



Short Communication

Effects of intrarectal ozone administration on back pain sensitivity in healthy horses

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Abstract

Ozone therapy has been commonly used as an integrative approach for pain management, despite limited scientific evidence in equine medicine. The aim of this study was to evaluate the effects of intra-rectal ozone administration on back sensitivity in healthy dressage horses using infrared thermography and pressure algometry. Four horses, clinically fit to work, were included in a sequential clinical design consisting of a four-week control phase with intra-rectal oxygen administration, a six-week washout period and a four-week treatment phase with intra-rectal ozone. Ozone was administered at a dose of 15 mg per session (1000 mL at 15 mg/L). Assessments were performed weekly before and after each administration at predefined thoracolumbar regions. Thermography did not show significant changes in superficial temperature following ozone administration. In contrast, pressure algometry revealed a significant increase in the mechanical nociceptive threshold after ozone treatment, as well as a cumulative effect throughout the treatment period. Our results suggest that the intra-rectal ozone therapy may have analgesic potential for the control of equine dorsal, objectively assessed by pressure algometry, despite the absence of detectable thermographic changes.

Keywords: Back pain, Horses, Infrared thermography, Ozone therapy, Pressure algometry.

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INTRODUCTION

The equine back is a complex structure, initially described as a bridge between the forelimbs and hindlimbs and currently characterized biomechanically by the “arch-and-cord” theory (Jeffcot and Dalin, 1980). Back pain in horses has multiple identified causes (muscle tension, ligament injuries, spondylosis, ankylosis) (Jeffcot, 1980; Meehan et al., 2009; Murray et al., 2010; Barstow and Dyson, 2015), which can compromise the athletic performance of horses across different sporting disciplines (Denoix and Dyson, 2011).

Due to the absence of a gold-standard examination for assessing thoracolumbar injuries—which have a significant prevalence—thermography has become a highly useful tool (Masko et al., 2021), allowing localization of spinal injuries as well as soft tissue and spinous process affections (Masko et al., 2019). However, it is important to note that this technique has limited specificity and does not detect the etiology of lesions (Turner, 2018; Masko et al., 2019), and should therefore be used alongside other diagnostic methods such as radiography and ultrasonography (Soroko-Dubrovina and Morel, 2016).

Another diagnostic option is pressure algometry, a useful tool for evaluating musculoskeletal pain and sensitivity, proven suitable in both humans and horses, though its reliability depends on device calibration and the examiner’s experience and technique (De Heus et al., 2010). Using a single examiner for all measurements and incorporating an independent observer to read the values can help reduce errors (Haussler, 2020). The general method for using a portable algometer involves applying slow, constant pressure at a rate of 1 kg/s with the examiner’s dominant hand (Haussler and Erb, 2006).

A promising approach to treating back pain involves ozone therapy (Sampaio et al., 2018). However, results, dosages, and application sites vary. In humans, Figueiredo et al. (2025) applied ozone subcutaneously and intra-rectally in nine patients with sciatic or low back pain, with only one individual not experiencing pain reduction; the remaining patients reported a decrease from 7.56 ± 0.44 to 3.88 ± 0.99 on the pain scale. They also reported reduced use of anti-inflammatory and analgesic medications. The effectiveness of ozone in controlling low back pain in horses has also been previously described in clinical contexts. Ballardini (2005) evaluated local application of an oxygen–ozone mixture to the interspinous and paravertebral muscles of horses with mechanical low back pain, observing resolution of pain on palpation after treatment. Similarly, Melo and Ferreira (2021) conducted a study using intralesional application of ozonized platelet-rich plasma (PRP) to the *Longissimus dorsi* muscle, observing regression of clinical signs of low back pain and reduced pain on palpation.

Given the scarcity of data in the literature, the present study aimed to investigate intra-rectal ozone therapy on back pain sensitivity in healthy dressage horses through thermographic analysis and pressure algometry response. Although this protocol is adopted by field veterinarians, scientific validation is still required. The hypothesis under study is that ozone exerts an analgesic effect, reflected by an increase in sensitivity threshold in the pressure algometer test, associated with an increase in surface temperature due to its pro-inflammatory effect (linked to increased local vascularization).

MATERIALS AND METHODS

This research project was submitted for review to the CEBEA-FMV, Lusófona University and was approved under number 34/2024. Each horse was monitored for a period of approximately 14 weeks. Data were collected during the therapeutic protocol established by the veterinary team that routinely monitors the animals, indicating treatment for the prevention of back pain.

This study is a pre-post comparison study (longitudinal design), in which four healthy Lusitano male horses were selected, with a mean age of 16.25 ± 4.60 years and an average weight of 540 kg. All horses underwent a standardized selection process that included a complete physical and orthopedic examination performed by an experienced equine veterinarian to confirm clinical suitability for inclusion in the study. Inclusion criteria comprised active participation in dressage training and competitions, similar body condition score (3/5), dark skin pigmentation, and overall good health status, with no previous history of back pain, orthopedic disease, surgery, or back trauma. Horses showing clinical signs of back pain or musculoskeletal abnormalities during examination were excluded.

All animals belonged to the same riding school and were maintained under standardized management, housing, feeding, and exercise conditions throughout the study period. Horses participated in comparable equitation activities, with similar training frequency, intensity, and workload, aiming to minimize variability associated with physical conditioning and athletic performance. In addition, all horses were accustomed to handling procedures, the experimental environment, and the study protocols before data collection.

The animals belong to an equestrian center located in Caneças, Portugal, and were subjected to the same feeding and sanitary management conditions. They were kept in individual stalls (3.0 × 3.0 m) and fed an oat hay diet twice daily (8am and 5pm), commercial concentrate feed (Easy Mix, Intacol; containing 10.5% protein and 2,750 kcal of digestible energy), provided three times daily (9am, 12pm, and 6pm), and bran once daily, adjusted according to each animal's individual needs. Water was supplied through an automatic drinker and was available at all times.

In general, the horses underwent lunging exercise once weekly for 30 minutes. On five days per week, they performed advanced-level ridden training sessions lasting approximately 45 minutes to 1 hour. Horses were also allowed access to an outdoor paddock for 1 hour daily, always during periods opposite to their training sessions. Sundays were designated as rest days.

For the protocol, horses were properly restrained in a specific stock for containment, where they received intra-rectal oxygen (O₂) administration once a week, at an approximate volume of 1,000 mL, for a period of 4 minutes. After a six-week washout period, the same animals, again restrained in the stock, received intra-rectal ozone (O₃) administration once a week, at a volume of 1,000 mL and a concentration of 15 mg/L, for 4 minutes. For both administrations, an ozone generator device, model O&L 1.5 - RM, manufactured by Ozone & Life (São Paulo, Brazil), was used.

Throughout the entire protocol period, the animals received clinical monitoring to assess their general condition and maintained their regular physical activity routine at the equestrian center (where they were used in riding lessons for equestrian training). All procedures were done by morning, before their routine at the training center.

