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**Original Research** 

# A Controlled, Blinded Study Investigating the Effect That a 20-Minute Cycloidal Vibration has on Whole Horse Locomotion and Thoracolumbar Profiles

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## ABSTRACT

In man, vibration therapy has been shown to be of benefit both in a clinical and sporting context, although in its infancy in the horse, similar benefits have been reported. To evaluate the immediate effect that cycloidal vibration therapy has on thoracolumbar range of motion and thoracolumbar muscular dimensions, 30 nonlame horses (mean  $\pm$  SD age 12  $\pm$  8.77 years, mean  $\pm$  SD height 1.65  $\pm$  0.94 m) were split into two groups: treatment group and placebo group. Horses were equipped with eight inertial sensors. A treatment group received a 20-minute cycloidal vibration therapy and a placebo group received no treatment. Differences in thoracolumbar epaxial musculature dimensions were obtained pre and post for both groups. A paired t test was carried out to determine differences in movement and thoracolumbar musculature dimensions (P < .05) for both groups. In the treatment group, an increase in range of motion of the wither in a vertical direction (Pre 69:00  $\pm$  8.77 mm, Post 70.84  $\pm$  8.79 mm, P = .04) and the 13th thoracic vertebral segment in a mediolateral direction (Pre 26.45 + 4.29 mm, Post 29.27 +5.29 mm, P = .01) was found. Thoracolumbar musculature dimensions increased at T10 (3 cm), (Pre 20.90  $\pm$  3.42 cm, Post 21.72  $\pm$  3.30 cm, P = .02) and T13 (3 cm), (Pre 27.01  $\pm$  5.11 cm Post 28.23  $\pm$  5.56 cm, P = .02). No significant differences (P>0.13) were found for any inertial measurement units-derived movement parameter and thoracolumbar musculature dimensions for those horses in the placebo group. Cycloidal vibration therapy applied in the region of the thoracic spine and hind quarters is associated with altered kinematics of the thoracolumbar.

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## 1. Introduction

The equine therapy market is extensive, with an array of products targeted at horse owners; for example, training aids, wearable

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massage systems, massage rugs etc. marketed to support horse owners in improving their horse's wellbeing, assisting with musculoskeletal function pre and post exercise and in some cases assisting with the rehabilitation of an injured horse. Training aids have received specific scientific attention [1,2] along with training rollers [3]; however, there is a paucity of objective evidence on products such as massage systems/rugs particularly on their use, effectiveness, and benefits for both short and long term. There are many benefits associated with horse owner friendly systems, being noninvasive, requiring little user training and when used regularly they may complement other therapy solutions.

Man and horse share similarities in their physiology and their response to interventions (i.e., dynamic posture exercises). Studies have shown that after a series of posture exercises, both man [4]







Animal welfare/ethical statement: The study was approved by the ethics and welfare committee of the fifth author's institution.

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and horse [5] showed similar musculature responses; with an increase in multifidi cross sectional area and function leading to greater thoracolumbar stability which, in the horse, has been demonstrated to enhance vertebral stability and optimize posture [5]. Given these parallels, it seems likely that similar responses will be seen in the horse as seen in man in regard to other forms of interventions.

An emerging therapeutic method is the use of vibration therapy, a form of therapy that has been established in man. Studies have shown a positive effect on upper arm function after focal muscle vibration post mastectomy surgery [6]. Improved muscle strength has been reported in hemiplegic poststroke patients after receiving high-frequency local muscle vibration [7], and in association with physiotherapy, vibration (100 Hz) therapy applied to the triceps brachii of a spastic upper limb showed a reduction in spasticity of the flexor agonist, biceps brachii, along with improved function after vibration therapy [8].

In the horse, although limited, the physiological response to vibration therapy has been reported. A recent study using whole body vibration (delivered in the form of a horse standing on a vibrating floor) found, when following a prescribed treatment program of 30 minutes, twice a day for five days per week over a total of 60 days, an increase in the cross sectional area of the multifidi muscle after 30 and 60 days of treatment. As well as this, it was found that multifidi symmetry improved (left-right) after 60 days [9]. Another study has reported that short duration of whole body vibration (30 days) may be of benefit to lame horses with various chronic musculoskeletal pathologies [10].

