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SHORT COMMUNICATION

Use of thermography and pressure sensors to evaluate the effect of load on pack mules

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ABSTRACT. Mules performing pack work can develop skin wounds and lesions on harness related areas of the body, but also muscular and bone damage that are not always visible during clinical examination. Thermographic imaging and pressure sensors have emerged as non-invasive diagnostic alternatives that can provide valuable information about the welfare of working equids. The aim of this study was to assess the effect of different loads on the back of mules through thermographic images and pressure sensors. A crossover design was used with twelve mules carrying three different loads (80, 105, and 130 kg) for two kilometers. Four pressure sensors were placed in the harnessing system to assess the pressure (N) of the loads. Thermographic images of the back were taken daily before and up to five days after the harnessing work. The results show that the heavy loads (105 and 130 kg) generated a significant increase of temperature in all the analysed areas of the mules' back, with no significant differences between anatomical areas. The pressure sensors did not reveal significant differences between treatments or between anatomical areas and no evidence of a correlation between pressure and temperature. Further studies including physiological and behavioral measures to assess the effect of different loads are required to better understand the effect on working equids welfare.

Keywords: mule, load, pressure, inflammation, welfare, working equid.

INTRODUCTION

One of the main problems reported in working equids that carry loads on their backs (i.e. pack work) are those associated with wounds, injuries, and pain caused by the harnessing (Burn *et al.*, 2010; Sells *et al.*, 2010) affecting the mule's welfare and decreasing their efficiency and work capacity. The most common factors involved in the presentation of harness related injuries are those associated with body condition, age, weight of load transported, type of load, work distance, cleanliness of the harness, work frequency, design, and adjustment of the harness (Biffa & Woldemeskel, 2006; Norris *et al.*, 2020; Pritchard *et al.*, 2005). Usually, studies in working equids focus on the evaluation of back wounds, but absence of visible wounds does not imply absence of lameness or pain (Lesimple *et al.*, 2013). Therefore, it is important to evaluate the effect of load work on back health and its effects on welfare and performance. However, detecting equids back problems is difficult to perform based only on behavioural modifications, even for veterinary specialists (Lesimple *et al.*, 2013), and the use of complementary methods, such as ultrasound, radiography, and scintigraphy, are not always possible to perform under field conditions or for a large number of animals because of the cost and availability (Cauvin, 1997). This may mean that existing back injuries in working equids are likely to be underestimated (Lesimple *et al.*, 2013).

Several studies have evaluated the influence of riders and saddles on the thoracolumbar area of horses in equestrian sports, finding that incorrect positioning and poor adjustment of the saddle cause greater pressure on the spinous processes, especially in the area of the withers, which is sensitive to pressure, causing pain (Cauvin, 1997; Greve & Dyson, 2015). In addition, the weight and ability of the rider to balance the weight correctly, added to the level of symmetry of the equine's back, affect the pressure on the thoracolumbar region in a complex interrelation (Dyson, 2017; Greve & Dyson, 2015). Broster *et al.* (2009) evaluated the presence of pain in the thoracolumbar area in working horses, finding behavioural signs of pain in 75% of them. Considering that this measurement was made based on palpation only, using the the support of any complementary technique, it is expected that the diagnostic sensitivity would increase.

In recent years, technological advances have been developed in the diagnosis of equine back pain. One of them is the incorporation of thermography as a complementary diagnostic technique in clinical practice (Soroko & Howell, 2018). This non-invasive technique allows identifying areas of increased heat, facilitating the identification of inflammatory processes in the thoracolumbar area in horses (Talas & Talas Jr., 2017). Another method used to evaluate the adjustment of the saddles, weight distribution and balance of the rider is the use of electronic sensors that allow quantification of the force exerted on the horse's back, which allows specific information about the places and times when the back receives greater pressure to be obtained (Desbrosses-Déléage *et al.*, 2019). These techniques have the advantage of providing information that can be useful to assess

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inflammation, saddle adjustment and load balance in working equids. Therefore, the objective of this study was to evaluate the effect of load weight on the back of working mules using thermography and pressure sensors.

