeo times, with the highest concentration found at T-Post Rodeo. Similar findings, for Chilean Creole horses before and after rodeo competition, were reported by Pérez et al. [2]. These authors associated their findings with a normal physiological endocrine response involved in the mobilization of substrates required during exercise.

Cortisol concentrations taken before the rodeo and immediately after it were higher than the values obtained the evening after rodeo and the next morning. Morning and

evening comparisons may be confounded by circadian rhythm acting as a confounding factor, as horses normally show cortisol peaks between 6 and 9 AM and a trough between 7 and 11 PM [23]. However, the difference between the morning samples taken before competition and at the same time the next day could be explained by the fact that at T-Pre Rodeo some horses had just arrived to the place (at least 1 hour before sampling) where the rodeo takes place, adding transportation as an additional stress, which is known to increase cortisol concentrations even when the horses are transported for short distances [24], and/or a psychological component where concentrations of cortisol increase in anticipation to exercise and the presence of unknown conspecifics nearby at the venue; this has been reported in humans as in horses [25,26]. Although T-Pre Rodeo cortisol values were still lower than those observed in this study at R sampling (Table 1) where horses had been at rest for more than 24 hours because they do not correspond to the same horses, there could be an individual factor influencing these results, with some horses having normal basal cortisol levels as compared with others.

The relatively high cortisol concentrations observed immediately after rodeo exercise were significantly lower by the evening and remained low the next day. This suggests a pattern of cortisol release in response to exercise, with a fast recovery of normal concentrations once the stress of the exercise has finished [27]. Again after exercise, cortisol mean values were higher after the NS and WS exercise (Table 1) than after T-Post Rodeo (Table 3).

The N:L ratio did not show significant differences between sampling times, but did show an overall tendency to decrease by T-Post Eve and T-Day After (Table 3). Five horses had an increased ratio already by T-Pre Rodeo, which could be a result of transport stress before the rodeo competition; the same number of horses showed increased ratios after exercise and during the following sampling times (Table 3), and the N:L ratio was threefold higher than at R sampling (Table 1). Rossdale et al. [28] reported significant changes in the N:L ratio by 180 minutes after a canter during training. Because changes in the ratio are a result of cortisol release, it would be difficult to identify whether there is a negative long-term consequence for the immune system of these horses, as exercise itself, and probably the excitement produced by the anticipation to the rodeo, could result in disturbances in leukocytes.

No significant increases were found for SAA acute phase protein after the rodeo exercise, although a tendency to increase was observed (Table 3). In the present study, only five horses had SAA levels within the range described for healthy horses [29] at the first sampling time (T-Pre Rodeo), and two had abnormally increased levels at this point. These horses also presented an increase in concentrations of plasma CK, and also a selenium deficiency with decreased activity of GSH-Px in the case of one horse. Although all horses were clinically sound at the beginning of the study, a subclinical pathology could have been present in these cases, because according to Moberg [7] an increase in some physiological variables could indicate a prepathological stage.

Although significant increase in SAA levels after rodeo exercise was not detected in this study, all horses had detectable levels of SAA at the T-Pre Rodeo sampling point. This could signify other underlying inflammatory pathologies present before competing. It is also possible that clinical signs of inflammation or pain were masked by the use of anti-inflammatory drugs because the use of flunixin meglumine and phenylbutazone are allowed by the Chilean Rodeo Federation [30]. Although this was not determined in the case of the horses considered in this study, it should be considered in future studies. Another reason for not detecting significant increases could be the sampling times used because many authors report peak increases at day 3 [29,31], and in this study, samples were only taken up to 16-18 hours after exercise, with a tendency of increasing SAA levels up to sample in T-Next Day (Table 3). These preliminary results could imply that SAA is probably not an accurate marker for assessing the specific effects of intensive short-term exercise in horses because of the time it takes to reach peak values. However, it may be a useful marker of recovery time and could help to inform decisions about when to resume training after an intensive competition.

From the enzymes associated with muscular function that were assessed, CK was the only one that showed significant increases immediately after exercise and approximately 6 hours after exercise when compared with values obtained at T-Next Day (Table 4). Pérez et al. [2] also reported significant increases of this enzyme in rodeo horses immediately after exercise, associating it to an increase in the muscular cell membrane permeability owing to hypoxia under anaerobic exercise conditions. In the present study, 9 of the 16 horses already had increased values before exercise (Table 4); transport, even for short distances (130-350 km), has been reported to increase CK plasma concentrations [18], and could explain these results.

From the eight horses with elevated CK values at T-Pre Rodeo, four had low concentrations of GSH-Px; this could also explain the increases in CK for these horses because selenium deficiency can result in a higher risk of muscular damage and increased cell membrane permeability. Also from these eight horses, three had high fibrinogen levels and two had elevated SAA at this same sampling, which could be related to an inflammatory process in these animals. AST did not show significant differences between sampling times. Pérez et al. [2] found significant increases for this enzyme 24 hours after rodeo exercise, although the AST values reported by them are lower than those reported in this study at every sampling time. Increases in means of AST activity have been previously described by Tyler-McGowan et al. [32] in overtrained standardbreds.

5. Conclusions

None of the variables analyzed showed changes that could signify a deleterious effect on the welfare of the horses studied. According to our results, it does appear that the rodeo involves many individual factors that can act as stressors for the horse; the presence of the steer itself seems to be one additional stressor during the rodeo, which is translated into changes in the blood variables when compared with the same exercise without the presence of a steer. Further studies on this possible effect are required.

Individual variations in blood variables seem to be more important than average group variations when assessing

animal welfare in horses at rodeo competition. This is the reason why, in the case of the present study, individuals with extreme values for some of the variables assessed were not excluded, because they represent those animals that although do not present clinical signs of pathology are participating in rodeo competitions under physiological conditions (eg, high blood concentration of CK and fibrinogen) that might represent a risk for their well-being. Although this might not be important at group level, it is important for those particular individuals.

At the rodeo venue, when observing the group mean values for each of the blood variables assessed, we obtained results that suggested normal physiological variation due to exercise, with rapid recovery of most parameters. This should not cause long-term welfare problems.

It has to be taken into account that this study was performed during two real situations, where other stressors indirectly related to the rodeo competition, such as transport, novel environment, and presence of unknown conspecifics and people, are present and could lead to the changes in the blood variables assessed. We cannot directly compare the results of this second part of our study to the first part, as different individual horses were involved; however, it is important to point out that horses assessed at farm level did not have cortisol levels at rest much lower than those observed at the rodeo venue; this could indicate some level of adaptation to this activity. In this study, the use of blood parameters was not sufficient to assess the overall effect of rodeo on horses, and further broader studies would be needed.

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