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## Original Research

## Effect of the Work Performed by Tourism Carriage Horses on Physiological and Blood Parameters



Fernando Vergara, Tamara A. Tadich\*

Departamento de Fomento de la Producción Animal, Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, La Pintana, Santiago, Chile

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## ABSTRACT

Welfare of working horses is a matter of public concern, with scarce information on their possible physiological coping mechanisms. The aim of this study was to assess changes in possible physiological welfare indicators in working horses, as a result of pulling tourism carriages under field conditions. A descriptive field study of the work performed by tourism carriage horses and their physiological, hematological, and blood biochemistry implications was performed. For this, 10 tourism carriage horses were studied under normal working conditions. For description of work, speed, distance, and force were calculated. To assess welfare, physiological variables including heart rate, respiratory rate, rectal temperature, and hematological and blood biochemistry parameters were evaluated before, during, and after work. Results show that tourism carriage horses exerted a sub-maximal effort in terms of speed, force, and physiological variables assessed. The heart and respiratory rate showed significant increases ( $P < .05$ ) after work, but recovered to basal values within the first 10 minutes. Blood variables did not show significant changes that could be related to poor welfare. Lactate and packed cell volume (PCV) were the only blood variables with significant differences across work ( $P < .05$ ) with lactate decreasing over time and PCV increasing with work and returning to basal levels at 10 minutes after work. Physiological variables showed a possible adaptation to work by the carriage horses but were not sufficient to diagnose a welfare problem. Management practices and other animal-based indicators should be included in further studies to obtain a holistic conclusion.

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## 1. Introduction

Working equines still play a crucial role in the provision of traction energy, and although it is difficult to find information on the economic impact of these animals in today's society, a large proportion of the world population still depends on them [1].

The management and use of carriage horses, as a tourism instrument in cities, has been recently highlighted

as a matter of public concern in terms of the welfare of these equines [2,3]. In many countries, animal rights advocates are pushing for the ban of activities that involve working horses, but often, no scientific evidence accompanies these petitions. There is scarce information on the welfare needs of this group of working horses, contrary to the available information on other types of working equids in developing countries [4–8], which encounter different welfare risks making it difficult to compare.

Normally, we would expect to see draught breeds pulling carriages for tourism, but in developing countries, it is common to see lighter crossbred horses performing this work [5]. This provides husbandry advantages for their owners who usually do not have incomes to maintain in proper conditions Percheron, Belgian, or other draught

\* Corresponding author at: Tamara A. Tadich, Departamento de Fomento de la Producción Animal, Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santa Rosa 11735, La Pintana, Santiago, Chile.

E-mail address: [tamaratadich@u.uchile.cl](mailto:tamaratadich@u.uchile.cl) (T.A. Tadich).

breeds as those reported by Rosser and Ardis [3] in South Carolina, or the Standardbreds, Morgans, and draught crossbreeds used by Amish communities [9].

Working horses in Chile have been described as crossbreeds with morphology corresponding to speed type, with live weights between 300 and 400 kg and height's to the withers between 140 and 145 cm [5,10]. The use of these lighter horses opens the question of whether they are physically adapted to perform this work without negatively affecting their welfare. The work performed by carriage horses develops physiological changes, as in any other physical activity, especially draught work that requires force and resistance for prolonged periods of time [11,12]. All these changes are in favor of coping with increases in the demand of oxygen by muscles under aerobic exercise and include changes in cardiac, respiratory, musculoskeletal, and endocrine systems [11–15] and consequently allow the individuals to promote their welfare. The use of objective physiological indicators associated with changes in these systems could allow veterinarians to assess the welfare of these horses and ensure provision of evidence-based feedback on good husbandry practices to the owners. At the same time, reference values obtained abroad may not be fully applicable under local conditions because factors such as breed, environment, management conditions, and type of work are not the same [16,17]; this is why it is important to have local data and when possible compare individuals with their own baselines.

This is why the aim of this study was to assess changes in classical physiological indicators of welfare as a result of the work performed by tourism carriage horses under real working conditions.

