Journal of Equine Veterinary Science 165 (2026) 106051

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.elsevier.com/locate/jevs

# Aqua training promotes changes of skeletal muscle mass in show jumping horses

C. Nascimento [a,b], C. Santos [a], M. Walton [a], T. Gonçalves [a], G. Freire [a,b], M. Nicolau [a,b], F. Silvestre [a,b], J. Simões [a,c], J. Borges [a], N. Pires [a], H. Manso Filho [d], H. Clayton [e], C. Coelho [a,c,f,*]

[a] *Faculty of Veterinary Medicine, Lusofona University, Campo Grande 376, Lisbon 1749-024, Portugal*
[b] *Hidrovet – Centro de Reabilitação Equina, Rua do Alecrim S/N, Janas 2710-295, Portugal*
[c] *Research in Veterinary Medicine (I-MVET), Faculty of Veterinary Medicine, Lusófona University, Lisbon University Centre, Campo Grande 376, Lisbon 1749-024, Portugal*
[d] *Núcleo de Pesquisa Equina, Universidade Federal Rural de Pernambuco (UFRPE), Rua Dom Manuel de Medeiros S/N, Recife 51171-900, Pernambuco, Brazil*
[e] *Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing 48824 MI, United States*
[f] *MED - Mediterranean Institute for Agriculture, Environment and Development, Universidade de Évora, Évora 7006-554, Portugal*

ARTICLE INFO

*Keywords:*
Biomechanics
Muscle mass
Thermography
Water treadmill

ABSTRACT

*Background:* The correct prescription of aquatic treadmill training for equine athletes depends on understanding the muscular changes generated by working in the water.
*Aims/Objectives:* The aim of this study was to test the hypothesis that the inclusion of a water treadmill in a routine training protocol would alter muscle mass of show jumping horses.
*Methods:* Six fit-to-compete horses were trained for 10 weeks with a water treadmill (twice a week, 20 minutes, water level at the carpus), in addition to regular physical exercise. Body composition, infrared thermography (IRT) of propulsive muscles (m. brachiocephalic, m. semitendinosus and m. semimembranosus) and field jumping tests for aspartate aminotransferase (AST) and creatine kinase (CK) analysis and kinematic parameters were assessed before and after the protocol. Data was analyzed using paired t-test ($p < 0.05$).
*Results:* Body weight altered (550.5 ± 14.5 *vs.* 587.0 ± 41.9 kg), related to a fat-free mass gain (485.1 ± 19.1 *vs.* 520.1 ± 42.2 kg). Fat mass remained unchanged (65.4 ± 11.4 *vs.* 66.9 ± 10.3 kg), as no significant changes were observed for rump fat thickness (0.69 ± 0.45 *vs.* 0.59 ± 0.40 cm). IRT temperature of the selected muscles and AST and CK levels increased after training. Improvements in strike power (2.53 ± 0.38 *vs.* 3.18 ± 0.38 G), take-off angle (20.0 ± 5.0° vs. 24.5 ± 5.4°) and the aerial phase duration (665.3 ± 179.2 vs. 765.3 ± 41.1 ms) for the vertical jumps and in take-off angle (21.7 ± 6.4° vs. 26.1 ± 5.8°) for oxer jumps.
*Conclusions:* These results suggest that the inclusion of the water treadmill promoted muscular recruitment and gain in muscle mass, contributing to a greater strength in the core muscles, to elevate the forehand, and in the propulsive hind limb muscles, to increase strike power.

## 1. Introduction

Training a horse for competition requires both physical conditioning and technical coaching. The latter is responsible for enhancing neuromuscular coordination and mental discipline, whereas physical conditioning induces physiological and structural adaptations in body tissues, to optimize performance and maintain the athlete's health [1–3]. Thus, a good training program ensures adequate physical fitness to perform a designated task efficiently, without excessive fatigue [4]. The adaptive response depends on the application of appropriate stimuli, organized in three variables: frequency, intensity and duration [2,3]. Exercise training improved many indicators of mitochondrial function and density, suggesting that exercise could be an extremely useful tool for improving the health and welfare of horses [5].

Physical conditioning methods are individualized, reflecting the diversity of equestrian disciplines and their specific physiological

* Corresponding author at: Faculty of Veterinary Medicine, Lusofona University, Campo Grande 376, Lisbon 1749-024, Portugal.
*E-mail address:* clarisse.coelho@ulusofona.pt (C. Coelho).

https://doi.org/10.1016/j.jevs.2026.106051
Received 31 March 2026; Received in revised form 27 May 2026; Accepted 5 June 2026
Available online 6 June 2026

demands [1–6]. In the show jumping discipline, training protocols should focus on improving aerobic capacity and the ability to generate force rapidly and explosively [7]. It is important that horses develop specific biomechanical patterns that enable them to clear obstacles without penalties, as one of the main challenges is to convert horizontal velocity into a sufficiently high vertical component during take-off [8, 9].

Currently, the use of the water treadmill has become increasingly common in training routines, particularly for sport horses [10–17]. Its application, whether for rehabilitation protocols or conditioning purposes, is based on three properties of the water – viscosity, buoyancy and hydrostatic pressure [16–18]. In addition to controlling speed, frequency and duration of the exercise, it is possible to adjust water depth and temperature [19]. Water depth, associated with the aforementioned physical properties of water influences physiological responses and significantly alters the movement pattern of the distal limb and pelvis [12,16,19–22].

Overall, exercising on the water treadmill provides a low-moderate impact controlled aerobic training, with greater muscle activation, thereby improving motor control and joint stability [10,18,19,22,23]. The goal of this type of training is to minimize the impact forces on the distal limbs, promoting controlled, straight-line exercise without axial loading [24,25]. As a result of distal limb joint flexion variation induced by changes in water depth, an increase in the amplitude of the dorsal kinematic parameters is expected to occur [26]. Regular working in the water induces muscle development and tone, causing an adaptation that includes increased protraction and decreased retraction of the fore and hind limbs [23]. These responses are primarily dependent upon water depth but also previous experience of the horses with the equipment, the number of sessions and type of imposed training program [23].

Much remains to be understood regarding the appropriate use of aquatic treadmill training in sport horses. A wide range of variables still needs to be defined and optimized, including the specific discipline, the desired athletic outcomes, and the individual characteristics of each horse, all while ensuring full compliance with animal welfare principles. In this context, the authors studied how the inclusion of the water treadmill in a routine training for 10 weeks alters body and muscle composition and locomotor biomechanics of show jumping horses. The authors hypothesized a gain in fat-free mass through the activation of muscle groups important for body propulsion and spinal stabilization. These would induce kinematic changes important for success in show jumping performance.

## 2. Material and methods

### 2.1. Ethical approval

This study was approved by CEBEA-FMV, Lusófona University, Lisbon University Center (protocols 28/2023 and 17/2025). The entire study was carried out within the clinical scope of a recommended training routine and monitored by veterinarians. Owners gave consent for the retrieval of clinical information.

### 2.2. Study design

This study is a pre-post comparison study (longitudinal design), which enrolled non-random group of horses with comparable physical conditioning, performing jumps of 1 meter in height. The horses were in full athletic activity, with several years of experience in show jumping and a consistent exercise routine. Also, all included animals were familiarized with the equipment and procedures. During the 10-week study, the inclusion of a water treadmill exercise was the only change made in their usual routine training. The protocol has already been described [27]

The study had a total duration of 23 weeks. All horses underwent a selection process, which included physical and orthopaedic examination by a specialist to ensure they were clinically fit to take part in the study according to an International Equestrian Federation pre-competition veterinary assessment (BASAL). During the following 10 weeks the horses maintained their usual training schedule and were monitored to ensure they remained clinically healthy. This was followed by one week of pre-training evaluation (PRETR) and an additional period of 10 weeks of training using the water treadmill, which was the only modification performed in their initial training routine. Finally, post-training evaluation (TR) was conducted five days after the final water treadmill session.

### 2.3. Animals

Six horses ($n = 5$ geldings, $n = 1$ mare; 16.7 ± 2.0 years; 578.3 ± 17.1 kg) of different breeds used in show jumping (Anglo-Arabian, French Saddle, Purebred Lusitano, BWP, and 2 German Warmbloods) were used in this case-control study. They were considered suitable for inclusion within the study after rigorous clinical examination including orthopedic evaluations. All animals belong to the same training facility located in Sintra, Portugal. They were fed lucerne (*Medicago sativa*), always available; and the commercial concentrate feed (Easy Mix, Intacol, with 10.5% protein and 2,750 kcal of digestible energy), at a rate of 0.7 kg/100 kg of body weight, three times a day (8am, 12pm, and 6pm). Fresh and clean water was supplied through an automatic drinker and was always available. The horses were housed in 3 × 3 m individual box stalls.

