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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),

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