



## Full Length Article

## Physiological responses in horses, donkeys and mules sold at livestock markets



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## ABSTRACT

The horse welfare at markets includes fatigue, fear, fasting, dehydration and injuries. However, the scientific literature contains no readily-available information on the physiology responses of equids sold in livestock markets. The objective of the present study was to evaluate the effect of holding in livestock markets on gas exchange, the acid-base balance, energy metabolism, and the mineral and water balance in 4 types of horses (*Equus caballus*), donkeys (*Equus asinus*) and mules (*Equus asinus* × *Equus caballus*). To this end, a total of 1,438 equine were utilized, after classification into six groups, as follows: mules, donkeys, culled horses, *charrería* horses (Mexican rodeo horses), foals, and ponies. The time intervals evaluated were 1 and 5 h. During the study, the animals received only water *ad libitum*. The physiological blood indicators assessed were: P<sub>r</sub>CO<sub>2</sub> (mm Hg) and P<sub>r</sub>O<sub>2</sub> (mm Hg) pressure, pH, glucose (mg/dL), lactate (mg/dL), bicarbonate levels (mmol/L), hematocrit (%), Na<sup>+</sup>, K<sup>+</sup> and Ca<sup>2+</sup> (mmol/L). The animals that presented the most marked physiological alterations were the mules and donkeys. The mules that remained in the corrals for 1 h presented increases ( $P < 0.0001$ ) in the values for pO<sub>2</sub>, glucose, hematocrit and blood pH, compared to reference values (RV), while the donkeys had increases ( $P < 0.0001$ ) in glucose and hematocrit values. By the end of the first hour at the exhibition corrals the equine were dehydrated, suffered fatigue, and suffered metabolic and compensatory problems, probably due to the combined effects of a series of factors that include transport and inadequate handling.

## 1. Introduction

Livestock markets can be defined as specific locations with dedicated facilities, where buyers and sellers come together to buy or sell live animals. They offer small producers the opportunity to commercialize animals for distinct zootechnical purposes [1–3]. However, in these settings animals are exposed to various stress factors that may include at least one more journey that will vary in length, additional periods of food and water restriction, unfamiliar noise, the mixing of animals from different origins, extreme climatic conditions, periods of fasting, and prolonged stays – sometimes amidst high stocking densities

– as well as poor handling by untrained personnel on these and other premises. Indeed, they be affected by the cumulative effects of all these factors [4,5]. Under such circumstances, the function of the stress response is to provide the energy required to cope with these challenges [6]. It involves activating two main physiological pathways: the hypothalamic-pituitary-adrenal cortex axis (HPA) and the sympathetic-adrenal medulla axis (SAM). Once the HPA and SAM axes are activated, they trigger specific corporal changes that can be measured to assess the degree of activation [7].

Also, many equids sold in livestock markets are acquired by dealers who travel from village-to-village, often crowding the animals

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purchased into inadequate vehicles for transport periods of up to 2 or 3 days to reach another market, where they are unloaded and sold [8]. Cattle and other animals are usually sent to market because they have reached either their slaughter weight or the end of their productive life. However, to the best of our knowledge, the scientific literature contains no readily-available information on the physiology of animals sold in livestock markets. Field studies provide useful data on the effects of commercial environments on animals (considering several interacting factors), and are particularly valuable where it is not possible to simulate all factors present in the environment in a controlled experimental setting [9,10]. Given this background, the objective of the present study was to evaluate the effect of holding times on gas exchange, the acid-base balance, energy metabolism, and the mineral and water balance in equine sold in livestock markets.

## 2. Materials and methods

### 2.1. Location

The study was conducted in July–September 2017 and involved 28 visits to a livestock market in central Mexico (90°14'20" N, 99°56'13" W) at an elevation of 2260 m, where the climate is temperate, with mean annual temperature and rainfall of 12.5 °C and 788 mm, respectively. The market covers a surface area of 80,000 m<sup>2</sup>, and commercializes an average of 3500 heads of livestock/week, including bovine, equids, goats, sheep, swine, dogs, poultry and rabbits. The site where all evaluations were conducted had an average temperature of 19.37 °C, relative humidity of 61.2%, a wind speed of 3.4 m/s, and luminosity of 262.16 lux, all measured using a portable digital weather-monitoring instrument (LT-LM-8010).

### 2.2. Ethical note

All animals were handled humanely throughout the study. All procedures related to the use and care of the animals strictly followed the Mexican regulation norm, NOM-062-ZOO-1999 of Mexico's Department of Agriculture, Ranching, Rural Development, Fishing and Alimentation for animal-based experimentation [11].

This study was carried out at a livestock market located in central Mexico, after obtaining approval from the Master Degree Commission of Agricultural Sciences of the Universidad Autónoma Metropolitana Iztapalapa-Xochimilco in Mexico City in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). All procedures were conducted in accordance with the guidelines for the ethical use of animals in applied ethological studies, described elsewhere [12].