Before and immediately after each application, whether O₂ or O₃, thermographic images (infrared thermometer, IRT) of the horses' dorsal region were obtained, subdivided into 11 regions of interest (ROI). Eight markers were placed along the thoracolumbar spine of the horses, making it possible to identify the 11 ROIs. The first point was positioned at T7, the second between T12 and T13, the third at T18, the fourth at L3–L4, the fifth at L6–S1, the sixth at S4, and the seventh and eighth at each of the coxal tuberosities (Figure 1).

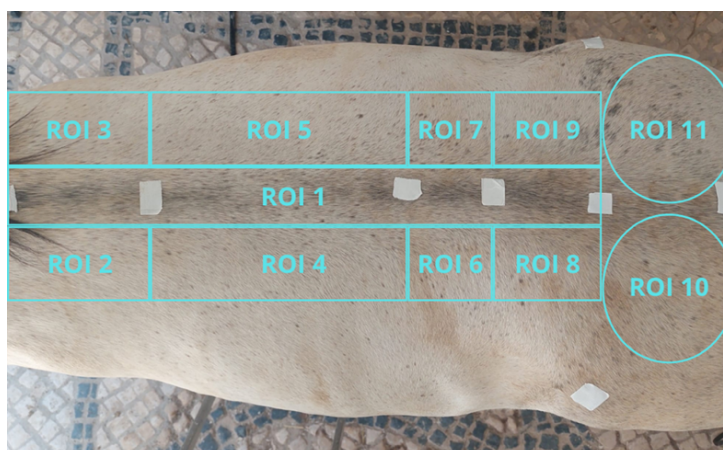


Figure 1 Image of the regions of interest (ROI) on the back of one of the horses used in the study.

ROI 1 was located vertically along the markers, in the region of the spinous processes. ROIs 2 and 3 were situated between the T7 and T12–T13 markers, identifying the spinalis and trapezius muscles. ROIs 4 and 5, delimited by the T12–T13 and T18 markers, corresponded to the *Longissimus dorsi* muscle. ROIs 6 and 7, delimited between the T18 and L3–L4 markers, identified the cranial portion of the middle gluteal muscle. ROIs 8 and 9, delimited between the L3–L4 and L6–S1 markers, identified the sacroiliac joint. Finally, ROIs 10 and 11, delimited between the L6–S1 markers and the coxal tuberosities, identified the middle gluteal muscles on each side.

The equipment used was the FLIR-C5 (FLIR Systems Incorporated, USA), with subsequent processing performed using FLIR Tools software. The program allows adjustment of the temperature scale of the images and extraction of minimum, mean, and maximum temperature values for each ROI. For the present thesis, only the mean temperatures were considered. The thermographic image was displayed through a thermal map, with temperatures shown by means of a color scale ranging from 16°C, in dark blue, to 38°C, in whitish coloration. The camera was positioned 1.5 m from the horse. The images were obtained with the horses in a quadrupedal position, relaxed and standing squarely, with all four limbs correctly positioned. Additionally, the images were taken always in the same place, at the left side of the animals, in an environment sheltered from direct sunlight and prior to any manipulation that could interfere with image recording, always by the same operator.

Immediately after obtaining the thermographic images, and using the same ROIs—with the exception of ROI 1—mechanical nociceptive thresholds were determined using a digital algometer (Wagner Pain Test FPX 100, Wagner, USA), equipped with a rubber tip measuring 1 cm² in diameter and calibrated within a range of 0–50 kgf/cm² (Haussler and Erb, 2006). Pressure was applied to the central point of each ROI at a constant rate of 10 kg/cm²/second for 2–3 seconds, until the first local reaction occurred that interrupted the measurement (local muscular contraction, cutaneous fasciculation, trunk movement, dorsal arching, body withdrawal, or other avoidance responses) and the device recorded the highest value (Figure 2). The readings were expressed in kgf/cm². Again, the evaluation was done always by the same operator and only once, as multiple measurements may cause habituation/desensitization or discomfort that interferes with the analysis (Pongratz and Licka, 2017).



Figure 2 Representative images of the use of the handheld algometer on the horse's back.

Statistical analysis was performed using computerized software (GraphPad Prism, version 10.3.0, GraphPad, Boston, USA). Variables were considered normally distributed based on the Shapiro–Wilk test ($p > 0.05$). To evaluate the effects of O_2 and O_3 administration on each ROI over time, data were analyzed using repeated-measures analysis of variance (ANOVA), considering treatment and time as fixed effects and horse as a repeated factor. When significant effects were detected, mean comparisons were performed using Tukey's post hoc test.

Comparisons of thermographic and pressure algometry measurements obtained before and after administration within the O_2 and O_3 groups were performed using the paired Student's t-test. In addition, comparisons between the overall mean values of the O_2 and O_3 groups were also conducted using the paired Student's t-test.

Results were expressed as mean $\pm$ standard deviation, and statistical significance was set at $p \leq 0.05$.

RESULTS

Important and significant changes in thermographic temperatures and pressure algometry were observed during the study period.

Temperature in each ROI was measured over the four weeks of monitoring during intra-rectal oxygen administration as shown in Table 1. There was no influence of oxygen treatment on the studied ROIs (PRE vs. POST) (Figure 3, A and B). However, over the weeks, it was possible to observe significant increases in the recorded temperatures, both in the PRE and POST measurements, with generally higher values between weeks 2 and 3 (time effect).

Table 1 IRT temperature (°C) (means and standard deviations) in the different regions of interest (ROI) over the four weeks of intra-rectal oxygen administration.

