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Original Research Article

Effects of work on physiological parameters and muscle stress markers in tourist carriage horses in Cartagena, Colombia

Efectos del trabajo sobre parámetros fisiológicos y marcadores de estrés muscular en caballos de carruaje turístico en Cartagena, Colombia

Efeitos do trabalho sobre parâmetros fisiológicos e marcadores de estresse muscular em cavalos de carruagem turísticos em Cartagena, Colômbia

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Abstract

Background: Cartagena de Indias, Colombia, is recognized as a tourist city, where carriage horses are a main means of transportation for visitors. However, overexploitation has led to horses being exposed to long work hours and unhealthy conditions, causing deleterious effects on the animal. **Objective:** To determine the effect of work on physiology, hematology, blood biochemistry, and muscle stress indicators of carriage horses. **Methods:** Sixty carriage horses were studied under normal working conditions. The evaluation of muscle stress indicators was carried out in a group of 12 animals (6 with prolonged rest and 6 with loading activity) with similar age, weight, sex, body condition, and clinical diagnosis. **Results:** Forty-eight percent of the animals presented morphometric measurements within normal ranges. The average values of total and direct bilirubin, GOT, GPT, and ALT in all animals were within the reference values, except for FAS, while 84% presented values of leukocytes and erythrocytes outside the reference values. When comparing between groups, there were significant changes in serum values ($P \leq 0.05$). The difference in plasma CK and LDH concentration peaked between 8 and 10 hours after carrying out the activities; however, 28 hours after work (rest period), these plasma values did not differ statistically. On the other hand, a correlation was observed between total CK and AST and ALT, and between AST and ALT ($P \leq 0.05$, $r = 0.44$; $P \leq 0.05$, $r = 0.43$; $P \leq 0.01$, $r=0.60$). **Conclusion:** The physiological and biochemical variables showed a possible adaptation to the work of carriage horses. This was not sufficient to diagnose a welfare problem in most of the animals.

Keywords: *animal health; animal welfare; biomarkers; blood biochemistry; carriage horse; draft horse; horses; muscle stress; workhorse.*

Resumen

Antecedentes: Cartagena de Indias, Colombia, es reconocida como una ciudad turística, donde los caballos cocheros o de carruaje son un importante medio de transporte para los visitantes. Sin embargo, la sobreexplotación ha llevado a que los caballos estén expuestos a largas jornadas de trabajo y condiciones inadecuadas de manejo, ocasionando efectos deletéreos sobre la salud animal. **Objetivo:** Determinar el efecto del trabajo realizado por los caballos cocheros sobre parámetros fisiológicos, hematológicos, bioquímicos, e indicadores de estrés muscular. **Metodología:** Se realizaron jornadas de salud en 60 caballos cocheros durante sus jornadas rutinarias de trabajo. La evaluación de los indicadores de estrés muscular se realizó en un grupo de 12 animales (6 con descanso prolongado y 6 con actividad de transporte) con edad, peso, sexo, condición corporal y diagnóstico clínico similares. **Resultados:** El 48% de los animales presentaron medidas morfométricas dentro de los rangos considerados normales. Los valores medios de bilirrubina total, bilirrubina directa, GOT, GPT y ALT en todos los animales estaban dentro de los valores de referencia, excepto para FAS, mientras que el 84% presentaron valores de leucocitos y eritrocitos fuera de los valores de referencia. Al comparar entre grupos, hubo cambios significativos en los valores séricos de los bioindicadores ($P \leq 0,05$). La diferencia en las concentraciones plasmáticas de CK y LDH alcanzó su punto máximo entre 8 y 10 horas después de realizar las actividades; sin embargo, 28 horas después del trabajo (período de descanso) estos valores plasmáticos no difirieron estadísticamente. Por otro lado, se encontró una correlación entre CK total y AST y ALT, y entre AST y ALT ($P \leq 0,05$, $r = 0.44$; $P \leq 0,05$, $r = 0,43$; $P \leq 0,01$, $r=0,60$). **Conclusiones:** Las variables fisiológicas y bioquímicas mostraron una posible adaptación de los caballos de carruaje al trabajo. Esto no fue suficiente para diagnosticar un problema de bienestar en la mayoría de los animales.