### 2.4. Submaximal exercise test to determine HRpeak

Prior to the training program, all horses underwent an incremental exercise test on the water treadmill (Aquatraining, Activo-Med Aqualine, Poland) to determinate their individual peak heart rate (HRpeak), using the Alogo Move Pro™ system (Alogo Swiss Technology, Renens, Switzerland) coupled with the H10 sensor (Polar Electro, Lake Success, NY, EUA).

For this assessment, the horses first walked on the treadmill at a speed of 4 km/h, for a 5-minute warm-up period. The tank was then filled with water for 3-4 minutes until it reached a depth of ~40 cm (at the level of the carpal-metacarpal joint area), while maintaining the same speed. Finally, the speed was increased by 1 km/h every 60 seconds until reaching 7 km/h, which was then maintained until a maximum duration of 15 minutes was achieved. The test was interrupted if any of the following conditions occurred: (1) backward movement despite human encouragement; (2) abrupt increases in heart rate and/or respiratory rate; (3) repeated stumbling or difficulty maintaining rhythm. If the test was interrupted or if it reached its maximum duration, treadmill speed was reduced and maintained at 4 km/h whilst the tank was drained.

The estimated HRpeak value of each horse determined by the Polar H10 in the submaximal exercise test described above was used to adjust individually the treadmill speed during the 10 weeks of training.

### 2.5. Training program using the water treadmill for 10 weeks

The horses were trained six days per week (40-60 minutes per day), during which they performed preparatory exercises for show jumping competitions (specific work), with moderate intensity (endurance work), alternating with peaks of greater demand (strength work), whilst respecting individual physical limits. The intensity imposed was adjusted to suit each individual.

On the first and third day of each week (considering Monday as the rest day and Tuesday as the first training day), a hydrotherapy session was conducted using a water treadmill, as described previously [27].

During the first five weeks of training sessions, the horses performed a 5-minute warm-up on the treadmill without any water at an average speed of 4 km/h. Subsequently, the tank was filled to level 3 (~35-40 cm, so that the water reaches the carpal-metacarpal joint area) over approximately 3-4 minutes. Then, the animals worked for 16-17 minutes at a working speed compatible with 60% of the HRpeak previously determined in the incremental effort test. At the end of each session, the tank was drained while the treadmill speed was reduced to approximately 4 km/h for an additional 3-4 minutes period, after which the equipment was turned off. The total duration of physical activity was ∼25 minutes. During the subsequent five weeks, the same protocol was maintained, but the working speed was adjusted to 80% of HRpeak, resulting in all horses trotting in the water. Throughout all training sessions, the horses were monitored using the Alogo Move Pro™ and Polar system to allow speed and HR monitoring.

Over the 10-week period, environment temperature, relative air humidity and water temperature in the treadmill were daily monitored (at the beginning, during and at the end of each session).

### 2.6. Assessment of the training protocol

In PRETR and TR, the horses were sequentially evaluated using infrared thermography, ultrasonography for body composition assessment, and field tests in show jumping.

#### 2.6.1. Infrared thermography (IRT)

IRT was performed with the animal at rest, in a covered area, without any direct solar radiation, and at an environment temperature close to 18°C, always by the same operator and following previous studies [28, 29]. IRT images were collected bilaterally from the brachiocephalicus, semitendinosus, and semimembranosus muscles, with the device positioned approximately 1 meter away. To assess the brachiocephalicus muscle, regions of interest (ROIs) 1 (left neck) and 2 (right neck) were delineated in the most distal portion of the muscle, near the marker placed at the clavicular intersection area (Fig. 1A). For evaluation of the semitendinosus and semimembranosus muscles, a marker was positioned delimiting both muscles, and ROIs 3, 4, 5, and 6 were established lateral and medial to the marker, respectively (Fig. 1B). The upper boundary was defined by drawing a horizontal line at the level of the ischial tuberosity.

The equipment used was a FLIR E4 (FLIR Systems Incorporated, USA), with subsequent image processing performed using FLIR Tools™ software (FLIR Systems Incorporated, USA). The FLIR E4 featured an IR resolution of 80 × 60 pixels, thermal sensitivity of <0.15°C at 30°C, image capture frequency of 9 Hz, fixed emissivity set at 0.99, a fixed-focus lens with a 45° × 34° field of view, and calibration performed according to the manufacturer's recommendations; additionally, the optimal image for the ROI analysis was selected based on image sharpness, animal positioning, and absence of motion artifacts.

The software allows adjustments to the temperature scale of the images, bilateral measurements with adaptations in images where the trunk or tail interfered, as well as the extraction of minimum, mean, and maximum temperature values for each ROI.

#### 2.6.2. Ultrasonography for body composition assessment

An ultrasound device (LOGIQ model, General Electric Healthcare, USA) with a linear probe (12L-RS model, General Electric Healthcare, USA) operating at 5 MHz in B-mode was used to determine the measurements of the *Longissimus dorsi* (LD) muscle and the thickness of the rump fat (RF). The procedure was done always by the same operator. Images were obtained with the horses at rest, in a quadrupedal stance, relaxed, and balanced, with all four limbs properly positioned.

As the animals were of high sporting value and intended for equestrian competitions, shaving of the evaluated region was not performed. Thus, the animals had the regions of interest (back and rump) cleaned with warm water to enhance ultrasound penetration and with neutral soap to remove particles that could interfere with image generation. Images were obtained after topical application of 70% alcohol.

The thickness of the LD was determined at the level of the 17th and 18th ribs, with the transducer positioned transversely to the thoracolumbar spine [30]. Images were obtained bilaterally, always at the thickest portion of the muscle in the transverse section, in a line parallel to the dorsal spinous process and tangent to the articular process (Fig. 2).

The thickness of the RF was determined at the intersection of two imaginary lines: the first drawn from the ischium to the base of the tail, and the second from the ilium to the lumbosacral spine (at the level of L6 to S1), adapted from a previously described methodology [31]. The thickness, in cm, of the fat over the intersection of the middle gluteal and superficial gluteal muscles was measured (Fig. 3). This assessment was performed only on the left side of the animals.

Based on the determination of RF, body fat percentage (BF%) was calculated using the formula BF% = 8.64 + (4.7 × RF)' described by Westervelt et al. [32]. Fat mass (FM) and fat-free mass (FFM) were calculated based on body weight and BF% [33].

#### 2.6.3. Showjumping field tests (JFT)

All animals performed two JFT, with the same course plane [27], one in PRETR and another in TR. In both tests, each horse was ridden by the same rider, who was instructed to complete the course in the shortest time possible without knocking down any obstacle. The tests were conducted in an outdoor arena with a high-performance track (silica sand), measuring 60 × 20 m. Prior to each test, all horses were ridden

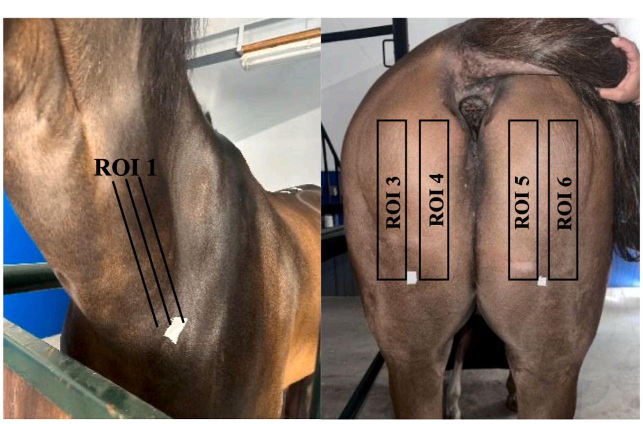


**Fig. 1.** Identification of the ROIs studied. (A) Marker at the left clavicular intersection and schematic representation of ROI 1. (B) Markers delineating the semitendinosus and semimembranosus muscles bilaterally and schematic representation of ROIs 3, 4, 5, and 6.