Following these findings, it is speculated that vibration therapy applied to the equine back might be of some benefit in the management of the ridden horse. During ridden activity, the equine back has to function with additional forces being applied as a result of the saddle and rider [11,12] for which evolution has not equipped the horse. The thoracolumbosacral in its entirety is responsible for force transfer. The thoracic spine is a site of great interest in respect to a horse's posture; it is an area of considerable muscular attachment aiding posture and control [13,14]. The musculature around the 13th thoracic vertebra has also been shown to alter throughout the year [15] due to climate, seasons, weight fluctuations, and exercise [16]. Changes in musculature in this region have been associated with saddle slip [17], confirming the importance of an optimal musculoskeletal system, particularly in the thoracolumbar region, enabling the horse to withstand the dynamic forces being applied as a result of horse-saddle-rider interaction and helping to provide resistance against injury and supporting the locomotor system.

Many horse owners use massage therapy before exercise in an attempt to optimize horse performance. To the authors' knowledge, there have been no peer-reviewed studies investigating the acute effect of cycloidal vibration on equine locomotion when applied to the thoracic spine and hind quarters. The aim of this study was to establish the immediate effect a roller containing a unit providing cycloidal vibration therapy, positioned on the thoracic spine, has on the equine locomotion and thoracolumbar epaxial musculature dimensions in a group of nonlame horses. The objective of this study was to identify any measurable changes in locomotion and thoracolumbar epaxial musculature dimensions pre and immediately after a 20-minute cycloidal vibration therapy treatment in a group of nonlame horses.

It is hypothesized that: 1) the treatment group will show an increased range of motion of the thoracic spine along with improved symmetry values compared to a placebo group; 2) in the treatment group, there will be an increase in thoracic epaxial musculature dimensions at the 13th thoracic vertebra segment.

### 2. Materials and Methods

The study was approved by the ethics and welfare committee of the fifth author's institution.

#### 2.1. Horses

Horses underwent a veterinary assessment performed by a veterinary surgeon and no lameness was observed subjectively. The horses' gait was also assessed quantitatively on a hard surface with a validated sensor-based system [18,19]. A convenience sample of 30 military horses that ranged in height at the withers from 1.63 to 1.80 m with a mean  $\pm$  SD of  $1.69 \pm 0.7$  m, body mass ranged from 495 to 550 kg with a mean  $\pm$  SD 523  $\pm$  47 kg, and age ranged from 6 to 12 years with a mean  $\pm$  SD 9  $\pm$  3 years. Horses were in regular work preceding the study and were deemed fit to perform their usual duties. All horses were housed at the same facility and had been there for a considerable time mean  $\pm$  SD 10  $\pm$  2 years. Participation was voluntary, and the veterinarian gave informed consent for all horses to be used in the study. The veterinarian could withdraw the horses at any point of the study.

### 2.2. Data Collection

#### 2.2.1. Kinematics—Inertial Measurement Units

Horses were instrumented with eight MTw inertial measurement units (IMU) (Xsens) using a validated sensor-based system (Equissage. NHC Technology Ltd, Colomendy Industrial Estate, Rhyl Road, Denbigh, LL16 5TS) [18,19]. These were attached over the poll, wither, vertebral segments of the 13th thoracic and third lumbar vertebra, sacrum, caudal sacrum, left and right tuber coxae, using custom built pouches and double-sided tape. The same technician applied each sensor throughout the study, and senor locations were referenced with white skin paint. To reduce variability, sensor pouches remained on the horse throughout. Sensor data were collected at 60 Hz per individual sensor channel and transmitted via proprietary wireless data transmission protocol (Xsens Technologies B.V., P.O. Box 559, 7500, An Enschede, Netherlands), to a receiver station (Awinda, Xsens) connected to a laptop computer running MTManager (Xsens) software. IMU specifications were as follows: internal sampling rate 1000 Hz, buffer time 30 seconds, dimensions, 47  $\times$  30  $\times$  13 mm, weight 16 grams, operating temperature range 0°C-50°C and dynamic accuracy 0.75° root mean square (RMS) (roll/pitch) and 1.5° RMS (heading).

Inertial measurement units data were processed following published protocols [20]. In brief, triaxial sensor acceleration data were rotated into a gravity (z: vertical) and horse-based (x: craniocaudal and y: mediolateral) reference frame and double integrated to displacement. Displacement data were segmented into individual strides based on vertical velocity of the sacrum sensor [21], and average values for the following kinematic variables were calculated over all strides for each exercise condition.