## 2. Materials and Methods

### 2.1. Animals

For the study, 10 carriage horses, five mares and five geldings, all light crossbreed with an average weight of 420 kg (380–500 kg), between 2 and 10 years of age were used and with a height to the withers of 148 cm in average. Body condition score (BCS) was assessed using a 0–5 scale, with two horses presenting a BCS of 2, and eight a BCS of 3. These horses are not allowed to work above 8 hours a day and must present a health certificate twice a year to the local authority (municipality). Horses were selected according to disposition of owners to participate and in coordination with the municipal authority from the city of Viña del Mar, Chile. The experiment was approved by the Animal Use and Care Ethical Committee of the Veterinary Faculty at the University of Chile and Fondecyt Iniciación 11121467.

### 2.2. Effort Test

The study was conducted during two normal working days of the tourism carriage horses in December 2013 (summer). The route used consisted in the one authorized by the Municipality of Viña del Mar, with one stop at the “Folk Museum” of less than 10 minutes. All samples were

taken between 11 AM and 5 PM. Before the effort test, each horse underwent a clinical examination to determine if they were sound. Lame horses or with any evident health issue were excluded from the study. For sampling, five times were established:

T<sub>0</sub>: at rest previous to a tour route

T<sub>1</sub>: at the first stop of the tour

T<sub>2</sub>: at initiation of the second part of the tour

T<sub>3</sub>: at final stop

T<sub>4</sub>: at 10 minutes after the tour was completed. Minimum time required by the municipality for drivers to wait until the next tour.

For each time, information on heart rate (HR), respiratory rate (RR), and rectal temperature (RT) was obtained. Blood samples were only taken at T<sub>0</sub>, T<sub>3</sub>, and T<sub>4</sub>.

Each tour was done with two passengers (the researcher and a helper) and the driver. For assessing the maximum force exerted by horses, a digital dynamometer (GSE Model 250) was attached between the breast band and the carriage. These devices store the information of the maximum force exerted by the horse during movement, which was then used for analysis. The unit measured is given in kilograms of force (kgf) that was then transformed to kN for the statistical analysis. For determining the speed and distance traveled by each horse, the Polar G3 GPS sensor W.I.N.D. was attached to the girth.

### 2.3. HR, RR, and RT

For HR, a Polar Equine H2 Heart Rate monitoring system was used during the whole effort test and the Polar Equine RS800CX training computer software was used for subsequent analysis. Respiratory rate was assessed by the researcher by observing the horse's torso for the movement of the rib cage and belly, and the RT was assessed with a digital thermometer at each sampling time.

### 2.4. Blood Hematology and Biochemistry

Blood samples were obtained by jugular puncture at T<sub>0</sub>, T<sub>3</sub>, and T<sub>4</sub>. A volume of 12 mL was withdrawn and divided into three tubes, one with EDTA for hematology (packed cell volume [PCV], fibrinogen, total proteins, plasmatic albumin, globulins, neutrophils, and lymphocytes), one with heparin for glutathione peroxidase (GPx), and one with no additives for obtaining serum and blood biochemistry (lactate dehydrogenase [LDH], aspartate aminotransferase [AST], creatine kinase [CK]) and hormones (cortisol). Lactate was assessed by the Accutrend BM-Lactate from Roche at the moment of sampling.

### 2.5. Statistical Analysis

All variables were inserted in a Microsoft Excel spreadsheet for descriptive statistics. The distance traveled, the time required for each effort test, speed, force, and latency time for recovery of HR were analyzed with descriptive statistics.

**Table 1**

Maximum force (kN) required to take the carriage out of inertia and force required to maintain movement (kN) in 10 carriage horses during normal tourism work.

Force	Carriage Horses									
	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10
Maximum force	1.02	0.75	0.91	0.78	0.67	0.41	0.59	0.47	1.07	0.80
Moving force	0.17	0.15	0.18	0.13	0.12	0.09	0.12	0.12	0.18	0.13

For HR, RR, and RT, a comparison between the five sampling times was performed with one-way analysis of variance or Kruskal–Wallis with a Tukey or all-wise comparison post hoc test depending on the normality of data; for hematological and biochemistry variables, the same tests were applied to compare between the three sampling times. The statistical software used was Statistix 8.0, and a significance level of  $P < .05$  was applied.

