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Advances in the application of technology for monitoring horse welfare and health

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Advances in the application of technology for monitoring horse welfare and health

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Editorial: Advances in the application of technology for monitoring horse welfare and health

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KEYWORDS

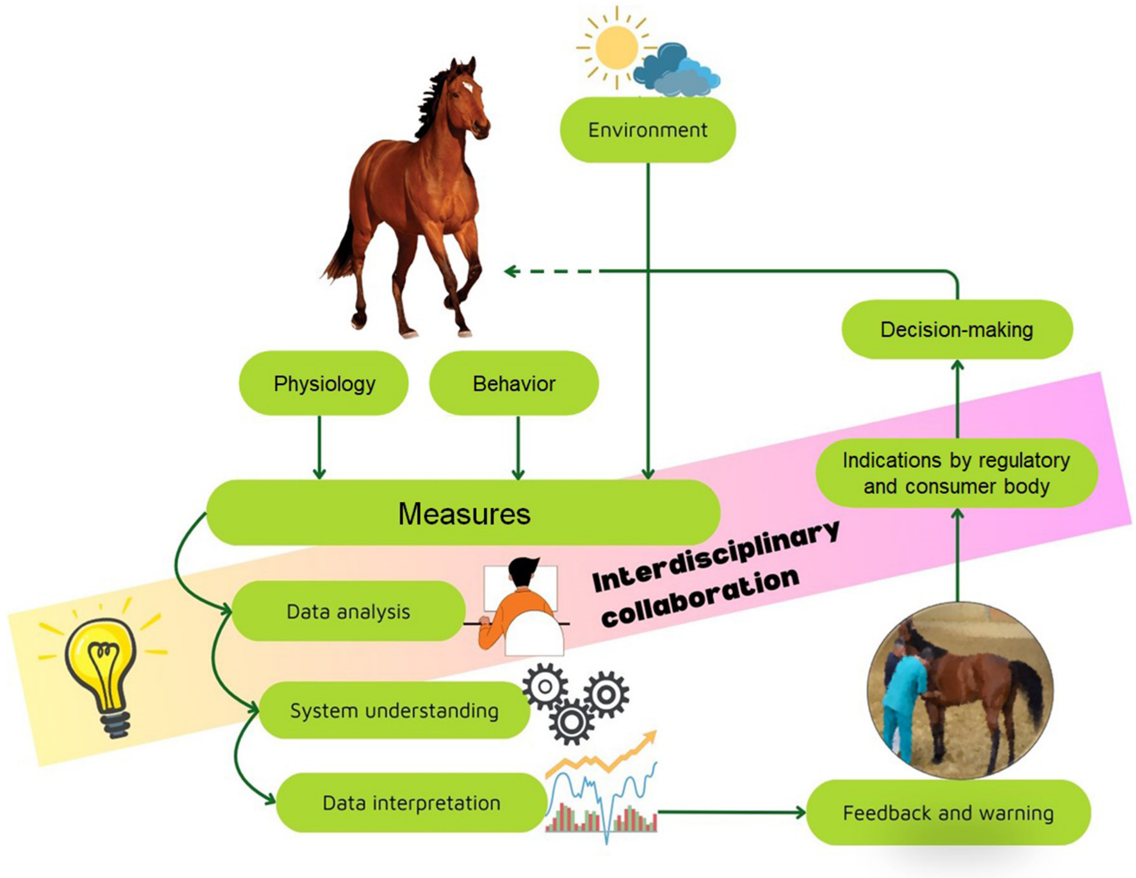
equine monitoring, wearable sensors, computer vision, horse behavior, PLF

Editorial on the Research Topic
Advances in the application of technology for monitoring horse welfare and health

The dynamic role of horses in today's society, from sports and leisure riding to therapeutic activities and companionship, underscores the critical need for effective monitoring of their health and welfare. Horses are highly sensitive animals, and, similar to other species, their wellbeing has a direct impact on their performance, longevity, and quality of life. In recent years, animal welfare has become a topic of increasing interest, evolving into an issue of scientific, regulatory, and ethical significance. This has stimulated intense research and development in various areas, including laboratories, farms, zoos, and the care of companion and working animals. This increased attention reflects a growing awareness of the need to improve the living conditions of animals while promoting environmental sustainability (1–4). In equines, welfare assessment is still a challenge, given the complexity of integrating multiple indicators into a representative framework (5–7).