### 2.3. Animals

A total of 1438 equids were evaluated, after classification into the following six groups: mules (*Equus asinus* × *Equus caballus*) (n = 176), donkeys (*Equus asinus*) (n = 244), cull horses (n = 466), *charrería* horses (n = 246), foals (aged 1–3 years) (n = 158), and ponies (n = 148). All animals achieved body condition scores of 2–4 (on a scale of 1–5), and were between 1 and 14 years of age. The equids arrived at the market from different production sites in the State of México, and the states of Guanajuato, Querétaro, Michoacán, Guerrero, Puebla and Hidalgo. Mean duration from loading on the farm of origin to unloading at the livestock market was approximately 6 h. Upon arriving at the market, the animals were unloaded and herded into exhibition corrals by handlers who were seen to use sticks, ropes, electrical prods, whistles and kicks (data not assessed). The corrals were built of metal tubes with roofs of galvanized metal sheeting, and equipped with a drinking apparatus. While in the corrals, the animals received only water *ad libitum*. They were not given any food during the time spent in the market, and were housed independently with no

mixing of the different equine groups mentioned above. All the equids, regardless of type, were housed in corrals with a total surface area of 40 m<sup>2</sup> and 0.50 m<sup>2</sup>/equid was provided. It is important to mention that the owners participated voluntarily in the study after consenting to the study protocol.

### 2.4. Blood sampling

All blood samples were obtained from the jugular vein of the equids in 1-mL hypodermic syringes, previously-heparinized with 100 mL of lithium heparin to avoid modifying the blood gas values. All personnel involved in sampling had received prior instruction and training. The researchers who drew the samples were able to collect blood on the first attempt in < 15 s to avoid altering the values through excessive handling of the animals. To eliminate the effects of handling on the blood-sampling procedure and be able to determine exclusively the effect of permanence in the livestock corrals at the market, an independent group of 120 equine (20 for each group) was sampled 24 h before being transported to a cattle market to establish reference values (RV). The blood samples in the livestock market were taken 1 and 5 h after the equids arrived. Once all the blood samples had been collected, they were placed in a bed of crushed ice. Samples were analyzed individually and immediately –i.e., within 1–3 min– using a portable blood gas and electrolyte parameter analyzer (GEM Premier, Instrumentation Laboratory Diagnostics, Milano, Italy/Lexington, USA). All analyses were performed on site by trained personnel to determine partial venous carbon dioxide [P<sub>v</sub>CO<sub>2</sub> (mm Hg)] and oxygen [P<sub>v</sub>O<sub>2</sub> (mm Hg)] pressure, pH, glucose (mg/dL), lactate (mg/dL), and bicarbonate levels (mmol/L), hematocrit (%), and plasma electrolyte concentrations [Na<sup>+</sup>, K<sup>+</sup> and Ca<sup>2+</sup> (mmol/L)]. All evaluations were based on 135 µL of the samples from all equine groups. The animals showed no signs of disease, and were apparently healthy at the time of sampling.

### 2.5. Statistical analysis

Normality was tested (PROC UNIVARIATE, JMP 8.0) for all the variables examined to verify: (1) that errors had a normal distribution; and (2) the existence of a null mean with (3) a typical deviation ( $\alpha$ ). All data showed a normal distribution. To test for the effect of holding times in the corrals at the livestock market on the different animals, an analysis of variance using a general linear model was performed (ANOVA JMP 8.0). When numerical differences were detected, a multiple-comparison Tukey test was used ( $\alpha = 0.05$ ) to compare the means among treatments. In the case of the variable pH, a *Kruskal-Wallis* analysis was run to compare the means to  $\alpha = 0.0001$ . The researchers who carried out the evaluation and collected the study outcomes were not aware of the treatments and did not participate in selecting the animals or in data analysis. Likewise, the researcher responsible for analyzing the data gathered was not aware of the treatments. In all tests, a two-tailed  $P < 0.05$  was considered significant.

## 3. Results

The results of this study show diverse imbalance in gas exchange, the acid-base balance, energy metabolism and the water balance, in all equine groups due to the effect of the time spent in pens at the livestock market. All results were collected in (Tables 1–8). Upon comparing each one of the two holding times considered, the mules that stayed in the corrals for 1 h had increases above the reference values (RV) ( $P < 0.0001$ ) for the following parameters: pO<sub>2</sub> (5 mmHg) (Table 1), glucose (42 mg/dL) (Table 5), hematocrit (13%) (Table 7), and blood pH (0.1) (Table 3). Also, test results showed reductions below the RV ( $P < 0.0001$ ) in the following values: pCO<sub>2</sub> (10 mmHg) (Table 1), lactate (17 mg/dL) (Table 6), and bicarbonate (4 mmol/dL) (Table 4). In contrast, after the 5-h Interval in the corrals, measurements revealed an increase above the RV in the values for pO<sub>2</sub> (4 mmHg) (Table 2),

**Table 1**Carbon dioxide concentration (pCO<sub>2</sub> mmHg) in different equine groups evaluated during 1 and 5 h of holding in a livestock market.