MATERIAL AND METHODS

Twelve adult working mules between 8 and 13 years and mean live body weight of 364 kg (292-497 kg), resulting from the cross of Baudet du Poitou, mixed Poitou, local cross breed Jack and mixed breed mares, were used for the study. All the mules belonged to the mountain detachment Nº3 "Yungay" of the Chilean army. Before the test, all mules were Clinically examined for health problems and body lesions. All individuals were sound at the time the evaluation was performed. Each mule used the same saddle harness model, a Schneider model (weight 23 kg), commonly used by the Chilean army. The harness had two belly webbing straps to keep it in place. A wool blanket and a piece of leather was used for padding. The saddle harness was checked and adjusted by a specialist from the Chilean army.

Three weigh-carrying treatments were used (80, 105 and 130 kg, including the harness weight). A crossover design was used where all mules carried each load for a 2 km outdoor circuit in a randomized order. The load consisted of gravel kept in sacks, distributed in two sacks for the 80 kg treatment evenly balanced on each side of the harness, and in three sacks for the 105 and 130 kg treatments, two sacks placed on either side of the harness and one on the top. The 2 km hand guided walk was conducted in November and December at the Army enclosure in a mountainous area of central Chile, with a temperature that fluctuated between 32 and 39ºC, average humidity of 24%, and at an altitude between 856 and 878 meters above sea level. The average speed was 4.6 km/h with a pace of 12.4 min/km. Obtained from a Garmin® forerunner 645 and an anemometer Benetech® GM816. There was a resting period of 10-12 days between different treatments.

Before and after each circuit, the mules were examined for the presence of wounds. Wounds bigger than 2 cm^2 or 1 x 3 cm on the harness area of skin contact were assessed (Popescu & Diugan 2013; Sells *et al.*, 2010). An image of the thoracolumbar region was taken using a thermographic camera Flir, E85 in an indoor area. The indoor area, the barn where mule stalls are located, was protected from solar radiation and wind. The camera was situated at 2 m over the mule's withers. Thermal images of the thoracolumbar region were taken at six sampling times: Time 0 (before work, at rest and before harnessing); Time 1 (ffter work, 10 min after unharnessing); Time 2 (2 h after unharnessing); Time 3 (24 h after unharnessing); Time 4 (72 h after unharnessing); and Time 5 (105 h after unharnessing).

During the complete sampling period, mules were kept inside the barn, in individual stalls.

For the thermographic analysis, the thoracolumbar region image was divided into four quadrants, two anterior/cranial (right and left) and two posterior/caudal (right and left), in order to evaluate symmetry. Furthermore, the average temperature of the dorsal midline and the wither were also analysed. The average, minimum, and maximum temperature of each area was obtained with the Flir® Software.

Four 5 x 5 cm pressure sensors were placed between the harness and the padding in each thoracolumbar quadrant, in order to assess the pressure of the carrying load and its balance. Before loading the mules, the sensors were calibrated to zero. Sensors registered the pressure every 3 to 3.5 seconds, then erratic data was eliminated and average, minimum and maximum pressure were analysed.

A field test was used to assess inflammation by using Serum Amyloid A (SAA) as a marker. For this, the semiquantitative EquiCheck™ ELISA test from Accuplex Diagnostics was used at three sampling points: before work, 72 h after work, and 120 h after work. A blood sample from the jugular vein was obtained at each sampling time and the sampler applicator from the kit was immediately used to add the blood to the test cassette as indicated in the instructions, the results were read after 10 minutes.

Statistical analyses were performed using SPSS Software (version 26, IBM, Armonk, NY, USA). Distribution of data was assessed with Shapiro Wilk Test. Analysis of variance was used to analyse differences in thoracolumbar temperature and an analysis of variance for repeated measures was performed to evaluate differences between sampling times and the post-hoc Dunn's test to identify differences with basal temperatures. To evaluate differences in pressure recorded in Newtons (N) between the four sensors analysis of variance was applied for each treatment and between treatments. In order to evaluate the relationship between the pressure recorded by the sensors and the thermography, a linear regression analysis was performed between the average pressure of the sensors and the images of each quadrant at times 1 and 2. Differences in inflammation degree within and between treatments were analysed with a Kruskal-Wallis non parametric test. Statistical significance was accepted for $P< 0.05$.