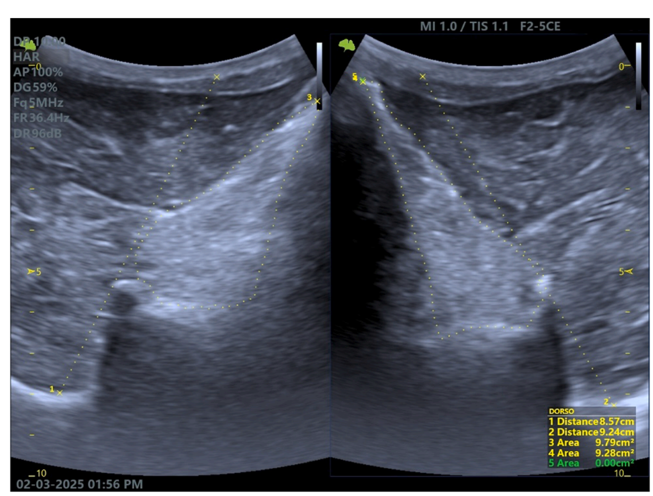


**Fig. 2.** Ultrasound image obtained from one of the horses used in the study, showing the measurement of the Longissimus dorsi muscle (lines 1 and 2).

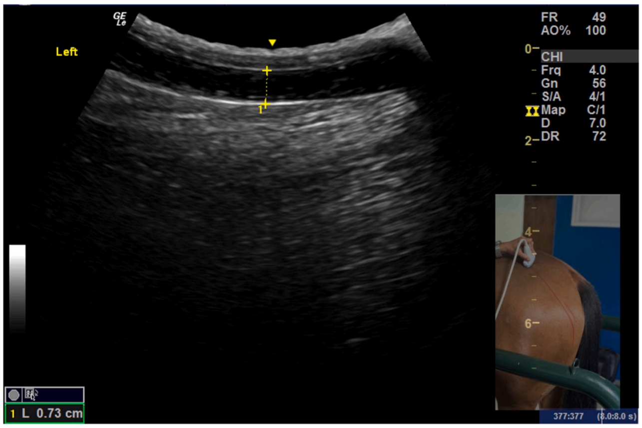


**Fig. 3.** Image obtained from one of the horses used in the study, showing the measurement area of the left rump fat.

and performed a warm-up consisting of approximately 10 minutes of walking, followed by 15-20 minutes of trotting and cantering, with 2-minute rest before beginning the obstacle course.

Environment temperature, air humidity and arena conditions were recorded. The show jumping course consisted of 300 meters, with 10 jumps of one meter height – 2 oxers and 8 verticals.

*2.6.3.1. Biomechanical analysis.* During the training sessions and both JFT, the horses were monitored with the Alogo Move Pro™ and Polar sensors. The device was attached to the ventral midline region of the girth using a protective mounting plate. The system does not restrict the horse's movement and weighs approximately 127 g [34]. The entire process followed the manufacturer's instructions, which can be found on their website (www.alogo.io).

The data obtained from JFTs during PRE and TR assessments were processed using the system's own software, analyzing simple vertical jumps and oxers separately, specifically the first three vertical jumps and the first oxer. After identifying these jumps, the following information was obtained from the system: jump height (m), vertical trunk displacement (cm), trunk angle at take-off (°) (Fig. 4), strike power (force exerted by the hind limbs at take-off, in G), speed (km/h), heart rate (beats/min) and aerial phase duration (ms). Additionally, during the second training sessions of weeks 4 and 9, data on the vertical trunk displacement (cm) were collected while the horses were walking and trotting on the water treadmill, respectively.

*2.6.3.2. AST and CK analysis.* Blood samples were obtained after local asepsis with 70% alcohol by jugular venipuncture at T0 (before physical exercise, with the animal at rest and still in the stall), T1 (within a maximum period of 1 minute after completion of physical exercise), T2 (after 30 minutes of recovery), and T3 (after 4 hours of recovery) in 9 mL tubes without anticoagulant. After collection, the samples were placed in a container with ice and taken to the laboratory. Within a period of 4-6 hours, they were centrifuged for 10 minutes at 2000 g, and the serum was stored in a freezer at −80°C for later analysis. In the obtained serum, AST and CK were analyzed using a DxC 700 AU Chemistry Analyzer (Beckman Coulter), with reagent kits from the same manufacturer. Additionally, serum CK determinations were performed during the second session of weeks 2, 4, 6, and 8 of training, using blood samples collected before exercise (Tr0) and after 4 hours of recovery (Tr3).

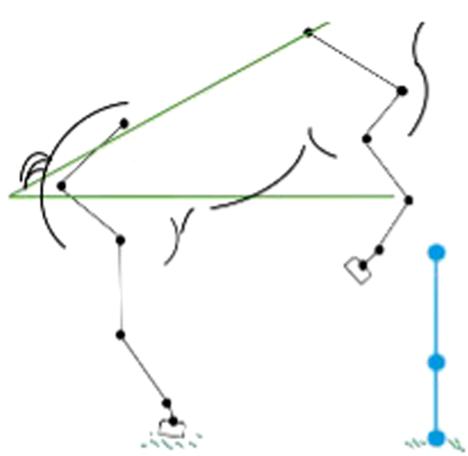

**Fig. 4.** Illustration of the trunk angle at take-off (°) during a jump obstacle.

### 2.7. Statistical analysis

Statistical analysis was performed using GraphPad Prism software (GraphPad Prism, version 10.3.0, GraphPad, Boston, USA). Data distribution had a normal distribution according to Shapiro-Wilk test ($p >$ 0,05). To evaluate the acute effects of exercise within each condition (PRETR and TR), a one-way repeated-measures ANOVA was performed with time (field test moments) as the within-subject factor and horse as the subject variable, followed by Tukey's post hoc test for multiple comparisons. To assess the effect of training (PRETR vs. TR), paired Student's t-tests were used to compare baseline values between conditions. Results were expressed as means ± standard deviations (SD), and statistical significance was set at $p \leq 0.05$ in all cases.

## 3. Results

The selected horses underwent a thorough physical examination performed by an EGAS (Equine Gait Analysis Society) specialist, which confirmed that they were fit to compete and to participate in this project.

Ultrasound analysis (Table 1) showed no significant changes in RF and LD thickness after the training program. However, significant increases in body weight and fat-free mass were observed after the 10-week training program on the water treadmill.

IRT evaluation of the three selected muscles for the study (brachiocephalic, semitendinosus, and semimembranosus muscles) showed a significant increase in minimum, mean, and maximum temperatures after the 10-week water treadmill training program (Table 2, illustrated in Figs. 5 and 6), except for ROI 4 (left-side semimembranosus muscle).

Both JFT altered AST levels, with no changes for CK. Furthermore, after the 10-week of training, significantly higher serum AST and CK values were observed (Table 3).

Throughout the training program, a significant increase in CK at baseline (Tr0) was observed, reaching its highest values in week 8 ($p =$ 0.0296). However, water treadmill activity did not induce significant acute changes in this serum enzyme ($p = 0.2306$) (Table 4).

Jump heights were recorded at 1.00 m. Significant differences were observed in trunk angle at take-off for both the simple vertical jumps and

**Table 1**
Body composition of horses subjected to a 10-week physical training program including water treadmill exercise.

| | PRETR | TR |
|---|---|---|
| Body weight (kg) | 550.5 ± 14.5[b] | 587.0 ± 41.9[a] |
| Left rump fat thickness (cm) | 0.69 ± 0.45 | 0.59 ± 0.40 |
| Body fat percentagem (%) | 11.9 ± 2.1 | 11.4 ± 1.9 |
| Fat mass (kg) | 65.4 ± 11.4 | 66.9 ± 10.3 |
| Fat-free mass (kg) | 485.1 ± 19.1[b] | 520.1 ± 42.2[a] |
| Right *Longissimus dorsi* thickness (cm) | 7.96 ± 1.00 | 8.33 ± 0.74 |
| Left *Longissimus dorsi* thickness (cm) | 8.32 ± 0.87 | 8.68 ± 0.98 |

*Note:* Different letters in the same line indicate a training effect according to the paired t-test ($p \leq 0.05$). PRETR, before the 10-week training program; TR, after the 10-week training program.