To date, equine welfare is assessed through direct observation of animal-based measures, such as body condition or the presence of injuries along with physiological indicators such as blood cortisol concentrations. However, these approaches have significant limitations, including the time needed for the assessment, the necessity of training new assessors to ensure reliable evaluations, and the inability to ensure continuous monitoring (8, 9).

These limitations have led to an increasing exploration of innovative technologies for monitoring equine welfare. Advances in livestock farming technologies, including wearables, environmental sensors, and computer vision, could transform the management practices for domestic animals, including horses. These technologies could provide not only real-time health monitoring but also early detection of anomalies that may indicate disease, which would be invaluable for research, veterinary care, and horse owners alike. While several promising technologies, such as accelerometers, pressure sensors, and video surveillance, have demonstrated high potential in research settings, many have yet to transition successfully into practical, everyday applications. In fact, the majority of these technologies have been tested and validated for research purposes, but few of them are, to date, applicable to daily management. In an operational environment, these technologies still face some limitations (3, 8). Thus, at least for now, the great theoretical potential of these new technologies has had little impact on reality.



GRAPHICAL ABSTRACT

Within this context, the present editorial summarizes the main contributions collected in this Research Topic, which aims to explore the evolution, validation, and on-the-ground application of technology for monitoring horse health and welfare (see Graphical Abstract). This Research Topic consists of a collection of ten studies that deal with advancing the integration of new technologies into routine use. This in order to ensure that new technologies meet the high standards required for accuracy and consistency, so bridging the gap between experimental research and real-world application. In the context of precision farming, wearable technologies are among the most widely studied innovations for monitoring equine welfare. These devices are typically fitted to harnesses, girths, or collars, and are designed to capture continuous physiological and biomechanical data. Accelerometers, gyroscopes, and GPS sensors record locomotion, gait patterns, and workload intensity, allowing trainers to tailor exercise regimens to individual horses. Such systems are critical for detecting subtle asymmetries or irregularities that may signal early lameness. Lameness remains one of the most pressing welfare concerns in horses, often resulting in a reduced quality of life and compromised performance. Technological advances in monitoring enable the automated detection and modification of gait abnormalities. Three studies investigated horse gaits and the relation to morphological aspects and work tasks (Zupan Šemrov et al.), kinematic modifications and training fatigue (Siegers et al.) and inertial measures for different breeds (Asti et al.). The ability to monitor changes in daily activity patterns provides valuable insight into welfare indicators such as stress, discomfort, or illness. Connected to the need to evaluate the effects of training sessions are the studies by Wonghanchao et al., monitoring the stress response via heart rate variability (HRV) during consecutive days of jumping competitions, and Garcia Carvalho et al., evaluating the response to a single whole-body vibration session as a recovery measure. The results provided in these articles highlight the need to be mindful of potential stress that could, at least in part, impact the welfare of horses participating in close-time competitions and propose robust methodologies to estimate it. In addition, Itoh et al. proposed and applied a novel method for non-invasively recording multichannel electroencephalography with the aim of demonstrating the feasibility and validity of this approach for investigating brain function in horses, paving the way for further applications, such as examining cognitive abilities or brain disorders in horses. The objective of the manuscript published by Tucker et al. was to apply computational fluid dynamics analysis to an equine head inhalation model that replicates recurrent laryngeal neuropathy (RLN): the authors evaluated fluid dynamics impedance for four surgical procedures. Troillet and Scharner provided a detailed description and exhaustive documentation of a cohort of five horses that were successfully treated with an incomplete bypass procedure, demonstrating positive long-term outcomes and the advancements in surgical techniques by implementing the closure of the mesenteric gap.