	Study period				p	
	S1	S2	S3	S4	Time	PRExPOST
ROI 1, °C						
PRE	28.0 ± 0.9 ^b	30.7 ± 1.0 ^a	30.8 ± 0.2 ^a	29.9 ± 0.8 ^a	0.0009	0.8989
POST	28.7 ± 0.9 ^c	29.5 ± 1.0 ^{bc}	31.0 ± 0.7 ^a	30.4 ± 1.0 ^{ab}	0.0037	
ROI 2, °C						
PRE	28.8 ± 0.6 ^b	30.2 ± 0.5 ^a	31.4 ± 0.5 ^a	30.3 ± 6.3 ^a	0.0006	0.9241
POST	29.5 ± 0.9 ^{bc}	29.4 ± 0.7 ^c	31.2 ± 0.5 ^a	30.5 ± 1.0 ^{ab}	0.0006	
ROI 3, °C						
PRE	28.1 ± 0.9 ^b	30.5 ± 0.8 ^a	30.5 ± 0.5 ^a	29.7 ± 0.9 ^a	0.0016	0.7970
POST	28.4 ± 1.2 ^b	29.2 ± 1.5 ^{ab}	30.8 ± 1.0 ^a	30.1 ± 0.8 ^{ab}	0.0221	
ROI 4, °C						
PRE	28.4 ± 1.0 ^b	31.5 ± 1.7 ^a	31.2 ± 0.5 ^a	30.4 ± 0.7 ^{ab}	0.0059	0.8290
POST	29.2 ± 0.8 ^b	30.3 ± 1.7 ^{ab}	31.2 ± 0.5 ^a	31.1 ± 1.1 ^{ab}	0.0353	
ROI 5, °C						
PRE	28.2 ± 1.0 ^b	31.3 ± 1.5 ^a	30.9 ± 0.4 ^a	30.2 ± 1.1 ^a	0.0033	0.8886
POST	28.9 ± 0.9 ^b	29.9 ± 1.4 ^{ab}	31.1 ± 0.9 ^a	31.0 ± 0.8 ^a	0.0180	
ROI 6, °C						
PRE	28.3 ± 1.1 ^b	31.3 ± 2.0 ^a	31.0 ± 0.5 ^a	30.2 ± 0.8 ^{ab}	0.0148	0.9632
POST	28.9 ± 0.9 ^b	30.0 ± 1.8 ^{ab}	31.0 ± 0.4 ^a	31.0 ± 0.8 ^a	0.0138	
ROI 7, °C						
PRE	28.0 ± 1.1 ^b	31.1 ± 1.8 ^a	30.6 ± 0.5 ^a	30.0 ± 1.0 ^{ab}	0.0053	0.9274
POST	28.6 ± 0.7 ^b	29.7 ± 1.1 ^{ab}	30.7 ± 1.0 ^a	30.6 ± 0.9 ^a	0.0238	
ROI 8, °C						
PRE	28.1 ± 0.9 ^b	31.1 ± 2.0 ^a	30.8 ± 0.3 ^a	29.9 ± 1.0 ^{ab}	0.0082	0.8295
POST	28.7 ± 0.7 ^b	29.6 ± 1.3 ^{ab}	31.0 ± 0.4 ^a	30.8 ± 0.7 ^a	0.0072	
ROI 9, °C						
PRE	27.7 ± 1.2 ^b	30.7 ± 1.8 ^a	30.5 ± 0.7 ^a	29.7 ± 1.0 ^{ab}	0.0038	0.8502
POST	28.2 ± 0.8 ^b	29.2 ± 1.0 ^{ab}	30.5 ± 0.8 ^a	30.4 ± 0.9 ^a	0.0010	
ROI 10, °C						
PRE	27.4 ± 0.6 ^b	30.0 ± 1.6 ^a	30.5 ± 0.3 ^a	29.4 ± 0.7 ^a	0.0008	0.8353
POST	27.9 ± 0.8 ^b	28.7 ± 1.2 ^{ab}	30.4 ± 0.8 ^a	30.0 ± 0.5 ^a	0.0080	
ROI 11, °C						
PRE	27.2 ± 0.9 ^b	30.1 ± 1.5 ^a	30.1 ± 0.7 ^a	29.4 ± 0.9 ^a	0.0005	0.8459
POST	27.9 ± 1.0 ^b	28.6 ± 0.9 ^{ab}	30.1 ± 0.9 ^a	29.9 ± 0.7 ^a	0.0105	

Note: Different letters in the same row indicate an effect of time (weeks) according to Tukey's test ($p \leq 0.05$); the "PRExPOST" column indicates significant differences between pre- and post-treatment evaluations according to the paired Student's t-test ($p \leq 0.05$). PRE, before the intra-rectal oxygen treatment protocol; POST, after the intra-rectal oxygen treatment protocol. S1: week 1; S2: week 2; S3: week 3; S4: week 4.

Table 2 presents the records of mean temperatures in each ROI over the four weeks of monitoring during intra-rectal ozone administration. Similar to what was observed with oxygen administration, ozone treatment did not significantly alter the mean temperature of the studied dorsal regions of the horses (PRE vs. POST) (Figure 3, C and D). However, over the weeks, significant reductions in temperatures were observed, both pre- and post-treatment, during weeks 2 and 3.

Table 2 IRT temperature (means and standard deviations) in the different regions of interest (ROI) over the four weeks of intra-rectal ozone administration.

	Study period				p	
	S1	S2	S3	S4	Time	PRExPOST
ROI 1, °C						
PRE	29.1 ± 0.6 ^{ab}	26.4 ± 2.1 ^b	28.4 ± 2.8 ^{ab}	30.5 ± 1.9 ^a	0.0245	0.9385
POST	30.3 ± 0.6 ^a	27.3 ± 0.8 ^b	27.3 ± 1.1 ^b	29.6 ± 0.5 ^a	0.0006	
ROI 2, °C						
PRE	29.5 ± 1.2 ^{ab}	27.6 ± 2.0 ^b	28.5 ± 1.5 ^{ab}	30.9 ± 1.9 ^a	0.0329	0.9816
POST	31.0 ± 0.7 ^a	28.3 ± 0.9 ^b	27.3 ± 0.8 ^b	29.8 ± 0.2 ^a	<0.0001	
ROI 3, °C						
PRE	28.9 ± 0.6	26.9 ± 2.3	27.8 ± 2.4	29.9 ± 1.9	0.0860	0.9683
POST	30.3 ± 0.6 ^a	27.3 ± 1.4 ^b	26.5 ± 0.7 ^b	29.5 ± 0.6 ^a	0.0002	
ROI 4, °C						
PRE	29.5 ± 0.6 ^{ab}	26.5 ± 2.5 ^b	28.7 ± 2.8 ^{ab}	31.1 ± 1.6 ^a	0.0209	0.9291
POST	30.6 ± 0.6 ^a	27.6 ± 0.6 ^b	27.4 ± 0.3 ^b	30.3 ± 0.8 ^a	<0.0001	
ROI 5, °C						
PRE	29.4 ± 0.5 ^{ab}	26.7 ± 2.1 ^b	28.4 ± 2.6 ^{ab}	30.3 ± 1.8 ^a	0.0376	0.9401
POST	30.5 ± 0.5 ^a	27.2 ± 1.2 ^b	27.3 ± 0.7 ^b	30.0 ± 0.7 ^a	0.0011	
ROI 6, °C						
PRE	29.3 ± 0.7 ^{ab}	26.8 ± 1.4 ^b	28.5 ± 2.5 ^{ab}	31.1 ± 1.7 ^a	0.0208	0.6363
POST	30.5 ± 0.6 ^a	27.3 ± 0.6 ^b	27.1 ± 0.6 ^b	29.8 ± 0.6 ^a	<0.0001	
ROI 7, °C						
PRE	29.0 ± 0.4	26.7 ± 1.2	27.7 ± 3.6	30.3 ± 1.9	0.0836	0.9066
POST	30.2 ± 0.7 ^a	27.1 ± 0.7 ^b	26.7 ± 1.0 ^b	29.4 ± 0.4 ^a	0.0004	
ROI 8, °C						
PRE	28.9 ± 0.9 ^{ab}	26.3 ± 1.2 ^b	28.1 ± 2.4 ^{ab}	30.6 ± 1.5 ^a	0.0136	0.5593
POST	30.1 ± 0.4 ^a	26.8 ± 0.4 ^b	26.7 ± 1.0 ^b	29.3 ± 0.6 ^a	0.0001	
ROI 9, °C						
PRE	28.7 ± 0.8 ^{ab}	26.0 ± 1.0 ^b	27.9 ± 2.2 ^{ab}	29.7 ± 1.0 ^a	0.0101	0.4681
POST	29.8 ± 0.5 ^a	26.4 ± 0.7 ^b	26.1 ± 1.1 ^b	28.9 ± 0.6 ^a	0.0001	
ROI 10, °C						
PRE	27.8 ± 0.9 ^{ab}	25.3 ± 1.1 ^b	27.3 ± 2.2 ^{ab}	29.8 ± 1.5 ^a	0.0041	0.7077
POST	29.3 ± 0.4 ^a	25.8 ± 0.6 ^b	25.9 ± 0.9 ^b	28.6 ± 1.0 ^a	<0.0001	
ROI 11, °C						
PRE	28.0 ± 0.8 ^a	25.2 ± 1.2 ^b	27.2 ± 2.2 ^{ab}	29.2 ± 1.5 ^a	0.0049	0.6496
POST	29.2 ± 0.3 ^a	25.7 ± 0.8 ^b	25.7 ± 0.8 ^b	28.2 ± 0.8 ^a	<0.0001	