Palabras clave: *bienestar animal; biomarcadores; bioquímica sanguínea; caballos; caballo de trabajo; caballo de tiro; estrés muscular; salud animal.*

Resumo

Antecedentes: Cartagena das Índias, Colombia, é reconhecida como uma cidade turística, onde os cavalos de carruagem são um dos principais meios de transporte para os visitantes. No entanto, a superexploração levou a que os cavalos fossem expostos a longas jornadas de trabalho e condições inadequadas de manejo, causando efeitos deletérios na saúde animal. **Objetivo:** Determinar o efeito que o trabalho realizado pelos cavalos de carruagem tem na fisiologia, hematologia, bioquímica sanguínea e indicadores de estresse muscular. **Metodologia:** Foram realizadas jornadas de saúde em 60 cavalos de carruagem em condições normais de trabalho. A avaliação dos indicadores de estresse muscular foi realizada em um grupo de 12 animais (6 com descanso prolongado e 6 com atividade de transporte) com idade, peso, sexo, condição corporal e diagnóstico clínico similares. **Resultados:** 48% dos animais avaliados apresentaram medidas morfométricas dentro dos intervalos considerados normais. Os valores médios de bilirrubina total, bilirrubina direta, GOT, GPT e ALT em 100% dos animais avaliados estavam dentro dos valores de referência, exceto para FAS, enquanto 84% apresentaram valores de leucócitos e eritrócitos fora dos valores de referência. Ao comparar entre grupos, houve mudanças significativas nos valores séricos dos indicadores de estresse muscular ($P \leq 0.05$). A diferença nas concentrações plasmáticas de CK e LDH atingiu o pico entre 8 e 10 horas após a realização das atividades; no entanto, 28 horas após o trabalho (período de descanso) esses valores plasmáticos não diferiram estatisticamente. Por outro lado, foi encontrada uma correlação entre CK total e AST e ALT, e entre AST e ALT ($P \leq 0,05$, $r = 0,44$; $P \leq 0,05$, $r = 0,43$; $P \leq 0,01$, $r=0,60$). **Conclusão:** As variáveis fisiológicas e bioquímicas mostraram uma possível adaptação ao trabalho dos cavalos de carruagem, embora não fossem suficientes para diagnosticar um problema de bem-estar na maioria dos animais.

Palavras-chave: *bem-estar animal; biomarcadores; bioquímica sanguínea; cavalos; cavalo de tração; cavalo de trabalho; estresse muscular; saúde animal.*

Introduction

Since domestication, horses have been used for traction and transportation in rural and urban settings. According to Suárez *et al.* (2005), the use of horses for traction in developing countries is widespread due to their affordability and cultural significance. Cartagena de Indias is a well-known tourist city, where horse-drawn carriages have become one of the main means of transportation for visitors. However, carriage horses frequently face numerous challenges during their daily routines, including long workdays (10-12 hours/day), inadequate management conditions, and various health problems (Rimbaud *et al.*, 2006; Burn *et al.*, 2010). Furthermore, factors associated with horse maintenance (Pearson and Krecek, 2006) often result in compromised immune function. Caretakers, who generally have limited financial resources, prioritize family needs over proper horse management, exposing the animals to dietary deficiencies and restricted veterinary care, thereby increasing their susceptibility to disease. Consequently, hematological, physiological, metabolic, and immunological parameters in stressed horses may become significantly altered.