### 3. Results

The tourism circuit traveled by the carriage horses ranged from 3.09 to 4.64 km, depending on the drivers decision on the route, with one driver not making the stop at the museum. These circuits were executed at a mean speed of 3.19 m/s (11.48 km/hr) being the highest speed achieved of 5.4 m/s (19.4 km/hr). The force required to take the carriage out of inertia and to maintain movement of the carriage differs and is shown in Table 1, being 1.07 kN (109 kgf) the maximum force required to initiate movement and 0.18 kN (18 kgf) the maximum force to maintain it.

#### 3.1. Physiological Variables

For HR and RR, a significant increase ( $P < .001$  for both variables) in  $T_1$  and  $T_3$  was observed; no differences were found for RT ( $P = .25$ ; Table 2). Three of the 10 horses studied required more than 10 minutes to recover basal HR after finishing the circuit ( $T_3$ ).

#### 3.2. Blood Variables

Lactate and PCV were the only two parameters that showed significant differences ( $P = .006$  and  $P = .0008$ , respectively) along the three sampling times, with lactate decreasing over time from  $T_0$  to  $T_4$ , whereas PCV showed a significant increase in  $T_3$ . White blood cells tended to stay

within range across sampling times with a slight increase in  $T_3$ . Median muscular enzyme concentrations (CK and AST) stayed within range along the work, whereas LDH median values were above normal range even before the start of the exercise ( $T_0$ ) and GPx median values were above normal range, with the exception of a few horses that showed selenium deficiency. Cortisol showed a nonsignificant increase ( $P = .78$ ) at  $T_3$ , returning to basal values at  $T_4$  (10 minutes after work; Table 3).

### 4. Discussion

Carriage horses, as other working horses, are usually associated with poor welfare and possible mistreatment by the general public. This perception by the community has led to petitions of replacement by motorized vehicles and bans on the use of working equines, such is the case for carriage horses in New York, USA [2], although there is a lack of research on horse-based tourism [19]. In the present study, we report a physiological adaptation to work in a group of carriage horses used for tourism in Viña del Mar, Chile.

To assess the effect of the use of equines for tourism, a description of the work was done. The route done under the study was not the same for all horses, showing a discrepancy with the route assigned by the municipality [20]. Despite this, in terms of speed and distance, they were similar, with an average speed of 3.19 m/s (maximum speed, 5.4 m/s) in distances ranging from 3.09 to 4.64 km. All routes included a pause, associated with a touristic attraction, with the exception of one horse, for which the driver did not stop. The speed at which horses are working is faster than those reported for draught horses in agricultural tasks ranging between 8.2 and 10.01 km/hr [21,22], but similar to those reported for wagons, open, and counting buggies in an Amish community in the United States (4.63–5.35 m/s) [9]. Speed has been associated with load where horses choose a speed that minimizes their metabolic cost to move a given distance [23,24].

The force required to move the carriage was always higher at the start in the moment that requires taking the carriage out of inertia by the horse. The force exerted by each horse, at start point and during movement, is shown in Table 1. The maximum force exerted to take the carriage out of inertia (1.07 kN) was lower than that required by draught horses performing light agricultural tasks (1.19 kN for moving 946 kg). To maintain movement tourism, horses generated a maximum force of 0.18 kN, less than the 0.48 kN required by draught horses in agricultural tasks [21,22]. The requirement of less force in the tourism horses

**Table 2**

Mean and standard deviation of heart rate (HR), respiratory rate (RR), and rectal temperature (RT) in 10 tourism carriage horses at five sampling times before, during, and after work ( $T_0$ ,  $T_1$ ,  $T_2$ ,  $T_3$ , and  $T_4$ ).

	$T_0$	$T_1$	$T_2^c$	$T_3$	$T_4$
HR	42.33 ± 5.70 <sup>b</sup>	90.89 ± 19.95 <sup>a</sup>	48.67 ± 8.87 <sup>b</sup>	102.33 ± 15.22 <sup>a</sup>	46.67 ± 9.80 <sup>b</sup>
RR	24.6 ± 5.74 <sup>b</sup>	45.25 ± 7.69 <sup>a</sup>	26 ± 6.78 <sup>b</sup>	38.8 ± 10.51 <sup>a</sup>	23.8 ± 8.97 <sup>b</sup>
RT	37.91 ± 0.49	38.09 ± 0.39	38.09 ± 0.39	38.14 ± 0.26	38.19 ± 0.41

Different superscript letters (a and b) in rows indicate significant difference between times ( $P < .05$ ).