Technologies are now also integrated into equine living environments to improve welfare. Stable-based sensors monitor critical air quality parameters such as temperature, humidity, and ammonia concentration, which are critical for respiratory health. Automated surveillance cameras, coupled with AI-based behavior recognition, can detect stereotypic behaviors such as cribbing or weaving, which often signal stress or poor welfare. IoT frameworks that combine individual wearables with environmental sensors create holistic welfare assessments at both the individual and group levels (10). In this dynamic context, Gobbo et al. explored the methods to assess positive horse behavior in relation to their environment to provide information that enhances animal welfare. With the adoption of accelerometers, the authors achieved a non-invasive continuous monitoring of lying behavior, thus suggesting a way to continuously monitor horse behavior in relation to their management and housing. Finally, in a recent article, Velineni et al. tested a new glucometer and reported that this new generation of tools represents an improvement over its predecessor and represents a reliable and cost-effective method for accurately monitoring blood glucose levels in horses in farm, clinic, or laboratory environments.

Author contributions

ED: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. MB: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Conflict of interest

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Accuracy and validation of a point-of-care blood glucose monitoring system for use in horses

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Abnormal blood glucose (BG) levels often seen in critically ill horses are significantly associated with adverse patient outcomes and increased mortality. Rapid and accurate BG monitoring is now considered an essential component of evidence-based equine practice and can provide critical information quickly for treatment. Although several point-of-care (POC) BG monitoring hand-held devices are commercially available for veterinary use, none contains a unique algorithm validated for use in horses. The AlphaTrak 3 (AT3) BG monitoring system is a first-of-its-kind device with an equine-specific algorithm that allows stall-side clinical decision making, and frequent monitoring at minimal cost. As such, AT3 is potentially a preferred alternative to more costly and time-consuming standard diagnostic reference laboratory methods. The objective of this study was to determine the accuracy of the AT3 device in measuring BG levels in equine whole blood samples in comparison to results obtained by the Beckman Coulter AU480 reference analyzer per ISO15197:2013 specifications. Accuracy of the AT3 equine algorithm were initially verified by testing equine blood samples with artificially adjusted blood glucose levels followed by its validation in a field study. Testing with artificially adjusted equine samples (n=129) showed that 98.9% of glucose measurements ranging from 29 to 479 mg/dL fell within ISO accuracy threshold of ±15mg/dL or ±15% of the average reference value. In addition, 100% of the AT3 measurements fell in consensus error grid (CEG) zone A, which indicates that test outcomes have a minimal likelihood of adverse clinical impact. In a follow-up field study involving 96 horses, 98.4% of AT3 measurements met the ISO accuracy threshold and 99.2% of AT3 measurements fell in CEG zone A. These results demonstrate that the AT3 glucometer has a high degree of accuracy in horses and is a dependable, convenient, and cost-effective device for accurately monitoring equine BG levels in farm or clinical settings.

KEYWORDS

AlphaTrak 3, blood glucose monitoring, equine, hypoglycemia, hyperglycemia, glucometer, point-of-care, ISO15197:2013

1 Introduction

Blood glucose (BG) is tightly regulated in healthy horses, with normal reference ranges variously established at 62–134 mg/mL (3.4–7.4 mm/L), 76–131 mg/dL (4.2–7.3 mm/L), and 70–135 mg/dL (3.9–7.4 mm/L) depending on the source (1, 2). Hypoglycemia, hyperglycemia and glucose variability, on the other hand, are associated with a variety of morbidities in horses. For example, prior studies found that septic and critically ill foals with relatively low or hypoglycemic BG concentrations at presentation had significantly reduced survival rates from birth to 96 h compared to foals with higher BG levels (3, 4). A study of critically ill neonatal foals ($n = 515$) presented at veterinary school hospitals found that >70% were either hypoglycemic (34.4%) or hyperglycemic (36.5%) at the time of admission (5). Glucose dysregulation in horses is known to be associated with acute gastrointestinal disease and suboptimal survival rates (1, 2). In one study, >50% of horses ($n = 269$) presenting with acute abdominal disease were hyperglycemic and had significantly reduced short-term ($p < 0.05$) and long-term ($p < 0.016$) survivability (2).