Biomarkers provide an economically feasible approach to monitoring horse health, as they reduce reliance on invasive procedures and can be assessed using blood samples collected during routine check-ups. Among the potential biomarkers for detecting chronic stress or pathology, creatinine kinase (CK) is widely used as an indicator of ATP hydrolysis in skeletal muscle fibers during sustained activity, reflecting intense oxidative and anaerobic metabolism, and serving to detect and monitor muscle damage in horses (Boffi *et al.*, 2010; Stopyra, 2002). Lactate dehydrogenase (LDH) is another valuable indicator of skeletal muscle integrity, with its elevation dependent on the type and intensity of exercise performed. Measurement of LDH facilitates differentiation between physiological and pathological responses to exercise (Guerrero *et al.*, 2009). Additionally, haptoglobin (HPT) has emerged as a sensitive and quantifiable protein marker, responding reliably to inflammatory stimuli in animals. Although previous studies have assessed physiological responses in horses during sporting activities (Hornemann *et al.*, 2003; Brancaccio *et al.*, 2007), data regarding traction horses under suboptimal management conditions remain limited.

Therefore, the present study aimed to evaluate the effects of work activity on physiological parameters and their association with muscle activity indicators in carriage horses from Cartagena de Indias.

Materials and methods

Ethical regulations

All animal care and handling procedures were approved by the Bioethical Management Committee in Experimentation and Practice with Animals of the Faculty of Agricultural Sciences, Universidad de Ciencias Aplicadas y Ambientales (UDCA), Bogotá, Colombia (Protocol No. 002/2022).

Location

The study was conducted in Cartagena de Indias, located at 10° 25' 30" N latitude and 75° 32' 25" W longitude. The city lies at sea level and has a humid tropical climate, with temperatures ranging from 28°C to 32°C. During data collection, average annual precipitation was 944.85 mm.

Population and sample collection

From 100 horses registered by the Municipal Unit for Agricultural Assistance in Cartagena, 60 were selected based on proper chip identification, adherence to work shifts (6–8 hours/day), and absence of lameness or clinical signs of pathology.

Sample collection was conducted during animal health sessions in September 2022 and May 2023. At the beginning of data collection, each horse was identified and underwent a clinical examination assessing variables such as body weight, body condition score (BCS), age,

and physiological parameters, including rectal temperature, heart rate (HR), respiratory rate (RR), arterial and venous pulses, and general evaluations of cardiocirculatory, respiratory, digestive, nervous, lymphatic, integumentary, mucous, and locomotor systems. All data were recorded in Microsoft Excel® for statistical analysis.

Hematological, biochemical, and immunological profile

Blood samples were collected via jugular venipuncture using Vacutainer® tubes with and without EDTA, stored on ice, and transported to the laboratory. Hematological profile (total erythrocyte count, hemoglobin concentration, mean corpuscular volume, mean corpuscular hemoglobin concentration, total leukocyte count, leukocyte formula, neutrophil/lymphocyte ratio, and platelet count) was determined using the Urit 3000 Plus automatic hematology analyzer. Biochemical parameters (total bilirubin, GOT, GPT, FAS, ALT) were analyzed using commercial diagnostic kits and the Hitachi 911 automatic analyzer (Roche Diagnostics, USA).

Plasma creatine kinase, lactate, lactate dehydrogenase, and haptoglobin

Two study groups of 12 animals each (6 control, 6 experimental) were formed, matched for age, weight, sex, body condition, and clinical status. The control group remained at rest to account for residual effect of tourist transport, while the experimental group continued their routine work. Venous blood samples were collected in heparin-coated tubes at six time points: H1 (rest, 0 h), H2 (3 h post-activity), H3 (7 h), H4 (10 h), H5 (16 h), and H6 (32 h). CK levels were measured by spectrophotometry, haptoglobin (HPT) concentrations using the Horse Haptoglobin ELISA Kit (Biotech Co., LTD), and lactate dehydrogenase (LDH) activity via the Stat Profile Critical Care Xpress automatic analyzer (Nova Biomedical Corp., Waltham, MA, USA).