RESULTS AND DISCUSSION

One of the main welfare problems of working equids are the presence of wounds and injuries caused by saddles when carrying heavy loads on their backs (Sánchez-Casanova *et al.*, 2014; Sells *et al.*, 2010). Therefore, it is important to determine how well load is positioned on the animals back and the amount of load the animal can carry without causing harm. In the present study, the clinical examination did not detect the presence of wounds in any of the three treatments associated to the contact areas of the harnessing system. This can be explained due to the short distance travelled (2 km). In addition, it is important to consider that most evaluations of injuries in working equids have been conducted in individuals that maintain a constant job over time and for several hours a day (Sells *et al.*, 2010), while army mules perform sporadic work. However, thermographic images showed a significant increase in temperature for the four quadrants evaluated with the 105 and 130 kg treatments at 2 h (T2) and 24 h (T3) after the 2 km circuit had ended, compared with the baseline temperature, indicating the presence of inflammation in the area, which was confirmed with the SAA inflammation test where a significant increase of this acute phase protein was observed for the 130 kg treatment at 72 h after work (Figure 2). The SAA is a positive major acute phase protein in equids with low or undetectable concentrations in sound individuals, but with a rapid increase after infectious or non-infectious inflammatory stimuli, reason why it is used to monitor inflammation during colic episodes, respiratory disease, and inflammatory response to exercise (Long & Nolen-Walston, 2020). The SAA can rapidly increase the concentration with peaks between 24 and 48 h post inflammatory stimuli and returning to basal levels 15 days after (Hultén *et al.*, 2002). Athlete equines can exhibit an inflammatory response after exercise (Page *et al.*, 2017) that can be monitored by an increase in SAA associated to glycogen depletion in working muscles (Fallon *et al.*, 2001). For example, Cywinska *et al.* (2013) found significant increases after training in racing horses and in inexperienced endurance horses. One limitation of the present study is that the first sample to monitor SAA was taken 72h after work and may not reflect a more acute response in all treatments at 24 or 48 hours after exercise. Also, the position of the gravel sacks on the back of the mule for the 105 and 130kg treatments could have an effect since part of the load was on the top. We reproduced the way in which the army would distribute loads, but further studies are needed to understand how different distributions may affect pressure.

Overloading is one of the main welfare issues reported in working equids (Bukhari *et al.*, 2021), and calculation of load limits is a challenge since it depends on both animal related factors (i.e. species, age fitness to work) and also on external factors (i.e. environmental temperature and humidity, type of terrain and quality of harnessing system) (Lagos *et al.*, 2022). One limitation of the present study is that the circuit travelled by the mules was short (2 km) compared to the work performed by most working equids worldwide and that we did not include more physiological and behavioural indicators (for a complete review see Bukhari *et al.*, 2021). Nevertheless, this short work already revealed significant increases of back temperature and SAA of mules, returning to basal temperatures five days after work (Figure 1 and 2).