<sup>c</sup> For  $T_2$ , only nine horses are considered because one did not make a stop in-between his route.

**Table 3**

Results of the hematology and blood biochemistry assessed at three sampling times (T<sub>0</sub>, T<sub>3</sub>, and T<sub>4</sub>) in tourism carriage horses (n = 10), and normal range for each parameter [18].

Blood Variable	Normal Range	T <sub>0</sub>		T <sub>3</sub>		T <sub>4</sub>	
		Median	Range	Median	Range	Median	Range
Lactate (mmol/L)	1.0–2.0	1.05 <sup>a</sup>	0.7–1.6	0.75 <sup>a</sup>	0.7–1.1	0.7 <sup>b</sup>	0.7–1.2
PCV, %	30–47	34.5 <sup>b</sup>	27–38	38.5 <sup>a</sup>	33–43	34 <sup>b</sup>	29–37
Hb, g/L	107–167	115	100–130	130	110–150	110	100–130
Leukocytes, $\mu$ L	5,000–11,000	7,565	5,680–10,680	10,430	7,290–12,840	8,240	6,200–11,790
Lymphocytes, $\mu$ L	1,500–6,500	2,302	1,590–3,322	2,846	2,218–5,521	2,573	1,860–4,716
Neutrophils, $\mu$ L	2,200–6,100	4,881	3,814–7,048	6,248	4,520–8,442	5,106.5	3,863–6,370
N:L	0.8–2.8	2.1	1.9–2.5	1.9	1.1–3.0	1.8	1.3–2.7
Plasma protein, g/L	65–73	72	56–86	74	59–84	71	57–84
Fibrinogen, g/L	<5	2	1–4	2	0.1–0.4	0.2	0.1–0.8
CK, U/L	<480	306	187–514	344	237–683	396	197–517
AST, U/L	<480	306	257–342	316	237–346	311.5	254–339
LDH, U/L	<700	757.5	533–900	740.5	629–993	781.5	507–942
GPx, U/g Hb	>130	192.5	67–268	191.5	45–254	202.5	57–259
Cortisol, nmol/L	32–240	236.9	144.5–459.6	272.3	155.4–514.0	259.9	149.7–430.3

Abbreviations: AST, aspartate aminotransferase; CK, creatine kinase; GPx, glutathione peroxidase; Hb, hemoglobin; LDH, lactate dehydrogenase; N:L, neutrophil-to-lymphocyte ratio; PCV, packed cell volume.

Different superscript letters (a and b) in rows indicate significant differences between times ( $P < .05$ ).

could be because of the type of surface over which they work (concrete) compared with soil in the case of draught horses, providing less resistance to movement; differences in harnessing system, load, and characteristics of wheels of the cart could be also an important factor (Fig. 1). Size of horses could also have made a difference because the ones assessed by Pérez et al [21,22] were crossbred draught horses of  $678 \pm 23$  kg compared with the light crossbred horses in the present study ( $428 \pm 47$  kg).

For the physiological variables assessed and in relation to welfare, it is expected that a horse adapted to work should show changes in response to exercise and an ability to cope with these changes, shown by the recovery of basal values within a short period of time. Heart rate and RR showed significant increases from T<sub>1</sub> to T<sub>3</sub> (Table 2). For HR, this response to adrenergic stimulus over the heart was expected [13], and was the equivalent to HR at submaximal work [25], because the HR tends to positively correlate to the level of effort required for work [26]. No other data on HR for working horses were found; however, comparison with trained horses could serve as a first approach.