Statistical analysis

For the population of 60 horses, descriptive statistics were calculated for age, body condition, body weight, heart rate, respiratory rate, rectal temperature, and hematological and biochemical parameters. For the 12 horses evaluated for stress indicators, chi-square tests assessed data distribution and prevalence, and Pearson correlation coefficients were determined via linear regression. Data normality was assessed using the Shapiro-Wilk test. For CK, LDH, and HPT, a completely randomized design was applied:

$$Y_{ijk} = \mu + D_i + (PC)_{ijk} + e_{ijk}$$

Where:

- Y_{ijk} = observed measurement
- μ = overall mean
- D_i = fixed effect of the ith sampling time
- $(PC)_{ijk}$ = fixed effect of body weight as covariate (when necessary)
- e_{ijke} = experimental random error

Least square means were compared using Fisher's least significant difference test. Results were considered significant at $P \leq 0.05$ and trending when $0.05 < P \leq 0.10$. All analyses were conducted using SAS 9.4 (Statistical Analysis System Institute, Inc., Cary, NC, USA).

Results

Physiological constants and morphometry

Descriptive statistics of the sampled animals are presented in Table 1. The mean values for height, age, body weight, and body condition score (BCS) were 148.04 cm, 6.45 years, 366.84 kg, and 3.07 points, respectively, showing moderate to high variability. The average height exceeded the minimum value considered necessary for traction work (140 cm), with only one animal falling below this reference. Regarding body condition, 14% of the horses exhibited a low BCS.

Table 1. Descriptive statistics of general characteristics and physiological variables of carriage horses in Cartagena.

	Mean	Minimum	Maximum	s.d	cv
General characteristics					
Height (cm)	148.04	115	158	6.43	4.34
Age (years)	6.45	3	14	2.1	32.52
Body Condition	3.07	2	4	0.37	12.31
Weight (kg)	366.84	280	484	46.02	12.54
Physiological Variables					
Heart rate[1]	41.19	32	58	7.08	17.2
Respiratory rate[2]	30.5	16	48	7.37	24.16
Rectal temperature[3]	37.74	36.6	38.7	0.48	1.29

[1]Measured in beats per min using a stethoscope placed on the left thorax.
[2]Measured in breaths per minusing a stethoscope on the trachea and hand near the nostrils.
[3]Measured with a digital thermometer inserted in the rectum for 1 min.

For HR, RR, and rectal temperature, the mean values were 41.19 bpm, 30.5 bpm, and 37.74 °C, respectively. No significant alterations were detected in most animals. Only 5% of the animals showed excitability during clinical examinations, which explained values above the normal range (32-40 bpm and 20-40 bpm for respiratory and heart rate, respectively) under the prevailing working and environmental conditions.

Biochemical profile and hepatic indicators

The hematological and hepatic profiles are shown in Table 2. Normality testing indicated that most hematological parameters followed a normal distribution, except segmented neutrophils, platelet count, and bilirubin. With respect to the biochemical profile, 84% of horses exhibited leukocyte and erythrocyte values outside the normal range.

Table 2. Hematological and biochemical values in carriage horses from Cartagena.

Parameters		Reference values*	Mean	s.d	Minimun	Maximum
Red Series	**Units**					
Hematocrit	%	32.11	33	3.19	28	40
Hemoglobin	g/dl	11.37	11.0	1.07	9.3	13.3
Erythrocytes	x 10^6/ ul	6.94	5.49	0.52	4.67	6.62
VCM	fl	50.23	60	0	60	60
CHCM	g/ dL	32.89	33.3	0	33.3	33.3
White Series						
Leukocytes	x 10^3/ ul	10.04	4.26	4.36	1.67	18.5
Segmented neutrophils	%	38.55	54.88	12.5	25	74