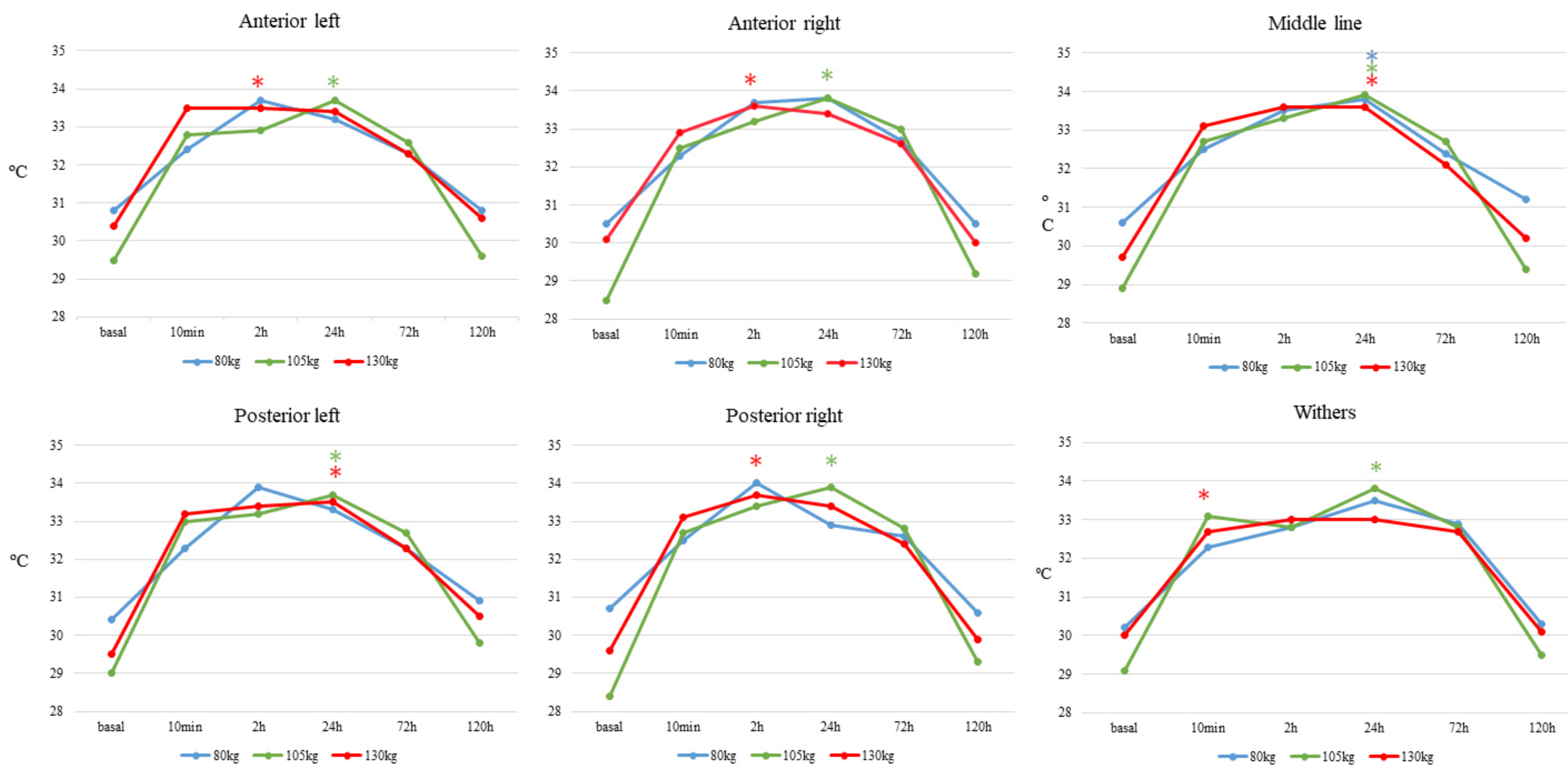
The asymmetric distribution of load on the back has been associated with difficulties in the functioning of the thoracic and lumbar area and the presence of pain or injuries (de Siqueira *et al.*, 2019; Dyson *et al.*, 2020). This asymmetric distribution often results from problems at the time of tacking, where the load is not well distributed or it is not well fixed, moving during the work. The results of the temperature and pressure analysis between the four quadrants in this study (Figure 1 and Table 1) did not report significant differences between them, suggesting that the weight distribution was symmetrical, which could be related to a correct strapping of the harness on each mule and to the fact that the loads located on each side had the same weight, were well tied and balanced, allowing a good weight distribution.

The withers area is one of the most injured areas in working equids (Sells *et al.*, 2010). In this study, although there were no visible wounds, an increase in temperature

Table 1. Mean, maximum and minimum pressure (N) detected by the pressure sensors according to their location on the back of the mules (FR: front right; FL: front left; PR: posterior right; PL: posterior left) and load treatment (80kg, 105kg and 130kg).