- Range of motion: maximum—minimum value over a stride cycle for x, y, and z for trot.
- Minimum difference (Min<sub>Diff</sub>): difference between the two minima in vertical (z) displacement observed during the two diagonal stance phases in trot [22].
- Maximum difference (Max<sub>Diff</sub>): difference between the two maxima in vertical (z) displacement observed after the two diagonal stance phases in trot [22].

#### 2.2.2. Thoracolumbar Epaxial Musculature Dimensions

Thoracolumbar epaxial musculature dimensions were obtained using a new Flexible Curve Ruler (Blundell Harling 600 mm), which was shaped around the dorsum, perpendicular to the dorsal midline at three levels of the vertebral column: T10, T13, and T18. For repeatability, T10, T13, and T18 were identified and referenced with skin paint. A single Society of Master Saddlers (SMS)—qualified saddle fitter performed all measurements, with the horse stood square on a hard, level surface. Measurements of dorsal thoracolumbar body shape of each horse were obtained following the SMS guidelines [23].The thoracolumbar dimensions (widths at 3 cm and 15 cm ventral to the dorsal midline) were measured at the level of the T10, T13, T18 [15,16] pre and post treatment then drawn on to A5 graph paper and the width measured at 3 cm and 15 cm ventral to the dorsal midline. Coefficient of variance was less than 0.04 for all measurements using three repeats per measurement.

#### 2.3. Study Protocol

Horses were randomly assigned in two groups: treatment group that received cycloidal vibration therapy and a placebo group that did not receive any vibration therapy. The two groups (treatment/ placebo) which the horses were assigned to were blinded from both the technician applying the sensors and the technician performing the thoracolumbar measurements. All horses had carried out normal exercise duties the day preceding the study, and on the day of the study, all horses were stabled in stalls. For both the treatment and placebo group, data were collected pre and post intervention, and IMU data were analyzed from 60 motion cycles mean  $\pm$  SD 60  $\pm$  3. Data collection protocol remained the same for both groups; horses were trotted in hand by the same handler on a concrete surface that had clear open ends and was the length of 20 meters. Six trials were carried out; data from six trials were automatically stride matched. Only straight motion cycles were included in the analysis omitting each turn along with two strides at the beginning and end of each trial; if the horse lost straightness (i.e., trotted on three or four tracks), altered its gait, or increased its speed, then the trial was repeated.

The treatment group consisted of 20 horses ranging in height at the withers from 1.63 to 1.80 m with a mean  $\pm$  SD of 1.50  $\pm$  0.1 m, body mass ranged from 495 to 590 kg with a mean  $\pm$  SD 500  $\pm$  42 kg, and age ranged from 6 to 12 years with a mean  $\pm$  SD 10  $\pm$  3 years. The placebo group consisted of 10 horses that ranged in height at the withers from 1.60 to 1.80 m with a mean  $\pm$  SD of 1.50  $\pm$  0.20 m, body mass ranged from 495 to 590 kg with a mean  $\pm$  SD 502  $\pm$  47 kg, and age ranged from 6 to 11 years with a mean  $\pm$  SD 9  $\pm$  2 years.

For the treatment group, horses were fitted with a custom roller (Fig. 1), positioned over thoracic vertebra 10-13 and secured with a belly and chest strap (Equissage Pulse Saddle, NHC Technology UK). Positioned on the dorsal aspect of the roller was a unit weighing 4.5 Kg, and 1400 mm in length, 300 mm width, and 80 mm head diameter (Fig. 2). On the ventral aspect of the roller, two pads were positioned creating a nontreed gullet ensuring clearance of the spinous process (Fig. 3). Each horse was cross tied in a stall and received one treatment lasting 20 minutes with a cycloidal vibration frequency set at 45 Hz. Alongside this, one operator provided manual vibration therapy for 20 minutes to the gluteals, hamstrings, lats, biceps femoral, triceps, brachio, trapezius and romboids. Using a handheld unit with the cycloidal vibration frequency set at 65 Hz, each region, where superficial and deeper muscles were located, received two and half minutes of massage with four strokes following the direction of the hair (Fig. 4).