Note: Different letters in the same row indicate an effect of time (weeks) according to Tukey's test ($p \leq 0.05$); the "PRExPOST" column indicates significant differences between pre- and post-treatment evaluations according to the paired Student's t-test ($p \leq 0.05$). PRE, before the intra-rectal ozone treatment protocol; POST, after the intra-rectal ozone treatment protocol. S1: week 1; S2: week 2; S3: week 3; S4: week 4.

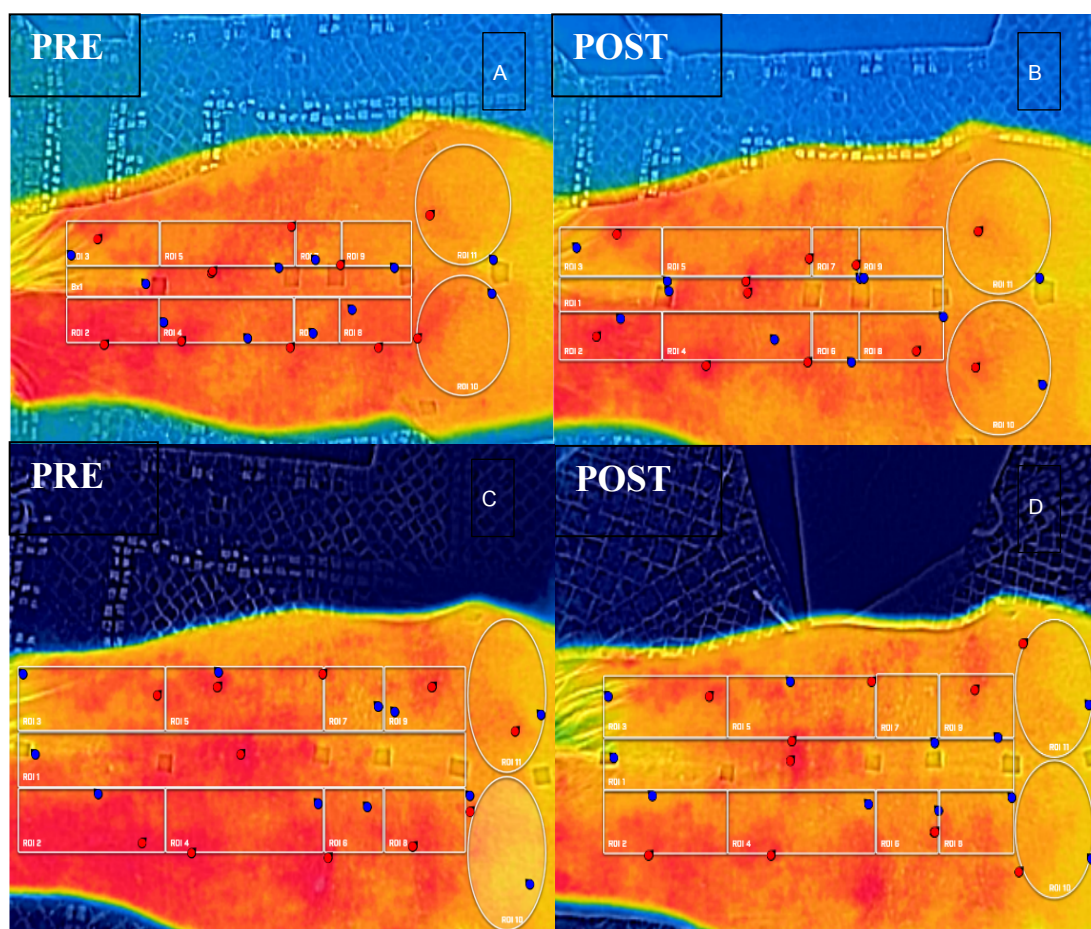


Figure 3 Representative thermographic images of the back of one of the horses, collected before and after intra-rectal oxygen administration (A and B) and ozone administration (C and D), during the first week of treatment, with the 11 ROIs identified and processed using FLIR Tools®.

Considering the overall mean of the ROIs, the findings reported in Tables 1 and 2 were confirmed; that is, the administration of oxygen and ozone did not significantly alter the mean dorsal temperature (Table 3). However, chronic changes were observed, with a significant increase in temperatures during weeks 2 and 3 of intra-rectal oxygen treatment, while ozone administration reduced temperatures during the same period (Figure 4, A and B).

Table 3 Overall mean temperatures of the regions of interest (ROI) and the environment (means and standard deviations) over the weeks of intra-rectal oxygen and ozone administration.

	Study period				p	
	S1	S2	S3	S4	Time	PRExPOST
Mean ROIs, °C - Oxygen						
PRE	28.0 ± 0.9 ^c	30.8 ± 1.4 ^a	30.8 ± 0.5 ^a	29.9 ± 0.8 ^b	<0.0001	0.9347
POST	28.6 ± 0.9 ^c	29.5 ± 1.2 ^b	30.8 ± 0.7 ^a	30.5 ± 0.8 ^a	<0.0001	
Local temperature, °C	28.0 ± 0.0	25.5 ± 0.6	29.0 ± 2.0	27.0 ± 2.6		
Mean ROIs, °C - Ozone						
PRE	28.9 ± 0.9 ^b	26.4 ± 1.7 ^d	28.0 ± 2.3 ^c	30.3 ± 1.6 ^a	<0.0001	0.4817
POST	30.2 ± 0.7 ^a	27.0 ± 1.1 ^c	26.7 ± 1.0 ^c	29.4 ± 0.8 ^b	<0.0001	
Local temperature, °C	25.0 ± 0.0	25.0 ± 0.0	25.0 ± 0.0	24.0 ± 0.0		

Note: Different letters in the same row indicate an effect of time (weeks) according to Tukey's test ($p \leq 0.05$); the "PRExPOST" column indicates significant differences between pre- and post-treatment evaluations according to the paired Student's t-test ($p \leq 0.05$). PRE, before the intra-rectal ozone treatment protocol; POST, after the intra-rectal oxygen treatment protocol. S1: week 1; S2: week 2; S3: week 3; S4: week 4.