Equids groups	Reference value (RV)	Time of remaining	Time of remaining	P-value
	Mean ± SE	1 h	5 h	
		Mean ± SE	Mean ± SE	
Mules (n = 176)	48.67 ± 0.0005 <sup>a,1</sup>	38.60 ± 0.57 <sup>b,5</sup>	30.02 ± 0.39 <sup>c,4</sup>	< 0.0001
Donkeys (n = 244)	43.40 ± 0.0008 <sup>a,3</sup>	40.77 ± 0.36 <sup>b,4</sup>	33.70 ± 0.91 <sup>c,3</sup>	< 0.0001
Cull horses (n = 466)	41.15 ± 0.0001 <sup>b,4</sup>	42.14 ± 0.26 <sup>a,3</sup>	41.02 ± 0.56 <sup>b,2</sup>	0.0006
Charrería horses (n = 246)	46.52 ± 0.50 <sup>b,2</sup>	48.35 ± 0.26 <sup>a,1</sup>	43.53 ± 0.88 <sup>c,1</sup>	< 0.0001
Foals (n = 158)	37.35 ± 0.79 <sup>b,5</sup>	42.38 ± 0.57 <sup>a,3</sup>	43.07 ± 0.82 <sup>a,1</sup>	< 0.0001
Ponies (n = 148)	43.35 ± 0.94 <sup>a,3</sup>	44.88 ± 0.51 <sup>a,2</sup>	45.00 ± 0.69 <sup>a,1</sup>	0.2106

<sup>a,b,c</sup>Letters in the same row indicate differences among treatments in the same species.<sup>1,2,3,4</sup>Numbers in the same column indicate differences among species in the same treatment. Tukey test ( $P < 0.05$ ); n, number of animals; SE, standard error. The same animals were sampled at one and five hours.**Table 2**Oxygen concentration (pO<sub>2</sub> mmHg) in different equine groups evaluated during 1 and 5 h of holding in a livestock market.

Equids groups	Reference value (RV)	Time of remaining 1 h	Time of remaining 5 h	P-value
	Mean ± SE	Mean ± SE	Mean ± SE	
Mules (n = 176)	38.0 ± 0.000b,4	43.42 ± 1.11a,1	42.28 ± 1.04a,1	< 0.0001
Donkeys (n = 244)	36.66 ± 0.0004a,5	38.18 ± 1.19a,2	30.97 ± 0.86b,3	< 0.0001
Cull horses (n = 466)	39.40 ± 0.001a,2,3	36.59 ± 0.56b,2	36.38 ± 0.92b,2	< 0.0001
Charrería horses (n = 246)	40.06 ± 1.19a,2	36.97 ± 0.49b,2	37.60 ± 0.90b,2	0.0168
Foals (n = 158)	44.65 ± 0.81a,1	36.10 ± 1.01b,2	34.82 ± 1.22b,2	< 0.0001
Ponies (n = 148)	38.40 ± 1.16a,3,4	35.88 ± 1.20a,2	35.95 ± 1.62a,2	0.3604

<sup>a,b,c</sup>Letters in the same row indicate differences among treatments in the same species.<sup>1,2,3,4</sup>Numbers in the same column indicate differences among species in the same treatment. Tukey test ( $P < 0.05$ ); n, number of animals; SE, standard error. The same animals were sampled at one and five hours.**Table 3**

Concentration of blood pH in different equine groups evaluated during 1 and 5 h of holding in a livestock market.

Equids groups	Reference value (RV)	Time of remaining 1 h	Time of remaining 5 h	P-value
	Mean ± SE	Mean ± SE	Mean ± SE	
Mules (n = 176)	7.32 ± 0.0005 <sup>c,4</sup>	7.42 ± 0.01 <sup>a,1</sup>	7.38 ± 0.008 <sup>b,1,2</sup>	< 0.0001
Donkeys (n = 244)	7.38 ± 0.003 <sup>a,3</sup>	7.38 ± 0.004 <sup>a,2</sup>	7.35 ± 0.006 <sup>b,4</sup>	0.0531
Cull horses (n = 466)	7.47 ± 0.0001 <sup>a,1</sup>	7.43 ± 0.002 <sup>b,1</sup>	7.40 ± 0.008 <sup>c,1</sup>	< 0.0001
Charrería horses (n = 246)	7.42 ± 0.004 <sup>a,2</sup>	7.36 ± 0.003 <sup>b,3</sup>	7.32 ± 0.01 <sup>c,5</sup>	< 0.0001
Foals (n = 158)	7.46 ± 0.014 <sup>a,1</sup>	7.36 ± 0.008 <sup>b,3</sup>	7.34 ± 0.011 <sup>b,4,5</sup>	< 0.0001
Ponies (n = 148)	7.41 ± 0.013 <sup>a,2</sup>	7.37 ± 0.005 <sup>b,2,3</sup>	7.36 ± 0.007 <sup>b,2,3</sup>	0.0007