For the placebo group, horses were fitted with a custom roller built to the same weight, dimensions and design as outlined



**Fig. 1.** Lateral view of the roller positioned on the thoracic spine in the region of thoracic 10-13. Positioned on the dorsal aspect of the roller is a unit weighing 4.5 Kg, and 1400 mm in length, 300 mm width, and 80 mm head diameter, providing a 20-minute cycloidal vibration set at a frequency of 45 Hz.

previously (Equissage Pulse Saddle, NHC Technology UK). Differing from the treatment group, the roller used in the placebo group provided no cycloidal vibration. Each horse was cross tied in stalls and received one dummy treatment lasting 20 minutes. In addition, as in the treatment group, one operator provided a dummy manual vibration therapy for 20 minutes to the gluteals, hamstrings, lats, biceps femoral, triceps, brachio, trapezius and romboids using a handheld unit, which, like the roller, did not provide any Cycloidal vibration therapy (Equissage Hand Unit, NHC Technology UK). The same protocol as outlined previously was carried out, with each



Fig. 2. On the ventral aspect of the roller, two pads are positioned to the left and right of the midline creating a nonrigid gullet ensuring clearance of the spinous process.



Fig. 3. Ventral view of the positioning of the roller on the thoracic spine.

region where superficial and deeper muscles were located, receiving two and half minutes of dummy massage with four strokes following the direction of the hair.

## 2.4. Data Analysis

## 2.4.1. Statistical Analysis

Statistical analysis was performed in SPSS (ver. 22, IBM, Armonk, USA). Thoracolumbar dimension and kinematic outcome



**Fig. 4.** Horses were given a manual massage lasting 20 minutes using a hand unit providing cycloidal vibration set at a frequency of 65 Hz. With the operator using the black hand grip, the white cylinder was positioned on the horse and following the direction of the hair cycloidal vibration was applied to the gluteals, hamstrings, lats, biceps femoral, triceps, brachio, trapezius and romboids.

parameters were assessed for normality using a Shapiro–Wilks test and found to be normally distributed. A mixed model was used to determine the influence of speed on outcome parameters. Thoracolumbar measurements and differences in range of motion in a craniocaudal (x), mediolateral (y), and vertical (z) direction for the wither, vertebral segments of the 13th thoracic and third lumbar vertebra, sacrum, caudal sacrum, left and right tuber coxae before and after treatment were assessed using a paired *t*-test with a significance level set at  $P \leq .05$ .

#### 2.4.2. Speed

Because many kinematic parameters are influenced by speed, differences in speed between different conditions were tested. A start and end point were identified with the time taken for the horse to reach each point being recorded. Time taken was recorded by the same technician. No significant difference was found in any of the outcome parameters when speed was included in the mixed model.

## 3. Results

Significant differences (mean  $\pm$  SD) in range of motion of wither (pre 69.00  $\pm$  8.77 mm, post 70.84  $\pm$  8.79 mm, P = .04) and sacrum (pre 76.63  $\pm$  10.10 mm, post 74.26  $\pm$  9.87 mm, P = .04) in a vertical direction and T13 (pre 26.45  $\pm$  4.29 mm, post 29.27  $\pm$  5.29 mm, P =.01) in a mediolateral direction were observed in horses that received a cycloidal vibration treatment along with a smaller difference between the left and right minima of the sacrum Min<sub>Diff</sub>. No significant differences (P $\ge$ 0.10) were found in any IMU-derived movement parameter for those horses in the placebo group (Table 1).

Significant changes (mean  $\pm$  SD) in thoracolumbar epaxial musculature profiles, increased at the level of T10 (3 cm ventral to the dorsum), (pre 20.90  $\pm$  3.42 cm post 21.72  $\pm$  3.30 cm, *P* = .02), T10 (15 cm ventral to the dorsum), (pre 33.50  $\pm$  3.22 cm post 34.81  $\pm$  3.22 cm, *P* = .02) and T13 (3 cm ventral to the dorsum), (pre 27.01  $\pm$  5.11 cm post 28.23  $\pm$  5.56 cm, *P* = .02). No significant changes (P $\ge$ 0.13) in thoracolumbar epaxial musculature profiles were observed in the placebo group (Table 2).