A discordant BG level by itself may not have been the primary cause of increased mortality in these studies. However, the authors collectively concluded that BG appears to be a strong and readily accessible surrogate indicator of various biological factors that contribute to equine mortality (1, 2). It is apparent that the extent and duration of equine BG concentrations outside the normal range are significantly associated with adverse patient outcomes, poor prognosis, reduced post-diagnosis duration of survival, and increased mortality.

As the role of BG dynamics in equine homeostasis and disease etiology is becoming better understood, rapid and accurate methods of glucose monitoring are considered an essential component of evidence-based equine practice. Particularly in critical care settings, serial on-site BG monitoring enables veterinarians to promptly identify hypo- and hyperglycemia in horses and initiate rapid intervention, including glucose regulation and other treatment protocols. The need for aggressive BG monitoring is increasingly relying on point-of-care (POC), stall-side glucometers as a preferred alternative to more costly and less timely analysis by diagnostic reference laboratories.

Portable, hand-held POC glucometers are the most commonly used devices for BG monitoring in clinical practice and at-home (6, 7). While portable POC glucometers have been in widespread use for diabetes management in human healthcare since the 1970s (8), these devices have been adopted more gradually in veterinary medicine. In the past two decades, studies have evaluated the accuracy of POC glucometers in cats, dogs, calves, laboratory animals, and non-human primates, with varying degrees of diagnostic accuracy (8–12). Particularly in equine critical-care cases where BG dysregulation is associated with increased mortality (1–4), the importance of accurate and prompt BG monitoring is being increasingly recognized. In horses, accuracy of portable POC glucometers has been reported in numerous studies in the U.S., Europe, and Australia (5, 9, 11, 13–19).

In most cases, POC glucometers evaluated in animals were originally developed for use in humans rather than in domestic animal species (14). However, expert opinion suggests that human glucometers should be avoided for measuring BG levels in animals because of the lack of equivalence in interspecies glucose distribution (14, 20). For example, glucose distribution in humans is closely divided between plasma (58%) and erythrocytes (42%), while plasma glucose distribution is 93% in cats, 87.5% in dogs, and 84% in rats (8, 14). Others have noted that species-specific pathophysiology may create bias in BG concentration (15), and that species differences in erythrocyte morphology and size may affect erythrocyte glucose concentration (8, 10). For these reasons, current canine and feline diabetes management guidelines go so far as to recommend against using human glucometers in dogs and cats (20). The preferred alternative is to use POC glucometers that have been specifically validated for each species in which they are used.

The on-market AlphaTrak 2 (AT2) BG monitoring system (Zoetis, Parsippany, NJ, USA) is an accurate, easy-to-use, handheld POC glucometer that requires a small sample volume (≥0.3 μL) to provide results in seconds, and developed for veterinary use, specifically for dogs and cats. AlphaTrak 3 (AT3) is a next-generation POC glucometer with applicability both in clinical and on-farm settings, with online connectivity capability and a first-of-its-kind equine-specific algorithm. The ability to accurately measure BG quickly will allow for early intervention to correct the issue and potentially improve outcomes in patients. The objective of this study was to determine the accuracy of the AT3 glucometer in measuring BG levels in horses using an equine algorithm and validating the AT3 results by comparing them with BG values obtained by a reference laboratory method.

2 Materials and methods

2.1 AlphaTrak 3 blood glucose monitoring system

AlphaTrak 3 (Zoetis, Parsippany, NJ, USA) is a veterinary BG monitoring system, which can selectively use algorithms for cats and dogs. This POC device can measure glucose levels from capillary or EDTA or heparin anti-coagulated venous whole blood (WB) sample volumes ≥0.3 μL with hematocrit levels ranging between 15 and 65%. A lancing device with single-use replaceable lancets included as part of the AT3 system ensures the safe collection of the capillary blood required to perform a test. When a test strip (TS) for capturing the blood sample is inserted into this glucometer, the device is activated. Following application of a blood sample, the TS draws the sample into the sample-receiving chamber for analysis. Accurate results for BG levels ranging from 20 to 750 mg/dL (1.1–41.7 mmol/L) are typically displayed within 5 min. The system includes Bluetooth-enabled, web-based, and mobile applications that facilitate the sharing, storing and transfer of test results via the internet to a veterinary clinic. The AT3 mobile app can also programmed to receive reminders for follow-up tests.