<sup>a,b,c</sup>Letters in the same row indicate differences among treatments in the same species.<sup>1,2,3,4</sup>Numbers in the same column indicate differences among species in the same treatment. Tukey test ( $P < 0.05$ ); n, number of animals; SE, standard error. The same animals were sampled at one and five hours.**Table 4**Bicarbonate concentration (HCO<sub>3</sub><sup>-</sup>) in different equine groups evaluated during 1 and 5 h of holding in a livestock market.

Equids groups	Reference value (RV)	Time of remaining 1 h	Time of remaining 5 h	P-value
	Mean ± SE	Mean ± SE	Mean ± SE	
Mules (n = 176)	30.10 ± 0.0005 <sup>a,2</sup>	27.27 ± 0.45 <sup>b,4</sup>	26.46 ± 0.71 <sup>b,2</sup>	< 0.0001
Donkeys (n = 244)	25.80 ± 0.0001 <sup>c,4</sup>	27.07 ± 0.37 <sup>b,4</sup>	28.42 ± 0.74 <sup>a,1</sup>	< 0.0001
Cull horses (n = 466)	22.20 ± 0.0006 <sup>b,5</sup>	28.19 ± 0.22 <sup>a,2,3</sup>	22.03 ± 0.42 <sup>a,1,2</sup>	< 0.0001
Charrería horses (n = 246)	30.90 ± 0.28 <sup>b,1</sup>	31.59 ± 0.16 <sup>a,1</sup>	27.67 ± 0.46 <sup>c,1,2</sup>	< 0.0001
Foals (n = 158)	26.31 ± 0.64 <sup>a,4</sup>	27.46 ± 0.49 <sup>a,3,4</sup>	24.40 ± 0.61 <sup>b,3</sup>	0.0010
Ponies (n = 148)	27.61 ± 0.65 <sup>a,3</sup>	28.84 ± 0.41 <sup>a,2</sup>	24.54 ± 0.58 <sup>b,3</sup>	< 0.0001

<sup>a,b,c</sup>Letters in the same row indicate differences among treatments in the same species.<sup>1,2,3,4</sup>Numbers in the same column indicate differences among species in the same treatment. Tukey test ( $P < 0.05$ ); n, number of animals; SE, standard error.

lactate (29 mg/dL) (Table 6), glucose (23 mg/dL) (Table 5), calcium (0.2 mmol/L) (Table 8), hematocrit (15%) (Table 7), and blood pH (0.06) (Table 3). Added to these changes, there were significant variations ( $P < 0.0001$ ) in the values for pCO<sub>2</sub> (18 mmHg) (Table 1), and bicarbonate (4 mmol/dL) (Table 4), below the RV.

Turning to the donkeys that remained in the corrals for 1 h, they showed increases above the RV ( $P < 0.0001$ ) in the values for

bicarbonate (1 mmol/dL), glucose (42 mg/dL) (Table 5) and hematocrit (4.5%) (Table 7). Associated with these changes were reductions ( $P < 0.0001$ ) in pCO<sub>2</sub> (Table 1), and calcium (Table 8), compared to the RV. In contrast, the donkeys that stayed in the corrals for 5 h showed increases ( $P < 0.0001$ ) in the values for bicarbonate (2 mmol/dL) (Table 4), glucose (73 mg/dL) (Table 5), and hematocrit (5%) (Table 7), above the RV. Analyses also found a reduction in the values

**Table 5**  
Blood glucose concentration (mg/dL) in different equine groups evaluated during 1 and 5 h of holding in a livestock market.

Equids groups	Reference value (RV) Mean ± SE	Time of remaining	Time of remaining	P-value
		1 h Mean ± SE	5 h Mean ± SE	
Mules (n = 176)	81.33 ± 0.0005 <sup>c,3</sup>	123.84 ± 2.48 <sup>a,1</sup>	104.23 ± 3.53 <sup>b,2</sup>	< 0.0001
Donkeys (n = 244)	68.2 ± 0.0001 <sup>c,4</sup>	110.59 ± 2.59 <sup>b,2</sup>	141.40 ± 4.10 <sup>a,1</sup>	< 0.0001
Cull horses (n = 466)	82.48 ± 0.0002 <sup>c,3</sup>	96.24 ± 1.44 <sup>b,3</sup>	103.51 ± 2.99 <sup>a,2</sup>	< 0.0001
Charrería horses (n = 246)	92.70 ± 1.49 <sup>a,1</sup>	91.73 ± 0.94 <sup>a,3</sup>	78.925 ± 1.40 <sup>b,4</sup>	< 0.0001
Foals (n = 158)	85.27 ± 2.30 <sup>b,2</sup>	94.75 ± 2.80 <sup>a,3</sup>	90.15 ± 3.15 <sup>ab,3</sup>	0.0737
Ponies (n = 148)	87.00 ± 1.32 <sup>ab,2</sup>	84.72 ± 1.97 <sup>b,4</sup>	92.80 ± 1.93 <sup>a,3</sup>	< 0.0001

<sup>a,b,c</sup>Letters in the same row indicate differences among treatments in the same species.