Variable	Sensor location	Load Treatment		
		80 kg	105 kg	130 kg
Mean pressure (N)	FR	2.23 ± 1.00	3.28 ± 1.24	2.74 ± 2.07
	FL	3.30 ± 2.11	2.78 ± 2.30	3.81 ± 2.80
	PR	2.62 ± 1.76	4.39 ± 2.96	3.49 ± 1.52
	PL	2.50 ± 1.46	1.89 ± 1.31	2.06 ± 0.78
Maximum pressure (N)	FR	5.42 ± 1.30	7.19 ± 3.42	7.53 ± 4.80
	FL	8.58 ± 1.84	10.0 ± 4.78	8.26 ± 2.53
	PR	13.68 ± 13.58	10.06 ± 6.14	10.01 ± 5.56
	PL	6.54 ± 4.33	7.78 ± 3.05	7.42 ± 4.14
Minimum pressure (N)	FR	0.78± 0.34	0.93 ± 0.10	0.72 ± 0.37
	FL	1.07 ± 0.60	0.58 ± 0.34	1.70 ± 2.98
	PR	0.69 ± 0.44	0.95 ± 0.61	0.64 ± 0.42
	PL	0.89 ± 0.27	0.66 ± 0.39	0.64 ± 0.38

Figure 1. Mean temperature (°C) for each anatomical region of the back of the assessed mules (anterior left, anterior right, posterior left, posterior right, middle line and withers) according to load treatment and time at which the thermographic image was obtained.



* Indicates sampling time at which a significant difference ($P<0.05$) with basal temperature was found. Color indicates the treatment for which the significant increase was reported.

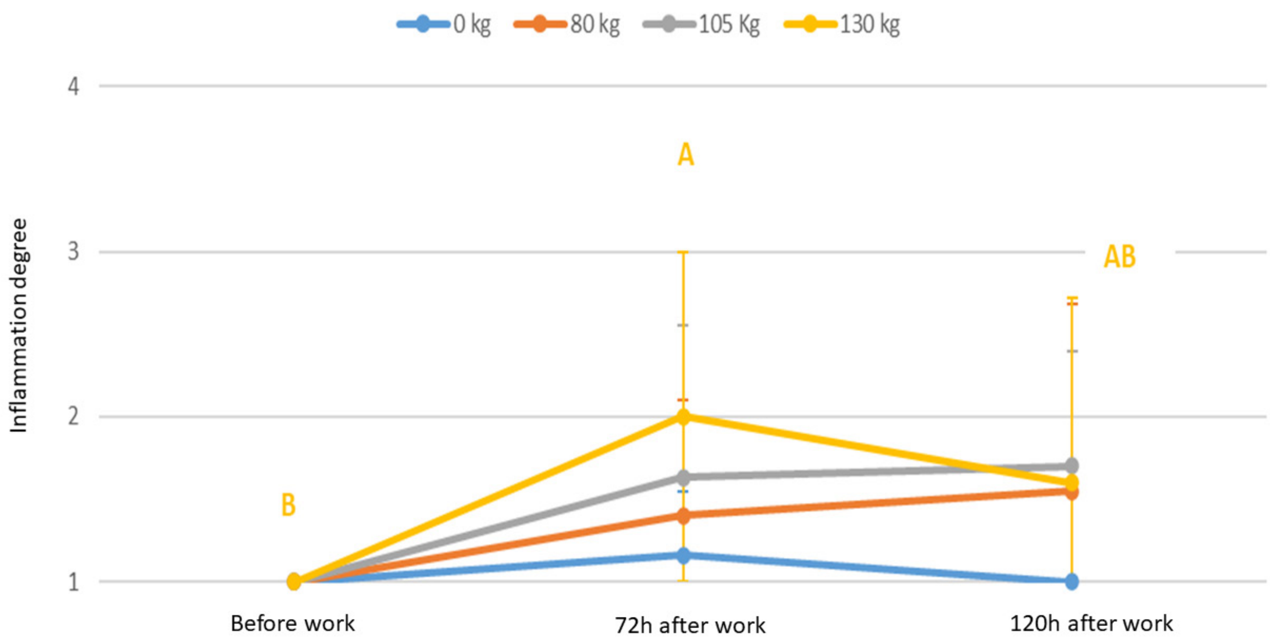


Figure 2. Average inflammation score (1= no inflammation; 4= severe inflammation) for the SAA semi-quantitative test according to time and treatment for the 12 mules in each load treatment. Different letters indicate significant differences (p<0.05) between sampling times for treatment 130kg compared to baseline before work.

for the 105 kg at 24 h (T3) and for the 130 kg treatment at 10 minutes (T1) after completing the circuit was found. These can be associated with an incorrect adjustment of the saddle too far ahead, generating greater friction (Arruda *et al.*, 2011) or being too loose making the load move towards the wither's area when walking downhill.