**Table 2**
Infrared thermography of horses subjected to a 10-week physical training program including water treadmill exercise.

| Temperature | Regions of interest | | | | | |
|---|---|---|---|---|---|---|
| | Brachiocephalicus m. | | Semitendinosus m. | | Semimembranosus m. | |
| | Left (ROI 1) | Right (ROI 2) | Left (ROI 3) | Right (ROI 6) | Left (ROI 4) | Right (ROI 5) |
| TMax, °C | | | | | | |
| PRETR | 28.9 ± 2.2$^{b}$ | 28.4 ± 2.9$^{b}$ | 29.1 ± 2.2$^{b}$ | 28.4 ± 2.3$^{b}$ | 31.9 ± 1.6 | 30.5 ± 2.1$^{b}$ |
| TR | 32.0 ± 1.0$^{a}$ | 32.2 ± 1.1$^{a}$ | 32.6 ± 1.4$^{a}$ | 32.4 ± 1.2$^{a}$ | 33.7 ± 1.1 | 33.7 ± 1.4$^{a}$ |
| TMin, °C | | | | | | |
| PRETR | 27.1 ± 2.6$^{b}$ | 26.9 ± 3.4$^{b}$ | 25.9 ± 2.1$^{b}$ | 25.2 ± 3.1$^{b}$ | 27.7 ± 2.6 | 27.1 ± 2.5$^{b}$ |
| TR | 30.9 ± 0.8$^{a}$ | 31.2 ± 1.3$^{a}$ | 30.6 ± 1.4$^{a}$ | 30.0 ± 1.0$^{a}$ | 30.8 ± 2.0 | 30.7 ± 2.1$^{a}$ |
| TMean, °C | | | | | | |
| PRETR | 27.9 ± 2.5$^{b}$ | 27.7 ± 3.2$^{b}$ | 27.8 ± 2.1$^{b}$ | 27.3 ± 2.4$^{b}$ | 29.9 ± 1.7 | 29.2 ± 2.3$^{b}$ |
| TR | 31.4 ± 0.8$^{a}$ | 31.7 ± 1.2$^{a}$ | 31.6 ± 1.5$^{a}$ | 31.4 ± 1.3$^{a}$ | 32.5 ± 1.2 | 32.4 ± 1.3$^{a}$ |

Note: Different lowercase letters in the same column for each variable indicate significant differences between PRETR and TR according to the paired t-test ($p \leq 0.05$). TMax, maximum temperature; TMin, minimum temperature; TMean, mean temperature; PRETR, before the 10-week training program; TR, after the 10-week training program.

the oxers, as well as in strike power for the simple vertical jumps (Table 5). As a preliminary evaluation, these analyses were conducted on the first three simple vertical jumps and the first oxer to minimize the cumulative fatigue effect commonly observed in the later jumps, which could potentially influence the measured variables.

The analysis of vertical trunk displacement during water exercise revealed no significant differences between the evaluated weeks, both at the walk and the trot (Table 6).

It is important to note that throughout the training protocol, the horses performed without any complications or signs of discomfort, demonstrating good physical fitness. The mean HRpeak recorded during the exercise test was 93.0 ± 22.8 beats/min, resulting in an average working HR range on the water treadmill between 55.8 and 74.4 beats/min. A progressive increase in individual and group water treadmill working speed was observed, starting at 8.3 km/h in week 2, 7.9 km/h in week 4, 11.0 km/h in week 6 and 11.2 km/h week 8.

The field test conducted at PRETR had a duration of 2.19 ± 0.29 minutes and a total distance of 335.0 ± 33.9 meters. At TR, the duration was 2.02 ± 0.12 minutes and a total distance of 348.3 ± 19.4 meters. All horses completed clearly the courses.

In general, the average environment temperature during PRETR and TR evaluation days was 13.2°C, with a relative air humidity of 74.9%. Throughout the training period, the mean environment temperature was 14.5°C and the relative air humidity of 79.1%. The average water temperature in the treadmill was 14.0 °C.

## 4. Discussion

The hypothesis of the present study was confirmed, as the inclusion of the aquatic treadmill promoted body weight gain, more specifically an increase in fat-free mass. Furthermore, IRT and CK findings showed changes consistent with muscle activation generated by drag work in water. The muscle strengthening produced by the training contributed to significant biomechanical changes, improving the elevation of the forelimbs and the propulsion of the hind limbs during the execution of the jump [35].

The drag force generated by working in water demands intense muscular effort, especially from the muscles involved in limb protraction and body mass propulsion [36–38]. In show jumping, these training protocols that increase muscle strength are crucial [39], as these animals must be able to generate the explosive power required to clear demanding obstacles at a relatively low speed and within a short period of time [7]. Moreover, the water height selection is critical when aiming to improve muscle activation, as well as duration of sessions and velocity of work [40,41]. Working in water at the selected height of ~35-40 cm (at the carpo-metacarpal joint area) recruited the propulsive musculature of the hindquarters, e.g., brachiocephalicus, semitendinosus, and semimembranosus muscles. As muscle activity increases, there is an increase in blood flow and capillarization to meet the higher metabolic demand [29,38,40,42,43] and this was reflected in the significant increases in surface temperatures recorded by IRT [28,44,45]. Although it is not considered a technique as objective as electromyography, IRT is a semi-qualitative technique frequently used to signalize inflammatory processes or muscle activation by exercise [28,42].

Increased lower limb muscle strength in humans has also been demonstrated through hydro gymnastics programs in elderly women [46]. Some studies have reported improvements of 7% in overall strength, 10.5% in hamstring muscle strength with water training in humans [47]. Furthermore, Bassett and Leach [48] reported a significant increase in core muscle strength after an 8-week aquatic training in gymnasts. In horses, there are few studies about muscle development and biomechanics patterns. Semitendinosus and semimembranosus muscles increase was observed after 8 weeks of aqua-training [49],

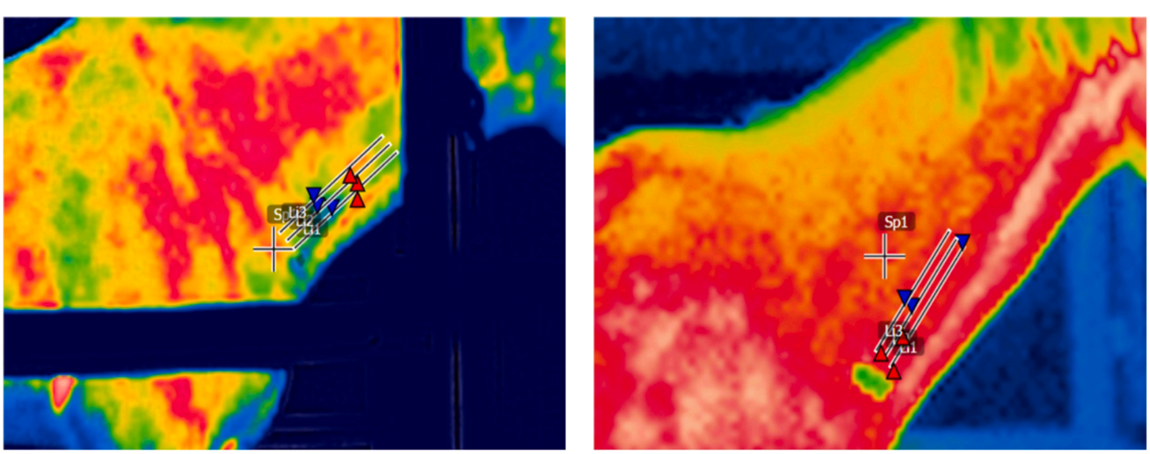


**Fig. 5.** IRT images of the right brachiocephalic muscle of one of the trained horses, collected before training (left) and after the 10-week training program (right), showing ROI 2. The thermographic images were displayed using a thermal color map, with temperatures represented by a scale ranging from 16°C (dark blue) to 38°C (whitish tones).

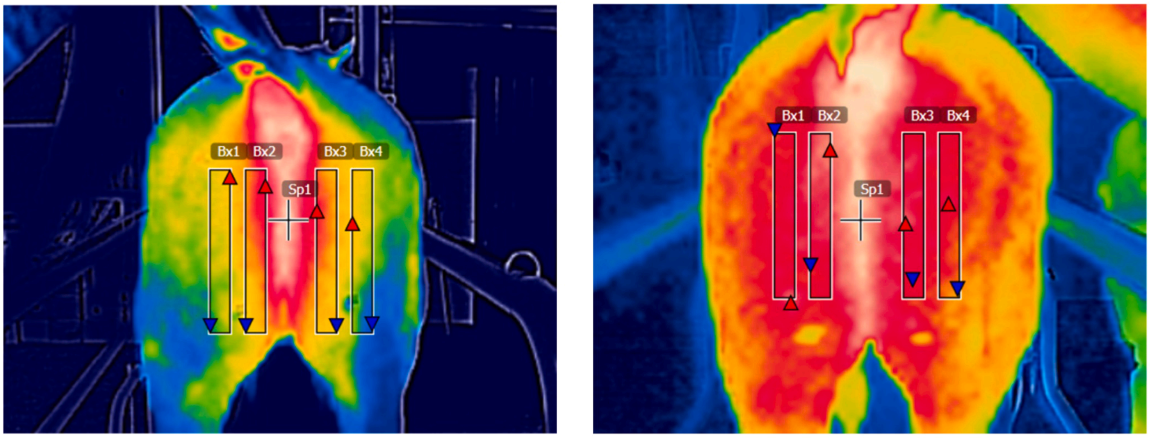


**Fig. 6.** IRT images of the semitendinosus and semimembranosus muscles of Horse 1, collected before training (left) and after training (right), showing ROIs 3, 4, 5, and 6, respectively. The thermographic images were displayed using a thermal color map, with temperatures represented by a scale ranging from 16°C (dark blue) to 38°C (whitish tones).