2.2 Accuracy of AT3 system in measuring artificially adjusted equine blood samples

Venous WB samples derived from 60 different horses (31 male and 29 females, age, 3–22 years, hematocrit levels, 23–40%) in vacutainer heparin tubes were utilized for this study. Following equilibration of the blood sample to room temperature, the basal level glucose concentration of each sample was measured on the YSI 2900 Biochemistry Analyzer per manufacturer's instructions by separating plasma by centrifugation at 3000 rpm for 5 min. The WB samples were artificially adjusted to generate a set of 129 blood samples with different glucose levels ranging from 29 to 479 mg/dL, which included (1) 60 unaltered blood samples in the normal glucose range, (2) 60 samples spiked with 20% condensed glucose solution to generate samples in the hyperglycemic range, and (3) 9 samples incubated at 37°C for ≤24 h to deplete their glucose levels to the hypoglycemic range. Glucose concentration of a given blood sample was measured using 2 test strips per each of three different lots on six AT3 devices. At the end of testing on the AT3 devices, leftover blood from the same sample were centrifuged immediately to separate plasma, which were stored at −80°C until shipped for reference testing. The same operator sequentially repeated the above testing protocol with each of the remaining blood samples with different glucose levels on AT3 devices generating a total of 774 measurements.

2.3 Validation of an equine-specific algorithm in a field study

As the prevalence of hypoglycemia is common in foals, the accuracy of the AT3 equine algorithm in measuring hypoglycemic equine samples was further validated in a field study involving 96 horses that belong to 18 different breeds (Arabian, American saddlebred, Thoroughbred, Quarter horse) of various ages (4–25 years). All horses were maintained at an equine farm at William Woods University, Fulton, Missouri. Venous WB samples collected in two vacutainer EDTA tubes (approximately 3.0 mL each) from each horse. An attending veterinarian supervised collection of equine venous blood samples and BG testing using the AT3 system. Immediately after collection, BG concentration of a given sample in tube 1 was concurrently measured in duplicate using three TS lots on three AT3 meters. Within 10 min after testing on AT3 devices, the leftover sample in tube 1 was centrifuged, and the plasma fraction was stored at −20°C until shipped for reference testing. Blood samples in tube 2 were subjected to glycolysis at 37°C for 1 h to deplete BG concentrations to a hypoglycemic range of approximately 66–105 mg/dL and tested on AT3 devices as described above using three strip lots. Immediately following AT3 testing, plasma was separated from the leftover glucose depleted sample in tube 2 by centrifugation and stored at −20°C until shipped for reference testing.

Testing of remaining WB samples and their corresponding glucose depleted samples was performed on the AT3 devices as described above. All plasma samples from both sets of collection tubes (fresh blood and glucose depleted) were then submitted for reference glucose testing. At the conclusion of the study, the AT3 testing data were submitted for biometric analyses.

At the end of each day of testing, all plasma samples were shipped overnight on ice to a Zoetis reference laboratory (San Diego, CA, USA) to measure glucose concentration on the reference Beckman Coulter AU480 Biochemistry Analyzer in triplicate.

2.4 Data analyses and acceptance criteria

ISO 15197:2013 guidelines were used to assess the accuracy of the AT3 device as compared to Beckman Coulter AU480 Chemistry analyzer reference measurements. The ISO 15197:2013 criteria stipulate that (1) 99% of all results are required to be in Zones A and B of the CEG, and (2) at glucose concentrations <100 mg/dL (< 5.55 mmol/L), 95% of the test results are required to be within ±15 mg/dL (±0.83 mmol/L) of the average reference value, and at glucose concentrations ≥100 mg/dL (≥5.55 mmol/L), 95% of the test results are required to be within ±15% of the average reference value. Clinical relevance of the AT3 system measurements were analyzed by Consensus Error Grid (CEG) distribution (21, 22). The CEG process is divided into five zones, which are defined by estimated risk to the animal if a result falls within a respective zone. Zones A and B indicate little or no adverse clinical effect of the test outcome. The deviation (bias) of BG values measured by AT3 versus the values measured by the reference analyzer were calculated. Bias plots displayed the BG values measured by AT3 in comparison to the average reference (6).