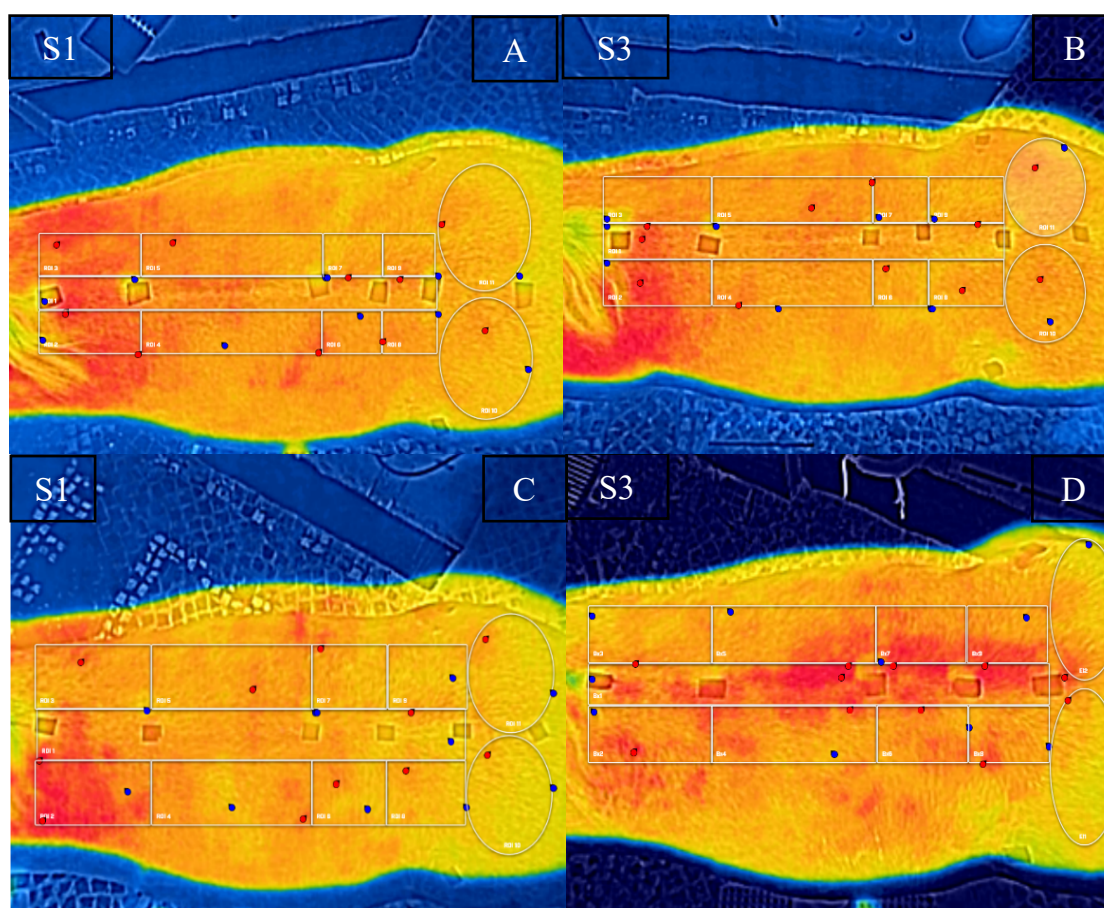


Figure 4 Representative thermographic images of the back of one of the horses, collected before the administration of oxygen (A and B) and ozone (C and D) via the intra-rectal route, during the 1st week (S1) and the 3rd week (S3) of treatment, showing the 11 identified ROIs and processed using FLIR Tools®.

Table 4 presents the records of pressure algometry performed in each ROI, with the exception of ROI 1, over the four weeks of monitoring during intra-rectal oxygen administration. Responses to applied pressure were altered by intra-rectal oxygen administration only in ROI 6 and ROI 11.

Table 4 Pressure algometry records (means and standard deviations), in kgf/cm², in the different regions of interest (ROI) over the four weeks of intra-rectal oxygen administration.

	Study period				p	
	S1	S2	S3	S4	Time	PRExPOST
ROI 2						
PRE	6.28 ± 0.78	6.43 ± 0.72	6.20 ± 0.66	6.28 ± 0.79	0.504	0.922
POST	6.35 ± 0.73	6.23 ± 0.86	6.40 ± 0.74	6.23 ± 0.77	0.518	
ROI 3						
PRE	6.50 ± 0.76	6.40 ± 0.78	6.28 ± 0.84	6.35 ± 0.99	0.719	0.191
POST	6.38 ± 0.85	6.35 ± 0.89	6.20 ± 0.82	6.40 ± 0.96	0.662	
ROI 4						
PRE	5.73 ± 1.10	5.65 ± 1.18	5.68 ± 1.32	5.80 ± 1.25	0.423	0.510
POST	5.65 ± 1.14	5.80 ± 1.28	5.63 ± 1.21	5.68 ± 1.22	0.140	
ROI 5						
PRE	5.73 ± 1.01	5.70 ± 1.16	5.73 ± 1.01	5.50 ± 1.06	0.063	0.411
POST	5.78 ± 1.00	5.73 ± 1.21	5.70 ± 1.15	5.55 ± 0.99	0.234	
ROI 6						
PRE	4.18 ± 1.07	4.30 ± 1.10	4.20 ± 1.05	4.35 ± 1.16	0.418	0.032
POST	4.30 ± 1.04	4.48 ± 0.97	4.20± 1.16	4.28 ± 1.19	0.135	
ROI 7						
PRE	4.10 ± 0.88	4.05 ± 1.04	4.08 ± 0.99	4.00 ± 0.94	0.759	0.108
POST	3.98 ± 0.92	4.08 ± 1.09	3.93 ± 0.98	4.03 ± 0.98	0.497	
ROI 8						
PRE	3.93 ± 0.70	4.15 ± 0.66	4.05 ± 0.70	4.10 ± 0.74	0.379	0.453
POST	4.00 ± 0.61	4.15 ± 0.74	3.93 ± 0.67	4.03 ± 0.70	0.313	
ROI 9						
PRE	3.98 ± 0.78	3.98 ± 0.66	4.08 ± 0.72	4.03 ± 0.70	0.357	0.510
POST	4.05 ± 0.62	4.05 ± 0.87	4.05 ± 0.66	4.00 ± 0.66	0.944	
ROI 10						
PRE	6.98 ± 0.74	6.98 ± 0.84	7.10 ± 0.72	7.08 ± 0.64	0.320	0.662
POST	7.10 ± 0.78	6.90 ± 0.88	7.13 ± 0.74	7.08 ± 0.52	0.472	
ROI 11						
PRE	7.15 ± 0.55	7.00 ± 0.73	7.08 ± 0.71	7.03 ± 0.59	0.271	0.048
POST	6.85 ± 0.58	6.93 ± 0.75	7.10 ± 0.65	6.93 ± 0.59	0.378	

Note: Different letters in the same row indicate an effect of time (weeks) according to the paired Tukey's test ($p \leq 0.05$); the "PRExPOST" column indicates significant differences between pre- and post-treatment evaluations according to the paired Student's t-test ($p \leq 0.05$). PRE, before the intra-rectal oxygen treatment protocol; POST, after the intra-rectal oxygen treatment protocol. S1: week 1; S2: week 2; S3: week 3; S4: week 4.