## 4. Discussion

The aim of this study was to determine the acute effect that vibration therapy has on equine locomotion and thoracolumbar epaxial musculature dimensions. Although some differences have been reported here, the authors appreciate that this study has only investigated acute effect and although statistically significant changes have been observed, some are small and could be as a result of biological variation. In an attempt to overcome biological variation, the horses in this study were housed and managed at the same facility, were of a similar type and fitness, in addition a placebo group was included in the study to further determine if the changes observed are as a result of biological variation or other factors.

In this group of horses, the results of the present study support the hypothesis that vibration therapy when applied to the mid thoracic vertebral segments and hind quarters has an effect on equine locomotion. In particular, range of motion of the withers and sacrum in a vertical direction, the 13th vertebral segment in a mediolateral direction and smaller differences between the two troughs (minima) of the vertical movement of the pelvis were observed along with an increase in thoracolumbar epaxial musculature dimensions at the wither and 13th vertebral segment. Furthermore, the placebo group who followed the same protocol Table 1

Kinematic data during trot pre and post cycloidal vibration treatment group (n = 20) and non-cycloidal vibration placebo group (n = 10), (ROMY = range of motion in mediolateral direction, ROMZ = range of motion in vertical direction, Sacrum Min<sub>Diff</sub> = difference between the two minima in vertical displacement).

IMU Parameter	Cycloidal Vibration Treatment Group ROM (mm)			Non-cycloidal Vibration Placebo Group ROM (mm)		
	Pre	Post	<i>P</i> Value <i>P</i> ≤0.05	Pre	Post	P Value
Wither ROMZ (mean ± SD) Thoracic 13 ROMY (mean ± SD) Sacrum ROMZ (mean ± SD) Sacrum Min <sub>Diff</sub> (mean ± SD)	$\begin{array}{c} 69.00 \pm 8.77 \\ 26.45 \pm 4.29 \\ 76.63 \pm 10.10 \\ 3.10 \pm 5.70 \end{array}$	$70.84 \pm 8.79 29.27 \pm 5.29 74.26 \pm 9.87 1.23 \pm 6.37$	0.04 0.01 0.04 0.05	$\begin{array}{c} 66 \pm 5.85 \\ 26 \pm 4.81 \\ 70.33 \pm 10.87 \\ .27 \pm 5.84 \end{array}$	$67.89 \pm 8.68$ 24.66 ± 5.04 71.00 ± 10.22 -3.73 ± 3.35	0.25 0.20 0.75 0.10

(excluding any vibration therapy) showed no changes in any movement or back-derived parameters.

The equine back is rigid, allowing for effective force transmission connecting the pectoral and pelvic limbs and aiding quadrupedal locomotion, which has been of recent scientific interest [12,24]. The region of 10th-13th thoracic vertebral segment is of great interest in optimizing equine back health and function [14]. In the ridden horse, it is an area where the twist of the saddle is positioned (narrowest point of seat) and it is the point at which the rider's center of mass aligns with the horse's center of mass, which has been shown to alter its movement while trotting with a rider in the seated and standing position [25]. The cranial portion of the thoracic vertebra has considerable muscular activity related to posture and control of movement, including the longissimus dorsi, largely responsible for control and stabilizing the vertebral column [13]. Using IMU-derived parameters, kinematics of the thoracolumbosacral have been reported where the greatest amplitude of dorsoventral movement occurred at thoracic 13 when trotting in a straight line, compared to trotting on a circle. In the ridden horse, it has been reported that reduction of saddle pressures on the thoracic vertebral segments (10-13) were associated with improved locomotor features and increased thoracolumbar epaxial musculature dimensions [26]. In the present study, within the treatment group, measurable changes have been observed after vibration therapy; it is speculated that the vibration therapy applied has affected the musculature associated with posture, control, and locomotion in particular the thoracic sling musculature and back stabilizers; further work is needed to confirm this, along with the use of other measuring systems which should aim to investigate muscle activity in response to vibration therapy. If the changes observed were as a result of vibration therapy, this could have an impact on the ridden horse in respect of training, saddle balance, saddle-rider interaction, and assisting with optimizing back health and function.