The significant increase in RR (Table 2) correlates with an increase in the demand of oxygen associated to the work performed and the need of a better alveolar ventilation to

eliminate the carbon dioxide produced [13]. During exercise, ventilation increases in response to increased metabolic demand, and the magnitude of this increase depends on the intensity and duration of exercise [27]. The RT did not show significant differences between sampling times (Table 2). During exercise of moderate intensity, the equine should be able to dissipate heat, keeping temperature near to basal values [28], which seems to be the case for the horses assessed. It is important to point out that, 10 minutes after the last stop, horses recovered to basal HR, RR, and RT values, with shorter periods for recovery indicating better adaptability to exercise [26].

For blood variables, only lactate and PCV showed significant differences between the three sampling times (Table 3). Lactate was greater, but within normal values, before the initiation of the tour and then decreased over time; this could be because of a good clearance capacity of the horses induced by submaximal resistance exercise [29] that also depends on the muscular blood flow, number of active muscles, muscular mass, and the transport of lactate toward other tissues such as the liver [30]. These results differ from those for urban draught horses in the south of Chile where mean lactate values (2.28 nmol/L) were above normal values [31]. These differences could be explained by the variability in the type of work performed by urban cart horses, with differences in intensity depending of the day and activity, whereas the tourism horses do the same work over time, allowing better adaptation.

The PCV showed a significant increase (Table 3), although always within normal values, at T<sub>3</sub>. This increase was expected because of the normal spleen contraction and subsequent release of erythrocytes during exercise or excitement, increase that normally returns to baseline values 2 hours after the stimulus has ceased [32], whereas in this case, a significant decrease was shown already 10 minutes after work. Dehydration does not seem to be a welfare problem for these horses during work, although a small increase was observed in plasmatic proteins no significant differences were found. These results could be explained by water loss through sweat, ventilation, and



**Fig. 1.** Typical carriage horse assessed in this study, used for tourism work in the city of Viña del Mar, Chile.

movement toward the musculoskeletal system to facilitate contraction [21]. In contrary to other studies in working horses where a decrease in hemoglobin values has been reported [17,31], horses in this study were all within normal range, although in the lower values which could be associated to the aerobic work performed.

Cortisol has typically been used as a stress hormone, associating increases to welfare problems. A normal increase of cortisol was found in T<sub>3</sub> because of activation of the hypothalamic-pituitary-adrenal axis in response to physical activity, increase that was not significant and returned to baseline values in T<sub>4</sub> (Table 3). Because of the variability in cortisol response under different stimulus, such as normal response to exercise, the neutrophil-to-lymphocyte ratio (N:L) has been pointed out as more reliable indicator of stress [33] where an increase in the ratio is expected under stressful conditions. It was expected that horses overexercised and with difficulty to cope with work would have this ratio altered. In the present study, the N:L ratio was within normal values at all sampling times (Table 3), and although neutrophilia was observed, the time until the last sampling might not have been enough to find a decrease in lymphocytes and a subsequent alteration of the N:L ratio.

Increases in muscular enzymes can be associated with muscular damage and be used as a welfare indicator. Increases in CK and AST have been reported for working horses [17,31] and were associated to low-grade chronic muscle damage [17]. In this case, only CK and LDH showed small increases, and these could be more related to an increase in permeability of the muscular cell during exercise than with real tissue damage related to overwork or mechanical damage [31]. The intensity and duration of the exercise are key on the expression of these enzymes in plasma because in light exercise, there is no significant change in CK [34]. This, together with the normal values found for AST, which has a longer half life (7–8 days) [34], and normal levels of fibrinogen, an inflammation marker, also confirm no important muscular damage was present before the study. Glutathione peroxidase was also evaluated to establish the nutritional state of selenium, mineral that acquires importance as an antioxidant together with vitamin E, and that can be associated to muscular degeneration [35]. This enzyme did not show significant changes between times and was always above normal minimum levels established for horses.

Although a small sample of tourism horses was evaluated and results should be interpreted with caution, this study provides a first approach to the effort exerted and coping capacity to work of these horses. Most of the horses were able to recover basal values of the variables assessed within the minimum time (10 minutes) asked by the municipality to rest between tours. In conclusion, the light crossbred tourism carriage horses studied seem to have adapted physiologically to their work activity, and the existence of a welfare problem cannot be determined with the variables assessed. Only one welfare aspect, related to the physical status, of these horses was assessed, and future studies should also include indicators of good mental and behavioral state to provide a more holistic view of their welfare state.

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