**Table 3**
Serum CK and AST (means ± standard deviations) of horses in show jumping field tests, used to evaluate a 10-week physical training program with the water treadmill.

| Parameters | Study period | | | | p | |
|---|---|---|---|---|---|---|
| | T0 | T1 | T2 | T3 | JFT | Training |
| CK, U/L | | | | | | |
| PRETR | 137.7 ± 25.9 | 153.3 ± 26.1 | 138.0 ± 23.7 | 150.0 ± 37.9 | 0.0841 | 0.0391 |
| TR | 180.6 ± 80.3 | 181.7 ± 69.5 | 158.5 ± 35.5 | 148.0 ± 37.5 | 0.3879 | |
| AST, U/L | | | | | | |
| PRETR | 285.0 ± 51.0$^{b}$ | 311.1 ± 44.4$^{a}$ | 284.8 ± 46.1$^{b}$ | 276.5 ± 40.8$^{b}$ | <0.0001 | <0.0001 |
| TR | 313.2 ± 57.2$^{bc}$ | 344.5 ± 56.5$^{a}$ | 316.2 ± 53.8$^{b}$ | 304.3 ± 53.4$^{c}$ | <0.0001 | |

*Note:* Different letters in the same line indicate the effect of physical activity (T0) according to Tukey's test ($p \leq 0.05$) and the effect of training according to the paired Student's t-test ($p \leq 0.05$). PRETR, before the training protocol; TR, after the training protocol. T0: rest; T1: immediately after exercise; T2: after 30 minutes of recovery; T3: after 240 minutes of recovery. AST, aspartate aminotransferase; CK, creatine kinase.

**Table 4**
Serum CK (means ± standard deviations) recorded over the 10-week water treadmill training program.

| | | S2 | S4 | S6 | S8 |
|---|---|---|---|---|---|
| CK, U/L | Tr0 | 127.7 ± 25.3$^{b}$ | 151.5 ± 48.2$^{ab}$ | 173.0 ± 41.4$^{ab}$ | 186.4 ± 72.9$^{a}$ |
| | Tr1 | 128.8 ± 33.2 | 160.2 ± 52.0 | 169.3 ± 57.7 | 128.8 ± 44.9 |

Note: Different letters in the same line indicate significant differences according to Tukey test ($p \leq 0.05$). S2, week 2; S4, week 4; S6, week 6; S8, week 8; Tr0, before treadmill training; Tr1, after 4 hours of recovery following the end of training.

**Table 5**
Biomechanical data recorded during show jumping field tests to evaluate a 10-week physical training program using the water treadmill.

| | PRETR | TR |
|---|---|---|
| | Simple vertical jumps | |
| Strike Power (G) | 2.53 ± 0.38$^{b}$ | 3.18 ± 0.38$^{a}$ |
| Trunk angle at take-off (°) | 20.0 ± 5.0$^{b}$ | 24.5 ± 5.4$^{a}$ |
| Vertical trunk displacement (cm) | 15.79 ± 2.07 | 16.28 ± 3.03 |
| Aerial phase duration (ms) | 665.3 ± 179.2$^{b}$ | 765.3 ± 41.1$^{a}$ |
| Speed (km/h) | 17.7 ± 2.9 | 16.1 ± 2.1 |
| HR (beats/min) | 110.2 ± 45.1 | 99.3 ± 22.8 |
| | Oxer jumps | |
| Strike Power (G) | 2.61 ± 0.43 | 3.17 ± 0.49 |
| Trunk angle at take-off (°) | 21.7 ± 6.4$^{b}$ | 26.1 ± 5.8$^{a}$ |
| Vertical trunk displacement (cm) | 16.67 ± 1.21 | 16.33 ± 2.50 |
| Aerial phase duration (ms) | 684.0 ± 205.3 | 793.3 ± 50.1 |
| Speed (km/h) | 17.2 ± 2.9 | 16.6 ± 1.7 |
| HR (beats/min) | 125.7 ± 35.8$^{a}$ | 114.0 ± 32.1$^{b}$ |

*Note:* Different letters in the same line indicate significant differences based on the paired Student's t-test ($p \leq 0.5$). PRETR, before the 10-week training program; TR, after the 10-week training program.

**Table 6**
Vertical trunk displacement recorded in horses during weeks 4 and 9 of water treadmill training.

| | S4 | S9 |
|---|---|---|
| At walk (cm) | 5.3 ± 2.6 | 3.6 ± 0.5 |
| At trot (cm) | 11.0 ± 3.7 | 8.6 ± 2.4 |

*Note:* Different letters in the same line indicate significant differences according to the paired Student's t-test ($p \leq 0.05$). S4, week 4 of training; S9, week 9 of training.

however evaluations were made using transcutaneous ultrasound differently from the present research. Conversely, a 4-week training with water at the level of the ventral abdomen did not improve muscle activity in horses of mixed breeds [41]. Differences can be attributed to the level of the imposed effort, including water level, speed, and duration of sessions and protocols [37].

It is important to emphasize that in the present study, there was an increase in work speed starting from the 6th week of training, respecting individual limits by using each animal's peak HR. In a previous study, it was suggested that exercise intensity and/or duration should be increased after 4 weeks of water treadmill inclusion [38]. This should be done to uphold the principle of training overreaching, aiming to maintain the stimulus that supports muscle development [2,3].

It is possible that muscle recruitment does not occur symmetrically. In a previous study [38], an asymmetric increase in muscle diameter of the semitendinosus and semimembranosus muscles was observed after aqua-training for 8 weeks, which may justify the absence of temperature changes in ROI 4. Additionally, some asymmetries may occur due to the animals' lateral preference during aquatic exercise. Unlike humans, more targeted muscle work in horses is challenging. To minimize such asymmetries, gait patterns during exercise should be evaluated, determining the best combination of water level and speed for each animal [50].

The muscle strengthening that occurred as a result of systematic and regular physical activity performed on the aquatic treadmill led to an increase in body weight, more specifically a gain in FFM [51]. This is an extremely advantageous result, since the main component of fat-free mass (FFM) is muscle mass and its increase is predictor of a greater potential force development and so it can improve athletic performance [52–55].

Another important finding was that the proposed protocol did not alter the thickness of the rump fat in the studied animals. Conversely, increases in lumbar subcutaneous fat thickness and at the base of the tail were described after 90 days of low-intensity aerobic exercise [53] and high-intensity exercises performed by Arabian horses led to a reduction in peripheral lumbar fat [30].

Longitudinal changes in body weight and composition, as observed after the inclusion of the aquatic treadmill, are not always found after training and they can be directly correlated with the intensity of the imposed effort [53,54], breed and environment conditions [55].

With increased strength, an enhancement of the propulsive forces of the stride would be expected. This strengthening of the hindlimbs propulsive muscles [44] was reflected in the increased strike power, a key parameter for optimal show jumping performance [56]. Previously, similar results showed an increased strike power in jumps performed on cross-country courses following water-based training [44]. However, in oxer jumps, strike power did not differ significantly, although the observed increase was clinically relevant for generating sufficient force at take-off. It should be noted that the take-off point cannot be identical for all horses due to anatomical differences [57].

Each obstacle, depending on its shape, type and height, requires specific combinations of approach speed, distance and trunk angle at take-off for the horse and rider to successfully clear it. Furthermore, the approach speed must be adjusted to the appropriate trunk position, allowing the hindlimbs to generate sufficient propulsion in the correct direction [57]. Although it has been suggested that higher speeds allow horses to adopt a more vertical body posture and to propel themselves from a greater distance relative to the obstacle [58], the improvement in body projection of the horses was not concomitant with an increase in speed in the present study. Possibly, the increase in angulation observed during the jumps, both simple vertical and oxer jumps, resulted from enhanced muscular strength developed through water-based training [44].