Table 5 presents the records of pressure algometry performed in each ROI, with the exception of ROI 1, over the four weeks of monitoring during intra-rectal ozone administration. Intra-rectal ozone application led to a significant increase in the pressure tolerated across all ROIs (PRE vs. POST). Additionally, a progressive increase in pressure resistance was observed for ROIs 6, 7, 8, 9, 10, and 11, with the highest values consistently recorded in the final week of the protocol.

Table 5 Pressure algometry records (means and standard deviations), in kgf/cm², in the different regions of interest (ROI) over the four weeks of intra-rectal ozone administration.

	Study period				p	
	S1	S2	S3	S4	Time	PRExPOST
ROI 2						
PRE	6.33 ± 0.70	6.35 ± 0.87	6.43 ± 0.77	6.53 ± 0.84	0.377	0.010
POST	6.38 ± 0.75	6.35 ± 0.83	6.60 ± 0.68	6.63 ± 1.02	0.277	
ROI 3						
PRE	6.50 ± 0.85	6.23 ± 1.03	6.75 ± 0.73	6.63 ± 0.83	0.448	0.036
POST	6.33 ± 0.79	6.30 ± 0.81	6.68 ± 0.85	6.70 ± 0.88	0.388	
ROI 4						
PRE	5.63 ± 1.16	5.78 ± 1.04	5.83 ± 0.97	6.00 ± 0.89	0.103	<0.001
POST	5.80 ± 1.08	5.75 ± 0.87	7.15 ± 0.87	6.05 ± 0.88	0.174	
ROI 5						
PRE	5.68 ± 1.06	5.80 ± 0.90	5.78 ± 0.85	5.95 ± 0.79	0.297	0.003
POST	5.83 ± 1.10	5.93 ± 0.88	6.08 ± 0.69	5.98 ± 0.92	0.668	
ROI 6						
PRE	4.25 ± 1.17 ^b	4.40 ± 1.06 ^{ab}	4.55 ± 0.99 ^{ab}	4.73 ± 1.04 ^a	0.013	0.007
POST	4.75 ± 0.83	4.78 ± 0.88	4.88 ± 0.83	4.88 ± 1.31	0.929	
ROI 7						
PRE	4.00 ± 0.91 ^b	4.35 ± 1.06 ^{ab}	4.48 ± 0.99 ^a	4.68 ± 1.02 ^a	0.005	0.017
POST	4.33 ± 0.77	4.65 ± 0.93	4.80 ± 0.79	4.80 ± 1.32	0.408	
ROI 8						
PRE	4.00 ± 0.59 ^b	4.40 ± 0.58 ^a	4.55 ± 0.45 ^a	4.73 ± 0.31 ^a	0.001	<0.001
POST	4.25 ± 0.41 ^b	4.75 ± 0.37 ^{ab}	5.03 ± 0.34 ^a	5.03 ± 0.73 ^a	0.020	
ROI 9						
PRE	4.03 ± 0.73 ^b	4.35 ± 0.60 ^{ab}	4.55 ± 0.45 ^{ab}	4.85 ± 0.19 ^a	0.007	<0.001
POST	4.18 ± 0.54 ^b	4.65 ± 0.45 ^{ab}	5.08 ± 0.13 ^a	5.20 ± 0.14 ^a	0.004	
ROI 10						
PRE	7.03 ± 0.82 ^b	7.10 ± 0.75 ^b	7.13 ± 0.68 ^b	7.43 ± 0.74 ^a	<0.001	0.018
POST	7.18 ± 0.59	7.38 ± 0.57	7.68 ± 0.95	7.55 ± 1.12	0.305	
ROI 11						
PRE	6.98 ± 0.75	7.13 ± 0.56	7.20 ± 0.67	7.30 ± 0.54	0.213	0.008
POST	7.05 ± 0.66 ^b	7.50 ± 0.55 ^{ab}	7.78 ± 0.83 ^a	7.45 ± 0.73 ^{ab}	0.016	

Note: Different letters in the same row indicate an effect of time (weeks) according to Tukey's test ($p \leq 0.05$); the "PRExPOST" column indicates significant differences between pre- and post-treatment evaluations according to the paired Student's t-test ($p \leq 0.05$). PRE, before the intra-rectal ozone treatment protocol; POST, after the intra-rectal ozone treatment protocol. S1: week 1; S2: week 2; S3: week 3; S4: week 4.

Intra-rectal ozone treatment led to an increase in pressure tolerance measured by the algometer, a result not observed during oxygen treatment (Table 6). Additionally, a progressive increase in dorsal algometry was observed over the course of the study.

Table 5 Pressure algometry records (means and standard deviations), in kgf/cm², in the different regions of interest (ROI) over the four weeks of intra-rectal ozone administration.

	Study period				p	
	S1	S2	S3	S4	Time	PRExPOST
Mean ROIs - Oxygen						
PRE	5.45 ± 1.45	5.46 ± 1.43	5.45 ± 1.43	5.44 ± 1.44	0.927	0.378
POST	5.44 ± 1.41	5.47 ± 1.41	5.43 ± 1.48	5.42 ± 1.43	0.593	
Mean ROIs - Ozone						
PRE	5.44 ± 1.45 ^d	5.59 ± 1.33 ^c	5.72 ± 1.28 ^b	5.88 ± 1.24 ^a	<0.001	<0.001
POST	5.82 ± 1.31 ^b	5.80 ± 1.23 ^b	6.07 ± 1.26 ^a	6.03 ± 1.29 ^a	<0.001	

Note: Different letters in the same row indicate an effect of time (weeks) according to Tukey's test ($p \leq 0.05$); the "PRExPOST" column indicates significant differences between pre- and post-treatment evaluations according to the paired Student's t-test ($p \leq 0.05$). PRE, before the intra-rectal treatment protocol; POST, after the intra-rectal treatment protocol. S1: week 1; S2: week 2; S3: week 3; S4: week 4.