3 Results

At the BG concentrations tested in this study, 98.96% (766 out of 774) of AT3 measurements fell within the ISO accuracy limits when analyzed with the equine algorithm for all three strip lots. A small subset 1.04% (8 out of 774 measurements) of equine measurements fell outside ISO accuracy threshold. Results confirm that accuracy of the AT3 system were maintained across a broad range of BG values (29–479 mg/dL) including equine blood samples with artificially adjusted BG levels. Bias plots of the AT3 accuracy study test results versus the laboratory reference standard show that all three TS lots provided a high degree of compliance with the ISO standard, and that all three TS lots produced comparable accuracy results (Figure 1). Out of 258 AT3 measurements per each of three TS lots, (three different production runs), the number of AT3 measurements fell outside the reference range were 3, 2, and 3, respectively. Consensus error grid plots for all three strip lots illustrate that 100% of BG test values fell within CEG zone A, indicating that deviation of AT3 test results from the reference standard represented no adverse clinical risk (Figure 2).

The accuracy of the AT3 equine algorithm in measuring hypoglycemic equine samples were further validated in a field study. Whole blood samples from 96 horses, including a subset of corresponding 87 glucose depleted samples, provided a total of 183 samples with BG levels ranging from 65 to 105 mg/dL for analysis. Testing 183 equine blood samples on duplicate test strips generated a total of 365 glucose measurements (one sample measurement was recorded incorrectly and eliminated from the analysis). Of the 365 AT3 measurements, 98.3% (345/351) of measurements at glucose concentrations <100 mg/dL were within the ISO accuracy threshold of ±15 mg/dL of the average reference value (Table 1). Additionally,

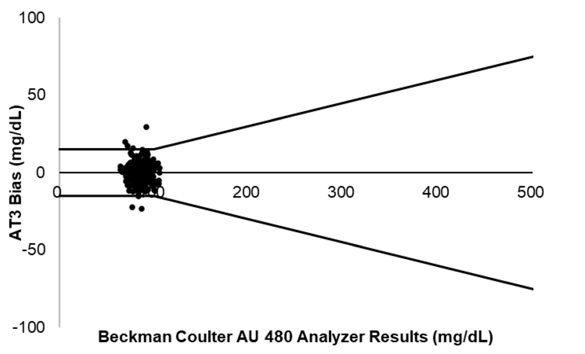

FIGURE 1
Bias plots for AT3 blood glucose results versus the Beckman Coulter AU480 laboratory reference method when tested with artificially adjusted equine blood samples (258 measurements per test strip lot). Upper and lower lines mark the upper and lower limits of ISO15197:2013 accuracy criteria, respectively.

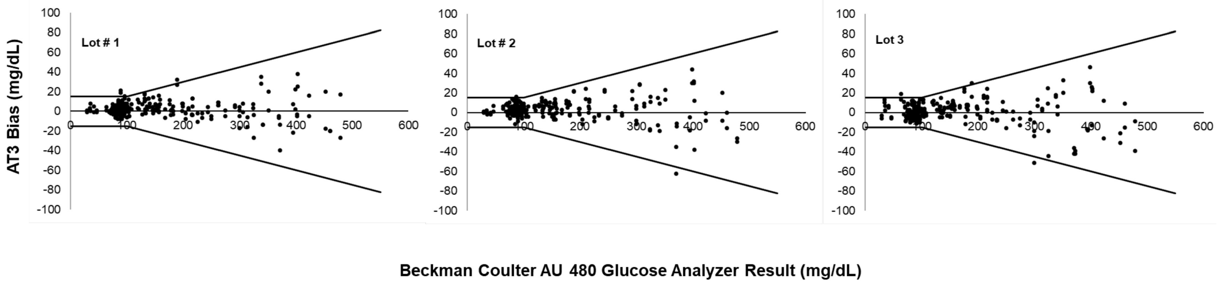

FIGURE 2
Consensus error grid (CEG) analysis of the AT3 system when tested with artificially adjusted equine blood samples (258 measurements per test strip lot).