Regarding the duration of the aerial phase, an increase was observed for simple vertical jumps, indicating a longer parabolic trajectory. According to Leach et al. [59], the take-off angle is directly related to the extension of the aerial phase during a jump. Thus, by increasing the take-off angle, the horse achieves greater height, with its body movement becoming more vertical and consequently prolonging the duration of the aerial phase, as the horse requires more time to reach its peak height. These findings are consistent with the responses recorded following the water treadmill training. The wider the obstacle (e.g., oxer jump), the smaller the vertical component at the take-off [57]. This implies that, although a greater take-off angle is required to efficiently clear the width of an oxer, it does not necessarily result in a longer suspension phase. On the contrary, the horse focuses on efficiently covering the horizontal distance, which may lead to a shorter suspension phase, as the jump tends to be more horizontal than vertical. This may explain the non-significant increase in aerial phase duration for oxer jumps after water-based training. However, depending on the optimization of the take-off, the highest point reached during the trajectory may not perfectly align with the type of obstacle [57].

The drag force generated by working in the water enhances the activation not only of propulsive muscles, but also of stabilizing muscles of the spine [15]. However, not all muscles show the same adaptation to a certain type of training as muscle groups primarily involved in locomotion may show a different pattern of adaptation when compared to muscles involved in posture [38]. In the present research, no changes were detected for *Longissimus dorsi* width at the level of the carpus. Recruitment of this muscle group generally occurs during gaits at higher speeds and/or during exercises involving circles or turns [36,60,61], and also with higher water levels [15]. A study described an increase of back muscles profiles using a flexible curve ruler after water treadmill exercises at the level of the metacarpus for only four weeks [15], a result that differs from that observed in the present study. However, the aforementioned study employed a 4% incline, which may have activated the dorsal musculature in order to stabilize the back of the horses used.

This, however, does not rule out that work in the water resulted in improved dynamic control during out-of-water performance. Exercise in water enhances the afferent stimulation of the motor neuron pools controlling the muscles responsible for stabilizing the thoracic and pelvic limbs [24]. It is important to highlight that flexion and extension of the vertebral column are primarily driven by locomotor forces and controlled by muscular forces that act mainly to stabilize the spine [23,62]. Although no significant reduction in trunk vertical displacement was observed during the second field test (TR), improved postural control of these horses can be inferred, as the trunk remained stable despite increases in other parameters such as strike power and take-off angle. This postural stability is crucial in show jumping performance and indicates a positive adaptive response to the proposed training [24].

In the kinematic analysis of vertical trunk displacement during water sessions, no significant changes were observed between S4 and S9, although those can be considered clinically relevant. Usually, horses adapt their locomotion in the presence of water to minimize energy expenditure. At water depths reaching the level of the carpus, this adaptation is characterized by increased pelvic flexion [20] and a greater range of flexion–extension of the thoracolumbar spine [63]. These changes are primarily driven by enhanced hindlimb activity required to overcome water resistance and lift the limbs through the water [23]. However, even with an increased workload applied during the second half of the training protocol, when the horses worked at 80% of HRpeak and all progressed to trotting, a trunk stabilization was observed, probably due an increased afferent excitation of the motor neuron pool promoted by the water treadmill effort [23]. This data is very important since few published reports describe the use of trot in water treadmill protocols for horses [64,65].

Monitoring the serum enzymes CK and AST during both JFT and throughout the training program was essential to track the muscular adaptive responses to training. While CK did not change in response to the field tests in PRETR and TR, the same was not observed for AST. Even so, the rapid recovery indicates good physical conditioning. These changes occur due to alterations in blood volume [66] and oxidative stress resulting from a higher rate of glycolytic metabolism, which may cause increased muscle membrane permeability in response to exercise, without causing significant muscle damage [67].

Both enzymes showed significant increases in TR, when compared to values observed in PRETR. These results corroborate the thermographic findings, which are suggestive of increased muscular metabolism [29,68]. Repetitive and prolonged training, such as the protocol defined in this study, and particularly the challenge imposed by aquatic exercise, induced musculoskeletal adaptations. Exercise increases skeletal muscle mass through stimulation of new myofibril formation and an increase in the individual diameter of muscle fibers [69].

The assessments performed during training sessions, that is, during exercise in water, did not promote changes in CK. This result was unexpected, particularly in weeks 6 and 8, when workload intensity was higher, with working speeds at 80% of peak heart rate (HRpeak). It is possible that, even under intense effort, this was not sufficient to induce changes in sarcolemma permeability; alternatively, water temperature may promote greater control of the inflammatory process generated by aquatic treadmill exercise, delaying the release of enzymes and muscle products into the bloodstream.

However, an increase in baseline CK values (Tr0) was observed throughout the training period, possibly indicating that muscle growth occurred progressively in accordance with the increasing workload. These findings suggest that the training was effective in promoting muscle development without causing injury. The absence of such alterations may indicate an efficient muscle recovery capacity, greater ability to remove metabolic waste, and better control of the inflammatory response [68].

There are some limitations of the study. Although the selected jump height does not correspond to high-performance levels, it reflects the most commonly used height in equestrian training facilities and riding centers. This approach ensured animal safety while maintaining the consistency and validity of the training protocol. Also, no control group was used, similarly to previous studies in horses [15,54]. Due to the logistical complexity, costs, and need for strict standardization in longitudinal equine training studies, the sample size was necessarily limited. To address this constraint, we adopted a paired within-horse design, in which each horse served as its own control, thereby improving precision and minimizing between-subject variability. Accordingly, we prioritized estimation—reporting mean paired changes and 95% confidence intervals—alongside conventional hypothesis testing. Nevertheless, outcomes with more moderate effect sizes may be underpowered at a sample size of six and should be interpreted with caution, warranting confirmation in larger cohorts where possible [27].

## 5. Conclusions

In conclusion, the inclusion of the water treadmill within a varied training protocol promoted changes on body composition, with a statistically significant gain in fat-free mass, and a strengthening of the muscles that substantially influenced the locomotory response.

After the 10 weeks of training that incorporated the water treadmill, it was observed a stronger core to elevate the forelimb and a greater strength in the propulsive hind limb muscles to increase strike power, highly relevant characteristics for jumping performance.

Future research is warranted to substantiate these results, ideally involving larger sample sizes, a broader range of equine disciplines and breeds, and training intensities (e.g., varying jump heights), and extended follow-up periods to provide a more comprehensive understanding of the effects of this approach, including the impact factors like age and gender.

## CRediT authorship contribution statement

**C. Nascimento:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **C. Santos:** Methodology, Investigation. **M. Walton:** Methodology, Investigation. **T. Gonçalves:** Methodology, Investigation. **G. Freire:** Methodology, Investigation. **M. Nicolau:** Methodology, Investigation. **F. Silvestre:** Methodology, Investigation. **J. Simões:** Writing – review & editing, Supervision. **J. Borges:** Methodology, Investigation. **N. Pires:** Writing – original draft, Conceptualization. **H. Manso Filho:** Writing – original draft, Conceptualization. **H. Clayton:** Formal analysis, Data curation. **C. Coelho:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

Funding sources: FMV – Lusofona University, n. TREINWATERTREADMILL

Preliminary results were presented as an Abstract at the British Equine Veterinary Association Congress, Birmingham, 10-13 September 2025.