The dorsal midline is an area with few soft tissues, and it should not have direct contact with the shoulder strap; however, the dorsal midline presented an increase in temperature in all treatments 24 h (T3) (figure 1). This can be the result of the padding used, which is intended to help distribute the loads over a larger surface (Pearson *et al.*, 2003), causing friction, greater pressure in that area because of the characteristics of the padding, because it is not correctly positioned, as well as not having the necessary thickness to provide adequate protection. Another point to consider in the effect of pressure distribution is the level of training, muscular development, and symmetry of the back (Kotschwar *et al.*, 2010).

The 105 and 130 kg treatments caused an increase in temperature in different areas of the mules' back, while for the 80 kg treatment an increase in temperature was observed only in the dorsal midline (Figure 1). Other studies have evaluated the relationship between the rider's weight and the temperature in the horses back, finding that heavier riders cause significant increases in average temperature compared to riders of lower weight (Wilk *et al.*, 2020), as well as greater asymmetry (Michelotto *et al.*, 2016; Soroko *et al.*, 2018), injuries and behavioural changes associated with pain (Dyson *et al.*, 2020).

No significant differences were found between the treatments in the average, maximum and minimum pressure, and in the pressure values between the four sensors (P>0.05). Therefore, it is not possible to conclude that the weight of the load affects the pressure force or magnitude and the balance. Nevertheless, due to technical problems such as errors in the registrations and damage to the sensors or their cables, it was possible to obtain data for only six mules for the 80 kg and 105 kg treatments and for nine mules in the 130 kg treatment, thus the smaller sample size per treatment may have affected the ability to detect small differences. Roost *et al.* (2020) found that light riders (between 10 and 12% of the horse's weight) generate significantly less pressure than heavier ones, while very heavy riders (more than 20% of the horse's weight) cause more pressure on the caudal than cranial area of the horse. In this study, mules carried between 16% and 44% of their live body weight, but it has to be considered that, unlike a rider, the load used here does not move by its own, while riders are in constant movement creating vertical ground reaction forces (von Peinen *et al.*, 2009). In addition, other studies on pressure sensors have found asymmetries in the distribution of weight on the back of saddle horses (Meschan *et al.*, 2007). The riders' ability to maintain balance and posture has a relevant role on pressure and thermal activity in the back of saddle horses (Dittmann *et al.*, 2021; Gunst *et al.*, 2019). On the other hand, load carrying depends on the distribution of weight and fixation that it has on the harness, so the effects of this "dead load" could be different from those in riding horses. A recent study by Haddy *et al.* (2021) found that equids used for carrying loads have more wounds than those used for riding, this suggests that there would be differences in the impact generated on the back by a saddle and a harness.

No association was found between the thermographic images and the pressure obtained by the sensors. Only one previous study was found where the relationship between thermal activity and pressure on the back was evaluated with sensors and, with similar results as in this study (Mackechnie-Guire *et al.*, 2021). Thermography and pressure sensors have been used as a practical tool to assess saddle fit in equines, by measuring the magnitude, pressure distribution, increased metabolic activity, and skin blood flow (Desbrosses-Déléage *et al.*, 2019; Soroko *et al.*, 2018). However, in working equids, up to our knowledge, there are no previous studies that use these technologies to evaluate the impact of the load on the back of working equids. Still, a limitation of the present study is the small sample size and the short length of the circuit done by mules, since working equids are usually subjected to long working hours, and with lower quality of harnessing systems than those used by the army. Nevertheless, the results can be used as a preliminary step to better understand how different amounts of load can induce inflammation after a short period of work.

In conclusion, heavier loads, of 105 and 130 kg for 2 km, resulted in a significant increase in the superficial temperature in all the areas of the back evaluated in relation to basal values. For the heaviest load, the increase in temperature was detected earlier and was also associated to an increase of acute phase protein SAA. Nevertheless, it was not possible to establish a relationship between the measurements obtained by pressure sensors and thermography.

ETHICAL STATEMENT

This study was approved by the Institutional Animal Care and Use Committee of the Universidad de Chile, authorization number 18185-VET-UCH.

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