Relative increases in nociceptive threshold values were calculated for the ROIs. The greatest responses were observed between the thoracolumbar and lumbosacral regions, with increases of 8.9% in ROI 6, 7.0% in ROI 7, 8.3% in ROI 8, and 7.8% in ROI 9. The most cranial regions of the back (cranial to T18) showed smaller relative increases, ranging from 1.6% to 2.7%. Finally, relative increases ranging from 3.9% to 4.2% were observed in the gluteal region (ROI 10 and 11).

All horses showed no side effects, pain or discomfort behavior before, during and after the procedures. Also, they performed their regular activities and their training protocol normally throughout the protocol.

DISCUSSION

It was proposed in the present investigation that intra-rectal ozone administration in horses would lead to increased pressure tolerance measured by algometry due to its analgesic effect, in addition to elevations in superficial temperatures in the same regions of interest, as a result of its inflammation-modulating action. However, only the increase in resistance to painful stimuli was confirmed.

Infrared thermography has been widely described as an effective, non-invasive method for detecting changes in superficial blood perfusion and tissue metabolic activity, indirectly reflecting alterations associated with inflammatory, muscular, and vascular processes (Soroko-Dubrovina and Morel, 2016; Masko et al., 2021). Nevertheless, it is a non-specific technique, since any change in microcirculation, muscle metabolism, tissue thickness, or sympathetic activation can result in variations in surface temperature (Turner, 2018). In the present study, thermography proved useful for reflecting thermal differences between the evaluated ROIs and for monitoring temporal variations throughout the treatment weeks. It is important to note that, as recommended by Soroko-Dubrovina and Morel (2016) and Turner (2018), all images were always obtained by the same operator, in an environment protected from direct sunlight, and prior to any physical manipulation of the animals, minimizing the influence of external factors on thermographic recordings and reinforcing the methodological validity of the protocol.

One of the study's hypotheses was that temperatures in the studied ROIs would increase with intra-rectal ozone administration. However, this did not occur. This absence of a thermal response may be explained by several factors. The intra-rectal route predominantly produces systemic effects through absorption via the hemorrhoidal plexus, which may not translate into peripheral changes intense enough to be detected by superficial thermography (Bocci, 2011). Second, in addition to inducing NO-mediated vasodilation, O₃ simultaneously activates

antioxidant and cytoprotective mechanisms via the Nrf2 pathway, which may modulate and limit the magnitude of a detectable peripheral inflammatory response (Re et al., 2014; Galiè et al., 2018). Additionally, interpretation of thermal patterns must consider individual physiological variability, as factors such as local perfusion, tissue metabolism, and anatomical characteristics can influence surface thermal distribution (Turner, 2018), attenuating the ability to detect subtle thermal changes induced by O₃. Similarly, intra-rectal O₂ administration also did not produce detectable changes in temperatures across the different ROIs.

Overall, an increase in surface temperature in the different ROIs was observed during weeks 2 and 3 of the intra-rectal O₂ phase, whereas there was a tendency toward thermal reduction during the same weeks of O₃ treatment. Since no significant environmental variations were recorded to justify these changes, this pattern may reflect physiological differences between the long-term systemic effects of O₂ and O₃. Intra-rectal O₂ administration can increase systemic oxygen availability, enhancing muscle metabolism and peripheral perfusion, which translates into increased surface temperature (Sagai and Bocci, 2011). Conversely, O₃ induces a progressive antioxidant and anti-inflammatory adaptive response via Nrf2 activation (Sagai and Bocci, 2011) and modulation of pro-inflammatory cytokines (Seyam et al., 2018). This regulatory effect may have led to metabolic and vascular stabilization over the weeks, resulting in a relative reduction in surface temperature. This suggests that ozone has a cumulative effect, effectively acting against inflammatory processes from the second week of treatment.

In contrast to thermography, pressure algometry revealed a significant positive response to O₃ treatment consistently in all four horses. In all ROIs, significant increases in pressure tolerance were detected after intra-rectal O₃ administration, representing an acute effect not observed during the O₂ treatment period. This result aligns with human literature, where ozone therapy has been shown to significantly reduce low back sensitivity as measured by subjective and functional scales (Biazzo et al., 2018; Llombart-Blanco et al., 2024; Figueiredo et al., 2025). Proposed mechanisms include modulation of pro-inflammatory cytokines (TNF- α , IL-1 β) and regulation of NO production (Re et al., 2008), activation of the Nrf2 pathway (Galiè et al., 2018), reduction of chronic oxidative stress (Sagai and Bocci, 2011), and modulation of nociceptive neurotransmission (Seyam et al., 2018). Literature indicates that ozone can modulate inflammatory mediators, including pro-inflammatory cytokines, contributing to the reduction of peripheral nociceptive sensitization (Re et al., 2008; Sagai and Bocci, 2011). Additionally, it induces mild, transient oxidative stress that activates endogenous antioxidant pathways via Nrf2, promoting progressive redox adaptations (Re et al., 2014). Regulation of NO production can improve microcirculation and tissue oxygenation (Sagai and Bocci, 2011), while peripheral inflammatory mechanisms contribute to pain modulation by attenuating nociceptive sensitization (Seyam et al., 2018).

The study by Jaramillo et al. (2020) provides relevant pathophysiological support for the present results, demonstrating that intra-rectal ozone can induce systemic effects associated with improved tissue oxygenation and metabolism. These mechanisms reinforce the physiological plausibility of the increased nociceptive threshold observed in algometry, even in the absence of detectable surface thermal changes. While not allowing for definitive clinical conclusions, this effect may help explain the growing interest in ozone therapy as a complementary strategy in pre-competition preparation of sport horses.

Interestingly, the region between T18 and L6–S1 showed the greatest percentage increases in back nociceptive threshold. Although the intra-rectal route is considered a systemic modality of ozone therapy, due to gas absorption through the hemorrhoidal plexus and subsequent systemic distribution, as previously described, the observed response was not homogeneous across all evaluated back regions. This finding suggests that the biological effects of ozone depend not only on its systemic distribution but also on the specific physiological and

neurofunctional characteristics of each anatomical region. Furthermore, the thoracolumbar and lumbosacral regions are frequently more predisposed to biomechanical overload, particularly in athletic horses, and may concentrate areas of muscular tension and hyperalgesia. Therefore, the increased nociceptive threshold observed in these areas may reflect a greater therapeutic responsiveness in previously sensitized tissues.

Beyond the acute effect, a progressive and significant increase in mechanical nociceptive thresholds was observed in the PRE evaluation measurements of each week for ROIs 6, 7, 8, 9, and 10, with maximal values reached in week 4, suggesting a cumulative analgesic effect. This is consistent with the concept of oxidative hormesis, in which repeated exposure to mild oxidative stimuli induces a progressive adaptive response, characterized by a sustained increase in endogenous antioxidant capacity and modulation of inflammatory processes (Sagai and Bocci, 2011; Re et al., 2014). In this context, O₃ acts not as a pro-inflammatory agent but as an inducer of cytoprotective mechanisms that promote redox homeostasis and regulate nociceptive response over time. Tsuzuki et al. (2015) demonstrated that ozonated autohemotherapy significantly increases systemic antioxidant capacity and reduces oxidative stress indices, with effects becoming evident only a few days after administration and persisting for a prolonged period. These results suggest that the biological effects of ozone are not exclusively immediate but result from progressive systemic adaptation, consistent with the gradual increase in nociceptive threshold observed in this study. Comparable results have been described in humans undergoing repeated ozone therapy protocols for low back pain, with improvements in clinical pain parameters (Biazzo et al., 2018; Figueiredo et al., 2025), while in horses subjected to systemic ozone therapies, progressive antioxidant adaptations compatible with cumulative biological responses have been observed (Tsuzuki et al., 2015).