<sup>1,2,3,4</sup>Numbers in the same column indicate differences among species in the same treatment. Tukey test ( $P < 0.05$ ); n, number of animals; SE, standard error.

**Table 6**  
Blood lactate concentration (mg/dL) in different equine groups evaluated during 1 and 5 h of holding in a livestock market.

Equids groups	Reference value (RV) Mean ± SE	Time of remaining	Time of remaining	P-value
		1 h Mean ± SE	5 h Mean ± SE	
Mules (n = 176)	4.33 ± 0.0007 <sup>c,6</sup>	22.01 ± 1.24 <sup>b,1</sup>	34.05 ± 1.05 <sup>a,1</sup>	< 0.0001
Donkeys (n = 244)	12.90 ± 0.0008 <sup>a,3</sup>	12.35 ± 0.84 <sup>a,3</sup>	12.64 ± 0.71 <sup>a,4</sup>	0.7999
Cull horses (n = 466)	11.25 ± 0.0000 <sup>c,4</sup>	13.45 ± 0.78 <sup>b,3</sup>	21.74 ± 2.28 <sup>a,3</sup>	< 0.0001
Charrería horses (n = 246)	6.14 ± 0.89 <sup>b,5</sup>	7.00 ± 0.32 <sup>b,4</sup>	10.92 ± 0.93 <sup>a,4</sup>	0.0001
Foals (n = 158)	16.02 ± 1.00 <sup>c,2</sup>	24.82 ± 1.17 <sup>b,2</sup>	29.40 ± 1.39 <sup>a,2</sup>	< 0.0001
Ponies (n = 148)	22.40 ± 1.28 <sup>b,1</sup>	23.94 ± 0.95 <sup>b,2</sup>	28.80 ± 1.40 <sup>a,2</sup>	0.0015

<sup>a,b,c</sup>Letters in the same row indicate differences among treatments in the same species.

<sup>1,2,3,4</sup>Numbers in the same column indicate differences among species in the same treatment. Tukey test ( $P < 0.05$ ); n, number of animals; SE, standard error.

**Table 7**  
Percentage of hematocrit in different equine groups evaluated during 1 and 5 h of holding in a livestock market.

Equids groups	Reference value (RV) Mean ± SE	Time of remaining	Time of remaining	P-value
		1 h Mean ± SE	5 h Mean ± SE	
Mules (n = 176)	32.30 ± 0.0008 <sup>b,3</sup>	45.54 ± 1.12 <sup>a,1</sup>	46.20 ± 1.11 <sup>a,1</sup>	< 0.0001
Donkeys (n = 244)	29.50 ± 0.0001 <sup>a,4</sup>	34.0 ± 0.87 <sup>a,3</sup>	32.23 ± 0.45 <sup>a,3</sup>	< 0.0001
Cull horses (n = 466)	38.17 ± 0.0003 <sup>a,1</sup>	33.67 ± 0.081 <sup>b,3</sup>	39.05 ± 0.07 <sup>a,2</sup>	< 0.0001
Charrería horses (n = 246)	36.41 ± 0.77 <sup>b,2</sup>	44.83 ± 0.84 <sup>a,1</sup>	44.95 ± 0.91 <sup>a,1</sup>	< 0.0001
Foals (n = 158)	34.70 ± 0.69 <sup>b,3</sup>	34.28 ± 0.49 <sup>b,3</sup>	38.15 ± 0.69 <sup>a,2</sup>	< 0.0001
Ponies (n = 148)	36.33 ± 1.34 <sup>a,2</sup>	38.30 ± 1.02 <sup>a,2</sup>	37.92 ± 1.33 <sup>a,2</sup>	0.4950

<sup>a,b,c</sup>Letters in the same row indicate differences among treatments in the same species.

<sup>1,2,3,4</sup>Numbers in the same column indicate differences among species in the same treatment. Tukey test ( $P < 0.05$ ); n, number of animals; SE, standard error.

**Table 8**  
Calcium concentration (mmol/L) in different equine groups evaluated during 1 and 5 h of holding in a livestock market.