## References

[1] Clayton HM. Conditioning sport horses. Sport Horse Publications, Michigan; 1991.
[2] Castejon-Riber C, Riber C, Rubio MD, Agüera E, Muñoz A. Objectives, principles, and methods of strength training for horses. J Eq Vet Sci 2017;56:93–103. https://doi.org/10.1016/j.jevs.2017.04.011.
[3] McKenzie E. Foundations of performance – factors that contribute to excellence in equine exercise. Comp Exerc Physiol 2017;13:127–35. https://doi.org/10.3920/CEP170022.
[4] Davies Z. Exercise physiology, functional anatomy and conformation. In: Mcilwraith CH, Merritt BT, editors. Equine science. 3rd ed. Wiley- Blackwell, New Jersey; 2017. p. 365–90.
[5] Latham CM, Owen RN, Dckson EC, Guy CP, White-Springer SH. Skeletal muscle adaptations to exercise training in young and aged horses. Front. Aging 2021;2:708918. https://doi.org/10.3389/fragi.2021.708918.
[6] Nightingale TE, Rouse PC, Walhin JP, Thompson D, Bilzon JLJ. Home-based exercise enhances health-related quality of life in persons with spinal cord injury: a randomized controlled trial. Arch Phys Med Rehabil 2018;99:1998–2006.e1. https://doi.org/10.1016/j.apmr.2018.05.008.
[7] Kirsch K, Fercher C, Horstmann S, von Reitzenstein C, Augustin J, Lagershausen H. Monitoring performance in show jumping horses: validity of non-specific and discipline-specific field exercise tests for a practicable assessment of aerobic performance. Front Physiol 2022;12:818381. https://doi.org/10.3389/fphys.2021.818381.
[8] Barrey E, Galloux P. Analysis of the equine jumping technique by accelerometry. Eq Vet J 1997;29(S23):45–9.
[9] Clayton HM. Time-motion analysis of show jumping competitions. J Eq Vet Sci 1996;16:262–6. https://doi.org/10.1016/S0737-0806(96)80195-X.
[10] Voss B, Mohr E, Krzywanek H. Effects of aqua-treadmill exercise on selected blood parameters and on heart-rate variability of horses. J Vet Med A 2002;49:137–43. https://doi.org/10.1046/j.1439-0442.2002.00420.x.
[11] Vincze A, Szabo C, Veres S, Uto D, Hevesi AT. Fitness improvement of show jumping horses with deep water treadmill training. Vet Med 2017;62:192–9. https://doi.org/10.17221/135/2016-VETMED.
[12] Greco-Otto P, Bond S, Sides R, Kwong GPS, Bayly W, Léguillette R. Workload of horses on a water treadmill: effect of speed and water height on oxygen consumption and cardiorespiratory parameters. BMC Vet Res 2017;13:360. https://doi.org/10.1186/s12917-017-1290-2.
[13] Scott R, Nankervis K, Stringer C, Westcott K, Marlin D. The effect of water height on stride frequency, stride length and heart rate during water treadmill exercise. Eq Vet J 2010;42:662–4. https://doi.org/10.1111/j.2042-3306.2010.00194.x.
[14] Murray RC, Hopkins E, Tacey JB, Nankervis KJ, Deckers I, MacKechnie-Guire R, Tranquille CA. Change in muscle development of horses undergoing 20 weeks of water treadmill exercise compared with control horses. Eq Vet J 2020;52:9. https://doi.org/10.1111/evj.09_13365.
[15] Fair N, Blake S, Blake R. Four weeks of incline water treadmill exercise can contribute to increase epaxial muscle profile in horses. Vet Med Int 2023;8:9090406. https://doi.org/10.1155/2023/9090406.
[16] Fraschetto C, Hatrise C, Miroud C, Beaumont A, Audigié F, Chateau H, Denoix JM, Jacquet S. Water depth and speed may have an opposite effect on the trunk vertical displacement in horses trotting on a water treadmill. Am J Vet Res 2023;85:1–8. https://doi.org/10.2460/ajvr.23.10.0236.
[17] Tranquille C, Nankervis K, Tacey J, Hopkins E, Deckers I, Walker V, MacKechnie-Guire R, Newton R, Murray R. Does muscle development of sport horses using water treadmill exercise as part of a long-term training programme differ from that of horses not using water treadmill exercise? Animals 2025;15:2426. https://doi.org/10.3390/ani15162426.
[18] Muñoz A, Becero M, Saitua A, Argüelles D, Medina A, Castejón-Riber C. Exercise in the water: should we incorporate it into training and rehabilitation programs for the sport horse? Atti Accad Peloritana dei Pericol Med-Biolog 2019;107:1–11. https://doi.org/10.6092/1828-6550/APMB.107.2.2019.SD3.
[19] Greco-Otto P, Baggaley M, Edwards WB, Léguillette R. Water treadmill exercise reduces equine limb segmental accelerations and increases shock attenuation. BMC Vet Res 2019;15:329. https://doi.org/10.1186/s12917-019-2075-6.
[20] Mooij MJW, Jans W, den Heijer GJL, de Pater M, Back W. Biomechanical responses of the back of riding horses to water treadmill exercise. The Vet J 2013;198:e120–3. https://doi.org/10.1016/j.tvjl.2013.09.045.
[21] Nankervis K, Lefrançois K. A comparison of protraction-retraction of the distal limb during treadmill and water treadmill walking in horses. J Eq Vet Sci 2018;70:57–62. https://doi.org/10.1016/j.jevs.2018.08.005.
[22] McCrae P, Bradley M, Rolian C, Léguillette R. Water height modifies forelimb kinematics of horses during water treadmill exercise. Comp Exerc Physiol 2021;17:91–8. https://doi.org/10.3920/CEP200013.
[23] Nankervis K, Tranquille C, Tacey J, Deckers I, MacKechnie-Guire R, Walker V, Hopkins E, Newton R, Murray R. Kinematic responses to water treadmill exercise when used regularly within a sport horse training programme: a longitudinal, observational study. Animals 2024;14:2393. https://doi.org/10.3390/ani14162393.

[24] King MR, Haussler KK, Kawcak CE, McIlwraith CW, Reiser RF. Mechanisms of aquatic therapy and its potential use in managing equine osteoarthritis. Eq Vet Educ 2013;25:204–9. https://doi.org/10.1111/j.2042-3292.2012.00389.x.

[25] Mendez-Angulo JL, Firshman AF, Groschen DM, Kieffer PJ, Trumble TN. Effect of water depth on amount of flexion and extension of joints of the distal aspects of the limbs in healthy horses walking on an underwater treadmill. Am J Vet Res 2013; 74:557–66. https://doi.org/10.2460/ajvr.74.4.557.

[26] Van Weeren PR. Kinematics of the equine back. In: Henson FMD, editor. Equine back pathology: diagnosis and treatment. New Jersey: Wiley-Blackwell; 2009. p. 41–2.

[27] Nascimento C, Braz AL, Barbosa I, Freire G, Nicolau M, Silvestre F, Manso Filho H, Simões J, Estepa JC, Clayton H, Coelho C. Cardiac morphologic changes induced by a 10-week water treadmill training for show jumping horses: a preliminary evaluation. The Vet J 2026;317:106642. https://doi.org/10.1016/j.tvjl.2026.106642.

[28] Witkowska-Pilaszewicz O, Masko M, Domino M, Winnicka A. Infrared thermography correlates with lactate concentration in blood during race training in horses. Animals 2020;10:2072. https://doi.org/10.3390/ani10112072.

[29] Soroko M, Spitalniak-Bajerska K, Zaborski D, Pozniak B, Dudek K, Janczarek I. Exercise-induced changes in skin temperature and blood parameters in horses. Arch Anim Breed 2019;62:205–13. https://doi.org/10.5194/aab-62-205-2019.

[30] D'Angelis FHF, Mota MDS, Freitas EVV, Ferraz GC, Abrahão AR, Lacerda-Neto JC, Queiroz-Neto A. Ultrassonography images of the longissimus dorsi muscle of Arabian horses in endurance training associated to chronically supplemented with creatine. Re Bras Ci Vet 2004;12:142–6. https://doi.org/10.4322/rbcv.2014.320.

[31] Gentry LR, Thompson DL, Gentry GT, Del Vecchio RP, Davis KA, Del Vecchio PM. The relationship between body condition score and ultrasonic fat measurements in mares of high versus low body condition. J Eq Vet Sci 2004;24:198–203. https://doi.org/10.1016/j.jevs.2004.04.009.

[32] Westervelt RG, Stouffer JR, Hintz HF, Schryver HF. Estimating fatness in horses and ponies. J Anim Sci 1976;43:781–5. https://doi.org/10.2527/jas1976.434781x.

[33] Manso HCF, Manso HECCC, Ferreira LMC, Santiago TA, Wanderley EK, Abreu JMG. Percentagem de gordura de cavalo criados em região tropical. Acta Sci Vet 2009;37:239–43. https://doi.org/10.22456/1679-9216.16336.

[34] Guyard KC, Montanvon S, Bertolaccini J, Deriaz M. Validation of alogo move pro: a GPS-based inertial measurement unit for the objective examination of gait and jumping in horses. Sensors 2023;23:4196. https://doi.org/10.3390/s23094196.

[35] De Oliveira K, Soutello RVG, Da Fonseca R, Costa C, Meirelles PRDL, Fachiolli DF, Clayton HM. Gymnastic training and dynamic mobilization exercises improve stride quality and increase epaxial muscle size in therapy horses. J Eq Vet Sci 2015; 35:888–93. https://doi.org/10.1016/j.jevs.2015.08.006.

[36] Prankel S. Hydrotherapy in practice. In Practice 2008:30-272-277.

[37] Nankervis KJ, Launder EJ, Murray RC. The use of treadmills within the rehabilitation of horses. J Eq Vet Sci 2017;53:108–15. https://doi.org/10.1016/j.jevs.2017.01.010.