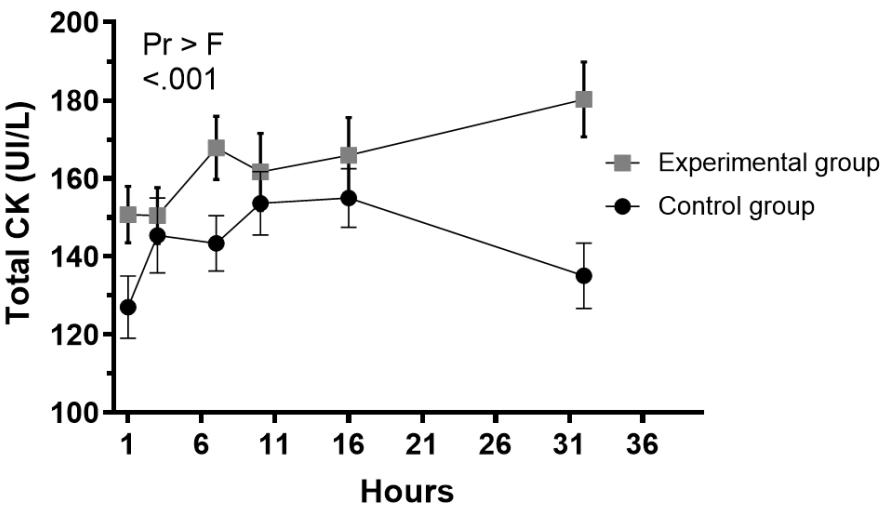
Lymphocytes	x 10^3/ ul	3.03	5.25	3.24	1.1	9.9
Monocytes	x 10^3/ ul	0.33	2.58	2.27	0.18	5.9
Eosinophils	x 10^3/ ul	0.37	3.65	2.99	0.1	8.8
Chemistry						
Total bilirubin	mg/ dL	1.33	3.8	1.86	2.44	9.95
GOT	UL/l	115.66	295.61	33.4	247	391
GPT	UL/l	15.94	13.22	3.17	9	21
FAS	UL/l	344.96	435.61	55.56	348	562
ALT	UL/l	2.57	16.83	5.96	9	30

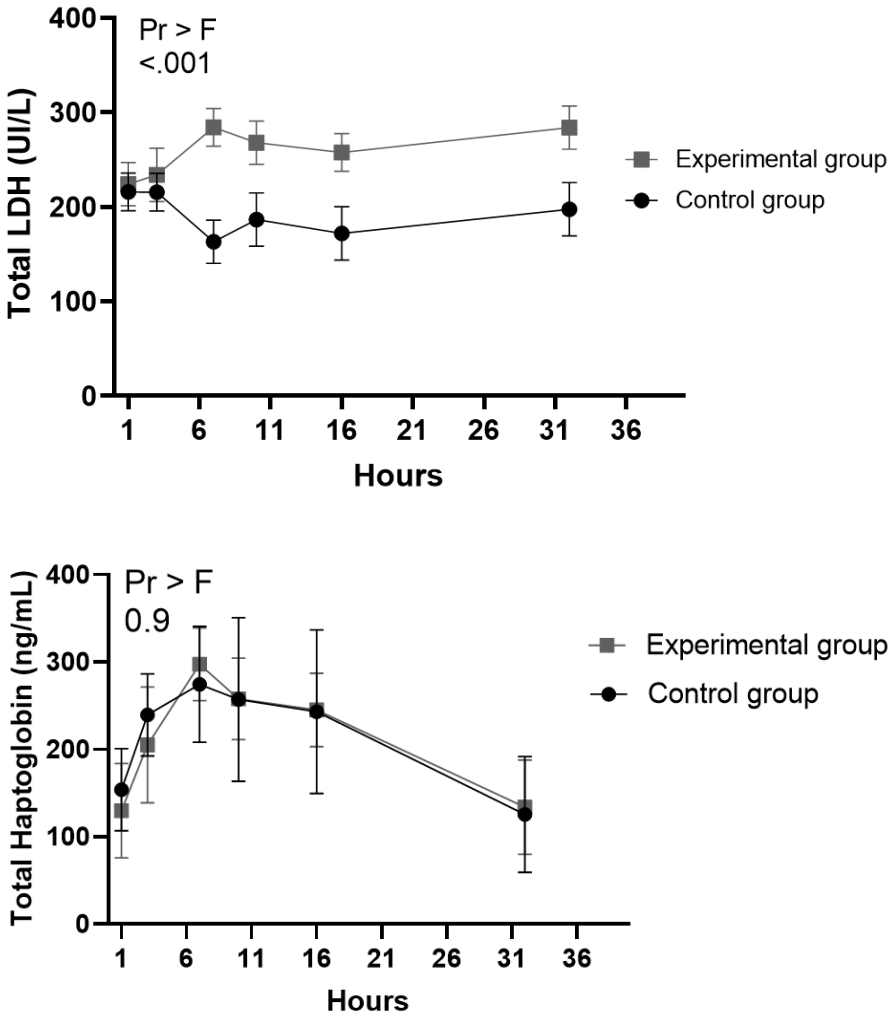
*Reference values for horses were obtained from Chica et al. (2022) and Chávez (2019).