Equids groups	Reference value (RV) Mean ± SE	Time of remaining	Time of remaining	P-value
		1 h Mean ± SE	5 h Mean ± SE	
Mules (n = 176)	1.54 ± 0.002 <sup>a,3</sup>	1.50 ± 0.01 <sup>b,1</sup>	1.66 ± 0.03 <sup>a,1</sup>	0.0185
Donkeys (n = 244)	2.78 ± 0.001 <sup>a,1</sup>	1.48 ± 0.01 <sup>b,1</sup>	1.46 ± 0.02 <sup>b,3</sup>	< 0.0001
Cull horses (n = 466)	1.29 ± 0.006 <sup>b,4</sup>	1.41 ± 0.008 <sup>a,2</sup>	1.57 ± 0.02 <sup>a,2</sup>	< 0.0001
Charrería horses (n = 246)	1.56 ± 0.01 <sup>a,2</sup>	1.41 ± 0.006 <sup>b,2</sup>	1.67 ± 0.03 <sup>a,1</sup>	< 0.0001
Foals (n = 158)	1.21 ± 0.02 <sup>b,4</sup>	1.37 ± 0.01 <sup>a,3</sup>	1.38 ± 0.02 <sup>a,4</sup>	< 0.0001
Ponies (n = 148)	1.21 ± 0.01 <sup>b,4</sup>	1.35 ± 0.09 <sup>a,3</sup>	1.35 ± 0.10 <sup>a,4</sup>	< 0.0001

<sup>a,b,c</sup>Letters in the same row indicate differences among treatments in the same species.

<sup>1,2,3,4</sup>Numbers in the same column indicate differences among species in the same treatment. Tukey test ( $P < 0.05$ ); n, number of animals; SE, standard error.

for pCO<sub>2</sub> (10 mmHg) (Table 1), pO<sub>2</sub> (5 mmHg) (Table 2), blood pH (0.03) (Table 3), and calcium (1 mmol/L) (Table 8), below the RV.

Results for the cull horses that stayed in the corrals for 1 h included increases ( $P < 0.0001$ ) in the values for pCO<sub>2</sub> (1 mmHg) (Table 1), bicarbonate (6 mmol/L) (Table 4), lactate (2 mg/dL) (Table 6), glucose (14 mg/dL) (Table 5), and calcium (0.2 mmol/L) (Table 8) above the RV, accompanied by reductions ( $P < 0.0001$ ) in the values for pO<sub>2</sub> (3 mmHg) (Table 2), blood pH (0.04) (Table 3), and hematocrit (4.5%)

(Table 7), below the RV. Meanwhile, the cull horses that remained in the corrals for 5 h presented increases above the RV ( $P < 0.0001$ ) for lactate (10 mg/dL) (Table 6), glucose (21 mg/dL) (Table 5), calcium (0.28 mmol/L) (Table 8), and hematocrit (1%) (Table 7). Also observed were reductions ( $P < 0.0001$ ) in the values for pO<sub>2</sub> (3 mmHg) (Table 2), bicarbonate (0.17 mmol/L) (Table 4), and blood pH (0.07) (Table 3) below the RV.

The *charrería* horses sampled 1 h after arrival, showed increases

above the RV ( $P < 0.0001$ ) in the values for  $p\text{CO}_2$  (3 mmHg) (Table 1), bicarbonate (0.69 mmol/L) (Table 4), and hematocrit (8%) (Table 7), but those changes were accompanied by reductions ( $P < 0.0001$ ) in the values for  $p\text{O}_2$  (3 mmHg) (Table 2), blood pH (0.06) (Table 3), and calcium (0.15 mmol/L) (Table 8), below the RV. In contrast, the *charrería* horses that stayed in the corrals for 5 h showed increases ( $P < 0.0001$ ) in the values for lactate (5 mg/dL) (Table 6), calcium (0.11 mmol/L) (Table 8), and hematocrit (7%) (Table 7), above the RV, but also reductions in the values for  $p\text{CO}_2$  (3 mmHg) (Table 1),  $p\text{O}_2$  (2 mmHg) (Table 2), bicarbonate (3 mmol/L) (Table 4), glucose (14 mg/dL) (Table 5), and blood pH (0.1) (Table 3), below the RV.

Foals sampled 1 after arrival exhibited increases ( $P < 0.0001$ ) in the values for  $p\text{CO}_2$  (5 mmHg) (Table 1), bicarbonate (1 mmol/L) (Table 4), lactate (9 mg/dL) (Table 6), glucose (9 mg/dL) (Table 5), and calcium (0.16 mmol/L) (Table 8). In this group, reductions below the RV were observed in the values for  $p\text{O}_2$  (8 mmHg) (Table 2), and blood pH (0.1) (Table 3). The foals that remained in the pens for 5 h, however, presented increases ( $P < 0.0001$ ) in the values for  $p\text{CO}_2$  (6 mmHg) (Table 1), lactate (13 mg/dL) (Table 6), and calcium (0.17 mmol/L) (Table 8), above the RV, together with reductions ( $p < 0.0001$ ) in the values for  $p\text{O}_2$  (10 mmHg) (Table 2), bicarbonate (2 mmol/L) (Table 4), and blood pH (0.12) (Table 3), below the RV.