The chosen protocol was based on adjustments from previous publications (Coelho et al., 2015), professional experience, and personal communications with veterinarians. According to the literature, O₃ biological effects depend on a specific therapeutic window (Bocci, 2011; Sciorsci et al., 2020), in which low to moderate doses induce mild, transient oxidative stress capable of triggering adaptive antioxidant responses and inflammatory modulation (Sagai and Bocci, 2011; Re et al., 2014), whereas excessive doses can be irritative or toxic (Bocci, 2011). Accordingly, a biologically safe range was selected, consistent with the aim of modulating musculoskeletal pain without inducing excessive inflammatory responses.

Regarding the administration route, intra-rectal application is described as one of the main methods of ozone therapy when a predominantly systemic effect is desired with minimal invasiveness (Bocci, 2011; Sciorsci et al., 2020). Through this route, absorption occurs via the intestinal mucosa and hemorrhoidal plexus, allowing O₃ reaction products—such as hydrogen peroxide and lipid peroxidation products—to enter systemic circulation and act as redox mediators capable of triggering biological responses at a distance (Bocci, 2011; Sagai and Bocci, 2011). In horses, available studies suggest good tolerability of the transrectal route, with a favorable safety profile. Jaramillo et al. (2020) reported no adverse clinical changes or significant systemic inflammation after repeated O₃ administrations via this route, reinforcing its applicability in experimental and clinical settings.

Despite these results, the present study has important methodological limitations that should be considered in data interpretation. The main limitation is the small sample size ($n = 4$), which compromises statistical power and limits generalization to the global population of dressage horses. Small-sample studies are particularly susceptible to inter-individual variability and uncontrolled confounding effects (Button et al., 2013). However, a within-subject design was adopted to provide greater internal validity, eliminate inter-subject variability, reduce sample size requirements, and allow direct comparison of pre- and post-

intervention states, which is crucial for isolating the specific effects of the studied therapy and for ensuring animal welfare.

Regarding intervention sequence, intra-rectal O₂ was administered before O₃, without a true placebo group or crossover randomization. This decision was made because the literature reports that O₃ can induce cumulative and prolonged effects associated with progressive activation of antioxidant and anti-inflammatory mechanisms (Sagai and Bocci, 2011; Re et al., 2014). This approach aimed to minimize carry-over effects of O₃ on the control phase, ensuring that data collected during O₂ administration predominantly reflected its isolated effect. Similarly, human studies on low back pain report progressive clinical improvements over repeated ozone therapy cycles (Biazzo et al., 2018; Llombart-Blanco et al., 2024), suggesting a cumulative component beyond the immediate effect. Therefore, the O₂ followed by O₃ sequence, combined with an adequate washout period, was chosen to ensure clearer interpretation of the observed effects.

The absence of a blinded evaluator is also a potential source of bias, particularly in pressure algometry, whose reliability partly depends on the technique and consistency of the examiner (De Heus et al., 2010; Haussler, 2020). This was mitigated by having the same trained operator perform all procedures, both in thermographic imaging and algometry assessments.

Additionally, the study evaluated only short-term effects, without a prolonged follow-up to determine the durability of the observed analgesic effect. The lack of correlation with other complementary assessment methods, such as imaging or functional movement analyses, also limits overall interpretation, as multimodal approaches are frequently recommended in assessing equine low back pain (Haussler, 2020). Finally, systemic inflammatory or oxidative biomarkers were not evaluated, which would have allowed a more direct characterization of the underlying pathophysiological mechanisms of the observed effects (Galiè et al., 2018).

Both thermography and pressure algometry provide indirect and semi-quantitative assessments of musculoskeletal discomfort and inflammation, primarily reflecting superficial or functional changes rather than direct measures of deep tissue pathology. However, these techniques were selected because they are non-invasive, repeatable, and field-applicable, allowing for objective assessment under real training conditions while ensuring animal welfare. At present, there are not clinically feasible, non-invasive tools capable of directly diagnosing active deep musculoskeletal inflammation in equine athletes under field conditions.

The use of oxygen administration as a control, together with an adequate washout period, helped minimize potential residual effects and reinforced the methodological robustness of the experimental design. The choice of the intra-rectal route, as well as the ozone volume and dose, proved safe and well-tolerated by the tested horses, in agreement with previous publications.

CONCLUSIONS

The present study aimed to evaluate the effects of intra-rectal ozone therapy on the back sensitivity threshold of healthy dressage horses, using infrared thermography and pressure algometry as complementary assessment methods. Thermographic analysis did not show evidence of significant changes in surface temperature in the regions of interest associated with ozone administration, failing to confirm the hypothesis of an acute peripheral thermal response. These findings suggest that the intra-rectal route predominantly produces systemic effects, which may not translate into detectable superficial thermal changes. However, the reduction in temperature observed after the second week of treatment points to potential chronic anti-inflammatory effects.

In contrast, pressure algometry demonstrated a significant increase in mechanical nociceptive threshold following intra-rectal ozone administration,

indicating an immediate (acute) analgesic effect. Additionally, a cumulative effect was observed over the weeks of treatment in some of the studied regions of interest, with progressive increases in nociceptive thresholds measured during pre-administration assessments, suggesting a chronic pain-modulating effect. These results indicate a possible analgesic action of intra-rectal ozone therapy, possibly mediated by inflammatory, antioxidant, and nociceptive modulation mechanisms described in the literature.

Despite the limitations related to the small number of animals included, the results of this study suggest that intrarectal ozone insufflation may increase the dorsal sensitivity threshold in healthy horses, indicating a potential modulatory effect on nociceptive response. Therefore, this approach may represent a promising integrative strategy for the management of back pain in horses. Nevertheless, further studies involving larger populations and animals with clinical back pain are necessary to confirm these findings and better establish the therapeutic applicability of this technique.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Ana Cristo: Investigation (Lead), Conceptualization (Equal), Formal Analysis (Equal), Methodology (Equal), Data curation (Equal), Writing – review & editing (Equal).

Maria Inês Farrim: Data curation (Supporting), Writing – original draft (Equal), Writing - review & editing (Supporting).

Inês Carvalho: Methodology (Supporting), Investigation (Supporting).

Mafalda Pardal: Methodology (Supporting), Investigation (Supporting).

Carolina Nascimento: Conceptualization (Equal), Methodology (Equal), Data curation (Equal).

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