The mean values of liver enzymes were 295.61 IU/L for GOT, 13.22 IU/L for GPT, 435.61 IU/L for FAS, and 16.83 IU/L for ALT. Eight animals had GOT and FAS values above normal ranges, though none presented clinical signs of organ dysfunction.

Muscle activity indicators

Mean serum concentrations of CK, LDH, and HPT in the control group (prolonged rest) were 138.38 ± 6.64, 228.69 ± 16.77, and 215.59 ± 29.26 U/L, respectively. In contrast, the experimental group (horses performing carriage work) showed mean values of 161.86 ± 6.64, 245.82 ± 16.77, and 211.54 ± 29.26 U/L, respectively. Significant differences between groups were observed when analyzed over time. Peaks in CK and LDH occurred 6–8 h after work (Figure 1). At 32 h post-activity, plasma values did not differ significantly from those of the control group. No significant differences ($p \geq 0.05$) were detected for HPT at any sampling time.

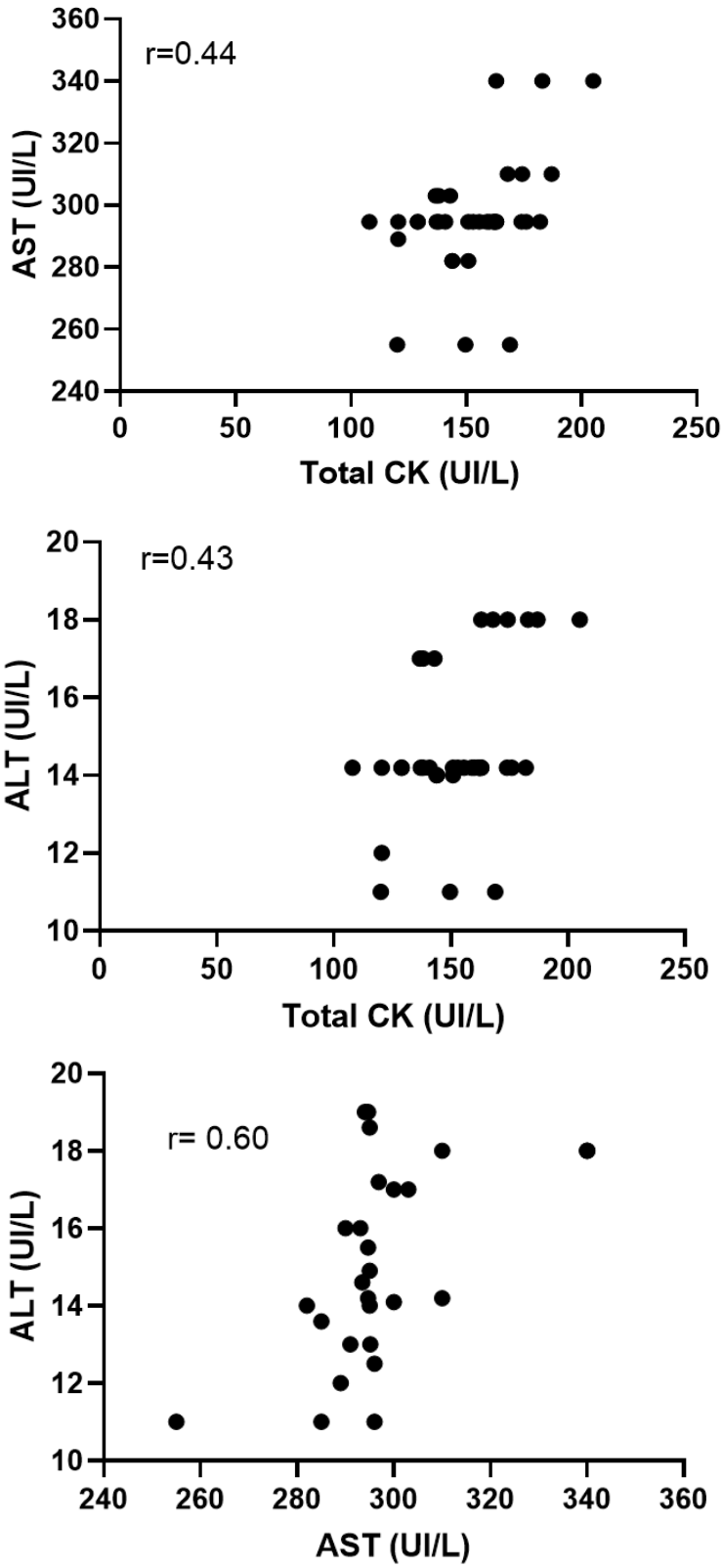




Abbreviations: *CK, creatine kinase; LDH, lactate dehydrogenase; Hp, haptoglobin.*

Figure 1.Concentration of CK, LDH, and Haptoglobin as indicators of muscle stress during work and rest days in carriage horses from Cartagena.

Correlation analyses revealed low-to-moderate positive associations between hepatic enzymes, skeletal muscle activity indicators, and biochemical parameters. Among the variables related to skeletal muscle activity, CK presented the strongest correlations with hepatic enzymes ($p \leq 0.05$). Multiple linear regression, with CK as the dependent variable, indicated moderate determination coefficients, but significant P values for AST and ALT ($P \leq 0.05$, $r = 0.44$ and $P \leq 0.05$) and between AST and ALT ($P \leq 0.01$, $r = 0.60$), respectively, supporting a meaningful relationship between the predictors and response variable (Figure 2).



Abbreviations: *ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine kinase.*
Figure 2.Correlation between creatine kinase (CK) and hepatic metabolites in carriage horses.

Discussion

Physiological constants and morphometry

The heart and respiratory rate values during the clinical examination (8 to 9 am) presented coefficients of variation considered high, which can be attributed in some animals to behavioral situations of excitement during their clinical assessment. It should be noted that these values were evaluated only once and not at the end of the animals' workdays. However, due to the adaptations to promote an adequate supply of oxygen to the muscular system, it would be expected that there was an increase between 3 and 4 times after the trips made and the car towing when compared with the values at the resting state.

Regarding morphometric measurements, 48% of the animals presented values between 150 and 160 cm. These data coincide with the parameters established by Garcia (2017) for animals carrying people or traction. In relation to body condition, 96% of the horses were evaluated as optimal, being similar to the values reported by Lagos *et al.* (2022). It should be noted that a good body condition reflects adequate nutritional management, based on chopped forage and supplements of corn grain with variable frequency, quantity, and quality. Despite the above, presenting this type of variable as optimal does not exempt animals from pathological alterations related to pain, injuries, and diseases (Garcia, 2017).