The influence that vibration therapy has on muscle function has been established in man [6,7,9,10,27]. Although in their infancy, investigations in the horse have shown that the cross sectional area of the multifidi muscles was increased after a prescribed treatment program requiring the horse to stand on a vibrating plate [9]. This study applied vibration therapy to the mid thoracic vertebra and hind quarters; in this group of horses, increase in thoracolumbar epaxial musculature dimensions was observed. Increase in thoracolumbar epaxial muscle dimensions have been reported and associated with the posture of the horse [15,26]. Although the differences are statistically significant, the differences reported here are small; studies should attempt to investigate these changes with a greater sample size. However, based on these changes, it is speculated that after treatment, the horse alters its thorax relative to the scapular because of increased musculature activity, hence a measurable change in movement associated with the 10th and 13th thoracic vertebral segment. Further work is needed to understand the direct mechanics behind these changes. With greater understanding, in the ridden horse, these changes could be desirable as it has previously been reported that improved locomotion was associated with increased thoracolumbar dimensions of the thoracic region (T13); further work is needed to investigate these possible benefits.

This study used IMUs positioned along the horse's thoracolumbosacral; the changes observed were localized to the positioning of the IMU on specific vertebral segments. In this study, IMUs were positioned at specific landmarks along the thoracolumbosacral wither, 13th thoracic and third lumbar vertebral segments and tuber sacral. To reduce variability, identification of anatomical landmarks and positioning of the sensors were performed by the same technician. A more detailed evaluation of back kinematics would improve the study in quantifying the terms thoracolumbar and sacrum. The IMUs have been used extensively in the field; however, performing data collection under laboratory conditions would help reduce some variables such as handler interaction. Although our study was not affected by speed and only trials where the horse maintained straightness were included in the analysis, using a treadmill would standardize speed and straightness for each horse.

In the present study, in respect of the treatment group, smaller differences were found between the two troughs (minima) of the vertical movement of the tubera sacrale (pelvis) indicating symmetry between left and right sides. Peak ground reaction forces have been associated with pelvic Min<sub>Diff</sub> [20]. It is speculated that more symmetric ground reaction forces were achieved after vibration therapy was applied; further work is needed to understand the mechanics behind this and these changes in symmetry values. It is possible that changes occurred because of altered back function

#### Table 2

Thoracolumbar epaxial musculature profiles at tenth (T10), thirteenth (T13), and eighteenth thoracic vertebra (T18) pre and post vibration treatment for both the treatment and placebo groups.

Condition	Group	T10 3 cm (cm)	T10 15 cm (cm)	T13 3 cm (cm)	T18 3 cm (cm)
Pre treatment (mean ± SD)	Treatment group	$20.90 \pm 3.42$	$33.50 \pm 3.22$	$27.01 \pm 5.11$	$33.17 \pm 3.95$
Post treatment (mean + SD)	Treatment group	$21.72 \pm 3.30$	34.81 + 3.22	28.23 + 5.56	34.20 + 2.94
<i>P</i> value $P \le 0.05$	Treatment group	0.02	0.02	0.02	0.19
Pre treatment (mean $\pm$ SD)	Placebo group	$21.86 \pm 0.94$	$34.34 \pm 2.79$	$26.23 \pm 3.23$	$34.92 \pm 2.66$
Post treatment (mean $\pm$ SD)	Placebo group	$21.08 \pm 3.16$	$33.52 \pm 2.78$	$25.95 \pm 3.21$	$33.23 \pm 3.15$
<i>P</i> value	Placebo group	0.39	0.53	0.73	0.13

assisting with force transmission; these findings warrant further investigation.

The present study investigated the effect that vibrational therapy has on locomotion of the horse while trotting in hand. Investigation of these parameters in walk and canter would be of interest. Evaluation of the horses was completed in hand with the same handler throughout to limit variability. Saddle-rider variables were reduced by evaluating the horses in hand as the influence the rider [11,28] and equipment have on the horse [26,29–31] has been reported previously. This study could be improved with equal number of horses in each group and greater sample size.

## 5. Conclusion

Vibration therapy in man has been shown to be of benefit in improving muscular activity, post exercise recovery, and in rehabilitation. In this group of horses, this study found that vibration therapy, when instrumented by means of positioning a roller on the horse and a handheld unit, housing a unit delivering cycloidal vibration, has an immediate effect on the horse by altering thoracic range of motion, thoracolumbar musculature, and pelvic symmetry. In the placebo group, who followed the same protocol excluding receiving any vibration therapy, no changes in any measured parameters were observed. Further work is needed to understand these changes and investigate the long-term effectiveness of vibration therapy and its clinical relevance and biological significance.

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