Finally, the ponies that remained in the corrals for 1 h showed increases ( $P < 0.0001$ ) in the values for calcium (0.14 mmol/L) (Table 8), above the RV, together with a reduction ( $P < 0.0001$ ) in the value for blood pH (0.04) (Table 3), below the RV. In contrast, the ponies that stayed in the pens for 5 h presented increases ( $P < 0.0001$ ) in the values for lactate (6 mg/dL) (Table 6), and calcium (0.14 mmol/L) (Table 8), above the RV. Finally, the study found a reduction ( $P < 0.0001$ ) in the values for bicarbonate (3 mmol/L) (Table 4), and blood pH (0.05) (Table 3), below the RV.

#### 4. Discussion

The results of this research indicate that, for the animals evaluated, the time spent in corrals at livestock markets causes various alterations in gas exchange, the acid-base balance, energy metabolism, and the water balance. These physiological alterations observed in the different types of equine assessed during two holding times at a cattle market can probably be explained by exposure to different, novel stimuli that the animals experienced as a consequence of the combination of diverse factors: hunger, fatigue, environmental conditions, the mixing of strange animals, and inadequate handling, that, together produced a metabolic disequilibrium [9,13].

The types of equine that had the highest concentrations of blood lactate were the mules, foals, and ponies, while the *charrería* horses, in contrast, presented the lowest blood lactate levels during the 5-h holding period. With respect to blood glucose levels, they were found to be highest in the donkeys after 5 h in the corrals, while the mules presented the highest values for glucose concentration, but after the 1-h holding period. The other groups of equine maintained similar blood glucose levels. As a result, the mules and donkeys presented the most accentuated metabolic problems during the holding period in the corrals, as they exhibited –simultaneously– states of hyperglycemia and hyperlactatemia. According to de Aluja [8], cortisol acts on the liver by increasing the synthesis of enzymes that convert amino acids into glucose inside the hepatocytes (gluconeogenesis). Most of this recently-formed glucose is released into the bloodstream, where it causes an increase in blood glucose values. In this way, cortisol facilitates an organism's adaptation to stressful situations [9], including time spent enclosed in corrals and inadequate handling, at least in all groups. de Aluja [8] further observed that culled equine transported for 59 min and 2 h, respectively, showed increases in blood lactate concentrations (hyperlactatemia), which were attributed to muscular fatigue. Guyton and Hall [14], however, observed that blood lactate concentrations were significantly higher after performance of a stressful procedure,

such as loading, unloading or entering the corrals, compared to those determined in steers that were not exposed to such handling methods. Meanwhile, the studies of Mckee and Mckee [15], and Cunningham and Bradley [16] found that lactate levels increased rapidly as a function of the physical exercise. Their findings may be due to an increase in catecholamines during inadequate handling that degraded muscle glycogen and so increased lactate concentrations [17]. In light of these findings, it is possible to suggest that the increases in both glucose and lactate could be caused by transport, since before arriving at the livestock market these groups of animals are exposed to different kinds of stressors that cause a high demand for energy. Moreover, the handling procedures performed represent another severe stressor for all these groups, because they tend to be inadequate and so provokes stress, which alters the species' energy metabolism.

The blood pH levels determined for the *charrería* horses, foals, ponies and donkeys were below those found for the cull horses and mules after 5 h of holding in the livestock market. This finding may be attributable to the action of physiological buffers that begin to act immediately by impeding large changes in the concentration of hydrogen ions, as well as to regulating mechanisms in the lungs and kidneys that are, ultimately, the factors responsible for maintaining pH [18].

The mules presented a higher concentration of oxygen than the donkeys, which had the lowest oxygen concentration during 5 h of holding in the livestock market. Added to these changes in partial pressure of gases, the *charrería* horses maintained higher  $p\text{CO}_2$  concentrations than the mules after 1 h in the corrals at the livestock market. In contrast, the *charrería* horses presented a higher concentration of  $\text{HCO}_3^-$  compared to the other equine groups evaluated after remaining in the livestock market for 1 h. This indicates that the mules tended to hyperventilate; that is, to break the respiratory equilibrium and begin to increase their  $p\text{O}_2$  levels, while  $p\text{CO}_2$  levels decreased. Mitchell, Hattingh [19] affirm that in unfavorable environments unfamiliar, stressed, anxious or fearful animals are exhausted, which triggers hyperventilation as an emergency thermolytic mechanism. Hyperventilation, or hypocapnia, induces respiratory alkalosis, defined as a clinical disorder caused by a reduced concentration of  $\text{H}^+$  ions, and characterized by high pH, low  $p\text{CO}_2$  and a variable reduction in blood  $\text{HCO}_3^-$ . If alveolar ventilation increases beyond the limits required to expel the daily load of  $\text{CO}_2$ ,  $p\text{CO}_2$  will decrease while systemic pH increases. When  $p\text{CO}_2$  decreases,  $\text{H}_2\text{CO}_3$  and  $\text{HCO}_3^-$  are also reduced. Together, they constitute the compensatory response [20,21]. In contrast, the donkeys suffered an increasing concentration of blood  $p\text{CO}_2$  and the consequent reduction of blood pH.