Health status in animals in Cartagena

Concerning biochemical and hepatic parameters, the values found support the idea that they may be signs of hemolytic anemia and leukopenia, possibly related to a nutrient imbalance (B complex vitamins, iron, among others) in the diet and a poor immune system. On the other hand, the values of each liver biomarker (except for FAS (435 ± 55.56) were found within the reference values considered normal reported for the species (Smith, 2010; Engelking, 2011). Furthermore, the data also coincide with those derived from Colombian Creole horses (Ruiz *et al.*, 2010).

Trend of biomarkers in horses

The use of inflammatory biomarkers in domestic animals has aroused great interest at the research level since they can help in the early diagnosis of different diseases (Eckersall and Bell, 2010). Therefore, the concentration of Creatine Kinase (CK), Lactate dehydrogenase (LDH) and Haptoglobin (HPT) was within the normal ranges reported by Tadichi *et al.* (1997) for carriage horses in Chile, which may be due to different variables such as environment, temperature, among others. When comparing the groups, it was found that serum CK and LDH values in animals subjected to routine work showed significant changes compared with animals that had prolonged rest. The 15% increase in serum activity for total CK in the routine work group ($\bar{x}$=180.2 IU/L) could indicate muscle stress or damage, given the severity of the exercise (due to the change in cell permeability) and metabolic acidosis related with the LDH concentrations found, as has been suggested in other studies (Rudolph *et al.*,1986).

Regarding LDH, several peaks were identified in animals subjected to routine work ($\bar{x}$= 284.09 IU/L), which decreased during rest periods ($P \leq 0.05$). These changes in LDH concentration indicate strong skeletal muscle metabolic activity caused by exceeding the muscle's energy supply capacity, forcing the tissue to resort to anaerobic glycolysis, producing lactic acid and thus raising its plasma concentration. Although LDH values return to normal 24 hours post-exercise, the high variation in the work intensity of the carriage horses could induce cellular damage, given the inefficiency of the compensation mechanism, which can also be supported by the increase in CK concentrations found in this study.

On the other hand, the HPT concentrations in both experimental groups were within the ranges considered normal for the species (200-1,000 µg/ml) according to Turlo *et al.* (2016). Although there was an increase in HPT concentration in the plasma between 8 and 10 hours in the animals subjected to work, there was no statistical difference when compared with those

maintained in prolonged rest ($P \geq 0.05$). This agrees with previous findings reported by Cywinska *et al.* (2014), who suggest that horses accustomed to performing frequent activities that generate physical effort tend to present significant increases in serum amyloid, while HPT levels vary little after activities. The above, added to the fact that there was no correlation between HPT and the hematological variables, supports the notion that the conditions of carriage horses are not stressful enough to detect an acute inflammatory response as previously reported (Cray and Belgrave, 2014).

The animals subjected to an activity after the rest period (experiment group) presented a slight increase in the variables related to hepatic enzymatic activity (10%, $P \leq 0.02$), slightly greater than that reported by Cywinska *et al.* (2014). This could be related to increased liver damage. However, it is considered that the variations in enzymatic activities between the two groups are not large enough to overcome the individual deviation of the enzymes, which may be caused by the conditioning/adaptation that the carriage horse population has to the activity carried out.

In conclusion, this study shows significant variability in the general and physiological characteristics of the horses evaluated, highlighting the importance of factors such as age, body weight, and body condition in their management and care. The biomarkers analyzed reflect a differential response to physical activity, emphasizing the adaptation of the horses to their work, although continuous monitoring is suggested to evaluate possible long-term effects on health and performance.

Declarations

Conflict of interest

No potential conflict of interest was reported by the author(s).

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Author contributions

JMM and MS were responsible for the design and conception of the study. JMM, JC, FG, PB, GO, AM, AF, TP, and DO were responsible for taking samples and data. JMM administered the project. JMM and DM wrote, reviewed, and edited the paper.

Use of artificial intelligence (AI)

No AI or AI-assisted technologies were used during the preparation of this work.

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