[38] Meêus d'Argenteuil C, Boshuizen B, Oosterlinck M, de Winkel D, De Spiegelaere W, Bruijn CM, Goethals K, Vanderperren K, Delesalle CJG. Flexibility of equine bioenergetics and muscle plasticity in response to different types of training: an integrative approach, questioning existing paradigms. PLoS ONE 2021;16: e0249922. https://doi.org/10.1371/journal.pone.0249922.

[39] Rose RJ, Evans DL. Training horses- art or science? Eq Vet J 1990;22:2–4.

[40] Leisson K, Jaakma Ü, Seene T. Adaptation of equine locomotor muscle Fiber types to endurance and intensive high speed training. J Eq Vet Sci 2008;28:395–401.

[41] Borgia LA, Valberg SJ. Essen-Gustavsson B. Differences in the metabolic properties of gluteus medius and superficial digital flexor muscles and the effect of water treadmill training in the horse. Eq Vet J 2010;42:665–70. https://doi.org/10.1111/j.2042-3306.2010.00229.x.

[42] Redaelli V, Luzi F, Mazzola S, Bariffi GD, Zappaterra M, Costa LN, Padalino B. The use of infrared thermography (IRT) as stress indicator in horses trained for endurance: a pilot study. Animals 2019;9:84. https://doi.org/10.3390/ani9030084.

[43] Vermeulen R, Meeûs C, Plancke L, Boshuizen B, Bruijn M, Delesalle C. Effects of training on equine muscle physiology and muscle adaptations in response to different training approaches. Vlaams Dierg Tijd 2017;86:224–30. https://doi.org/10.21825/vdt.v86i4.16183.

[44] Tranquille CA, Nankervis KJ, Walker VA, Tacey J, Murray R. Current knowledge of equine water treadmill exercise: what can we learn from human and canine studies? J Eq Vet Sci 2017;50:76–83. https://doi.org/10.1016/j.jevs.2016.10.011.

[45] Mescher AL. Muscle tissue. Junqueira's basic histology: text and atlas. McGraw-Hill Education, New York; 2018.

[46] Costa RR, Kanitz AC, Reichert T;, Prado AKG, Coconcelli L, Buttelli ACK, Pereira LF, Masiero MPB, Meinerz AP, Conceição MO, Sbeghen IL, Kruel LFM. Water-based aerobic training improves strength parameters and cardiorespiratory outcomes in elderly women. Exp Gerontol 2018;108:231–9. https://doi.org/10.1016/j.exger.2018.04.022.

[47] Pöyhönen T, Sipilä S, Keskinen KL, Hautala ARTO, Savoilainen J, Mälkiä ESKO. Effects of aquatic resistance training on neuromuscular performance in healthy women. Med Sci Sports Exerc 2002;34:2103–9. https://doi.org/10.1249/01.MSS.0000039291.46836.86.

[48] Bassett SH, Leach LL. The effect of an eight-week training programme on core stability in junior female elite gymnasts. Afr J Phys Health Edu Recreat Dance 2011;17:567. https://doi.org/10.4314/AJPHERD.V17I3.68067.

[49] de Winkel D, Bruijn M, Touwen N, Duchateau L, Goethals K, Oosterlinck M, Pille F, Vanderperren K, Delesalle C. Morphological changes in 15 skeletal muscles of horses after 8 weeks of aquatraining. Eq Vet J 2016;48:30.

[50] Nankervis K, Tranquille C, McCrae P, York J, Lashley M, Baumann M, King M, Sykes E, Lambourn J, Miskimmin KA, et al. Consensus for the general use of equine water treadmills for healthy horses. Animals 2021;11:305. https://doi.org/10.3390/ani11020305.

[51] Abreu JMG, Manso Filho HC, Manso HECC. Body composition on working horses. Cienc Animal Bras 2009;10:1122–7. https://doi.org/10.5216/cab.v10i4.5709.

[52] Kearns CF, McKeever KH, Kumagai K, Abe T. Fat-free mass is related to one-mile race performance in elite standardbred horses. The Vet J 2002;163:260–6. https://doi.org/10.1053/tvjl.2001.0656.

[53] Gobesso AAO, Martins RADT, Gil PCN, Françoso R, Gonzaga IVF. Avaliação de escore corporal em equinos através da ultrassonografia. Braz J Res Animal Sci 2014;51:136–41. https://doi.org/10.11606/issn.2318-3659.v51i2p136-141.

[54] Klein DJ, Anthony TG, McKeever KH. Changes in maximal aerobic capacity, body composition, and running capacity with prolonged training and detraining in standardbred horses. Comp Exerc Physiol 2020;16:187–96. https://doi.org/10.3920/CEP190046.

[55] Padilha FGF, Dimache LAG, Almeida FQ, Ferreira AMR. Blood biochemical parameters of Brazilian sport horses under training in tropical climate. R Bras Zootec 2017;46:678–82. https://doi.org/10.1590/S1806-92902017000800008.

[56] Schlup E, Godoi FN, Andrade AM, Bergmann JAG, Almeida FQ. Efeito do treinamento sobre a cinemática de equinos no salto de obstáculos. Arq Bras Med Vet Zootec 2014;66:1647–54. https://doi.org/10.1590/1678-7442.

[57] Fercher C. The biomechanics of movement of horses engaged in jumping over different obstacles in competition and training. J Eq Vet Sci 2017;49:69–80. https://doi.org/10.1016/j.jevs.2016.10.002.

[58] Powers PNR, Harrison AJ, Storey NB. Kinematic analysis of take off parameters during loose jumping in young untrained horses. In: Proceedings of the 1999 International Symposium on Biomechanics in Sports. Perth: ISBS; 1999. p. 101–4.

[59] Leach DH, Ormrod K, Clayton HM. Stride characteristics of horses competing in Grand Prix jumping. Am J Vet Res 1984;45:888–92.

[60] Wakeling JM, Barnett K, Price S, Nankervis K. Effects of manipulative therapy on the longissimus dorsi in the equine back. Equine Comp Exerc Physiol 2006;3: 153–60.

[61] Cottriall S, Ritruechai P, Wakeling JM. The effects of training aids on the longissimus dorsi in the equine back. Comp Exerc Physiol 2008;5:111. https://doi.org/10.1017/S1478061509342346.

[62] Robert C, Valette JP, Poucelot P, Audigié F, Denoix JM. Effects of treadmill speed on the mechanics of the back in trotting saddlehorses. Eq Vet J 2001;33:154–9. https://doi.org/10.1111/j.2042-3306.2001.tb05380.x.

[63] Nankervis K, Finney P, Launder L. Water depth modifies back kinematics of horses during water treadmill exercise. Equine Vet J 2016;48:732–6. https://doi.org/10.1111/evj.12519.

[64] Moiroud C, Jacquet S, Parmentier M, et al. Influence de la hauteur d'eau et de la vitesse sur la locomotion du cheval au pas et au trot sur un tapis roulant immergé. In: Proceedings of the 2018 Journées Annuelles de l'AVEF (Association des Vétérinaires Équins Français). Bordeaux: AVEF; 2018. p. 96–7.

[65] York J, Walker A. Vertical displacement of the equine pelvis when trotting on an aqua treadmill. Equine Vet J 2016;48:34–5. https://doi.org/10.1111/evj.69_12595. –55.

[66] Silva CJFL, Trindade KLG, Cruz RKS, Vilela CF, Coelho CS, Ribeiro Filho JD, Manso HECCC, Manso Filho HC. Association between infrared thermography, blood count and creatine kinase in the evaluation of the welfare of Vaquejada horses. Open J Vet Med 2023;13:53–67. https://doi.org/10.4236/ojvm.2023.136006.

[67] Allen KJ, van Erck-Westergren E, Franklin SH. Exercise testing in the athlete. Eq Vet Educ 2016;28:89–98. https://doi.org/10.1111/eve.12410.

[68] Valberg SJ. Muscle anatomy, physiology, and adaptations to exercise and training. In: Hodgson D, McGowan C, McKeever K, editors. The athletic horse: principles and practice of equine sports medicine. Saunders: Elsevier; 2014. p. 174–201.

[69] Bryan K, McGivney BA, Farries G, McGettigan PA, McGivney CL, Gough KF, MacHugh DE, Katz LM, Hill EW. Equine skeletal muscle adaptations to exercise and training: evidence of differential regulation of autophagosomal and mitochondrial components. BMC Genom 2017;18:595. https://doi.org/10.1186/s12864-017-4007-9.