In the case of the donkeys, then, the phenomenon of hypoventilation, the condition of hypercapnia, and the increase in the  $\text{HCO}_3^-$  concentration reveal a process of metabolic acidosis. Respiratory acidosis, or primary hypercapnia, appears when the production of carbon dioxide exceeds its rate of elimination by the lungs. It is almost always the result of a condition of respiratory insufficiency accompanied by alveolar hypoventilation that results in, and is characterized by, an increase of  $p\text{CO}_2$ , reduced pH, and a compensatory increase in the concentration of blood  $\text{HCO}_3^-$  [22]. When  $\text{CO}_2$  bonds to water through carbonic anhydrase, it is converted into carbonic acid, a weak acid that partially dissociates into bicarbonate and hydrogen cations. These hydrogen ions are what cause the increase of plasma acidity. The excess hydrogen reduces pH and, therefore, the amount of bicarbonate, leading to a condition of metabolic acidosis [23]. The metabolic consequences of hypercapnia include retention of sodium and water, possibly as a result of an increased release of the antidiuretic hormone, increased cortisol secretion, and activation of the renin-angiotensin system [24].

De Aluja et al. [25] evaluated 32 donkeys before and after subjecting them to distinct intervals of work. That study found that the pH, bicarbonate ( $\text{HCO}_3^-$ ) and total  $\text{CO}_2$  ( $t\text{CO}_2 = [\text{HCO}_3^-] + a * p\text{CO}_2$ ) values increased ( $p > 0.05$ ) after 1, 2 and 3 h of work, compared to the values recorded prior to the work. Observations also showed a decrease

( $p > 0.05$ ) in  $p\text{CO}_2$  and hematocrit values after the work periods. Those authors concluded that the Mexican donkey (*burro*) has a satisfactory compensation to prolonged exercise for one, two and three hours, despite transporting heavy loads.

In horses, hematocrit is normally utilized as an indicator of dehydration [26]. Observations during the present study revealed that both the *charrería* horses and the mules presented high percentages of hematocrit, compared to the other groups evaluated. Stress produces specific effects in such organs as the spleen, stomach, mesenteries and skin, where it generates contractions and reduced motility while diverting blood towards the muscles, which leads to variations in hematocrit (VGA) [27]. The increase in hematocrit was probably due to the increase in blood viscosity caused by extreme dehydration or due to physiologic compensation [28]. This, in turn, increases the number of erythrocytes (erythrocytosis) in circulation, which increases the organism's carrying capacity for oxygen [22]. de Aluja [24], mention that, in general, the longer that animals are deprived of food, during transport or simply while holding in a commercial establishment or slaughterhouse, the higher the probability that they will present stress due to hunger, thirst and/or adverse environmental situations. Gul et al. [29] found that Hb (hemoglobin) was highest in horses, followed by mules and donkeys, while total leukocyte counts (TLC) values were highest in donkeys, followed by mules and horses.

The differences shown in the blood profile between mules, horses and donkeys, may be due to their body condition, physical condition, fasting period and age [30].

## 5. Conclusions

The results of this research indicate that both time intervals assessed (1 and 5 h) caused diverse imbalances in gas exchange, the acid-base balance, energy metabolism, and the water balance in all 6 groups of equine evaluated. The equine groups that suffered the greatest physiological alterations were the mules and donkeys. It is important to mention that after remaining in the exhibition corrals for just 1 h, the equine assessed were found to be dehydrated and fatigued, and manifested metabolic and compensatory problems, caused by a combination of factors that include fasting, loading, transport, unloading, and inadequate handling.

At present, the commercialization of equine occurs for different purposes. The most important one in Latin America is slaughtering to obtain meat to be marketed, though most of the animals involved are not raised for this objective; rather, they tend to be culled equine whose productive life has ended. Of course, equine may be bought and sold to perform other activities, such as sports, or may simply be put to work. At this time, there is no information on the question of how holding times at livestock markets affect the physiological blood profile of these animals, despite the great importance of this approach, due to the heated controversy over the inadequate handling that animals receive in these exhibition facilities. An estimated 39 million donkeys, 40.5 million horses and 12.3 million mules live in developing countries, constituting over 85% of the world's equids. In developing countries, equids are mostly used as working animals, often carrying out tasks under harsh and impoverished conditions for long hours each day.

The findings in this study illustrate the need for further research into the effects of chronic exercise stress and longitudinal studies of muscle damage in equids.

## Competing interests

The author declares no conflicts of interest regarding the publication of this paper.

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