TABLE 1 Accuracy of AlphaTrak 3 blood glucose monitoring system in measuring equine glucose levels in a field study.

Host BG level	AT3 system accuracy results		
	Within ± 5 mg/dL or ± 5%	Within ± 10 mg/dL or ± 10%	Within ± 15 mg/dL or ± 15%
<100 mg/dL	65.8% (231/351)	90.9% (319/351)	98.3% (345/351)
≥100 mg/dL	28.6% (4/14)	85.7% (12/14)	100.0% (14/14)
All samples	64.4% (235/365)	90.7% (331/365)	98.4% (359/365)

100.0% (14/14) of measurements at glucose concentrations ≥100 mg/dL were within the ±15% of the average reference value. The bias plot for the field study shows that all BG values measured by the AT3 system were within the ISO accuracy limits (Figure 3). A CEG plot for (Figure 4) shows that 99.2% (362/365) of BG samples measured by the AT3 system were within zone A and the remaining 3 samples were within zone B (Figure 4), thus meeting ISO criteria.

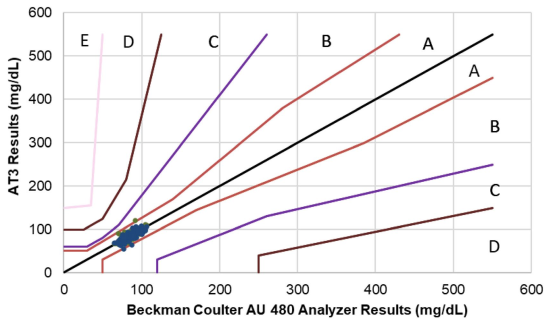

FIGURE 3
Bias plots for AT3 blood glucose results versus the Beckman Coulter AU480 laboratory reference method measured in the field study.

4 Discussion

The AT2 system were developed for veterinary use with validated algorithms for accurate measurements of glucose levels in canine and feline blood samples. However, the AT2 feline algorithm was determined to be appropriate in testing equine blood samples. When used in horses ($n = 50$) and foals ($n = 50$) in an earlier study, the AT2 system had a median bias of 6.1% in adult horses and 5.0% in foals when BG levels were compared to Hitachi 917 Blood Chemistry System results, and 97% of BG values were within CEG zone A (14). The AT3 system is the next generation version of AT2 glucometer with connectivity capability and a first-of-its-kind equine-specific algorithm.

The principal outcome of this study was the confirmation that equine algorithm used in the AT3 system was well within the ISO 15197:2013 accuracy limits when compared to the BG concentrations measured by Beckman Coulter AU480 analyzer and YSI 2900 analyzer (unpublished data). Overall, at the glucose concentrations tested in this study, >98% of AT3 measurements fell within the ISO accuracy limits when analyzed with the equine algorithm for three different strip lots. Additionally, both AT3 feline and canine algorithms performed equivalently in measuring equine blood samples, but their accuracy was slightly lower as compared to the performance of AT3 equine algorithm (unpublished data).

As a measure of glucometer performance, the CEG was developed as an alternative to a straightforward bias percentage of BG samples that meet the ISO standard (21, 22). Each of the five CEG zones represents a degree of clinical risk posed by the BG test deviation from the reference method, progressing from zone A (accurate measurement with no effect on clinical outcome) to zone E (measurement error could have dangerous consequences). The AT# device, with the equine algorithm, performed reliably for reporting BG concentrations. In this study, 100.0% of AT3 measurements were within CEG zones A and B, indicating that any AT3 measurement deviation from the reference standard pose little or no clinical risk to the horse. Zone B indicates benign measurement error with little or no effect on clinical outcome (6, 21, 22).

Although nearly all horses in this study had BG values in zone A of the CEG, individual deviation from the reference standard tended to increase as BG levels rose (Figure 1). This was consistent with results of other studies measuring BG levels and glucometer

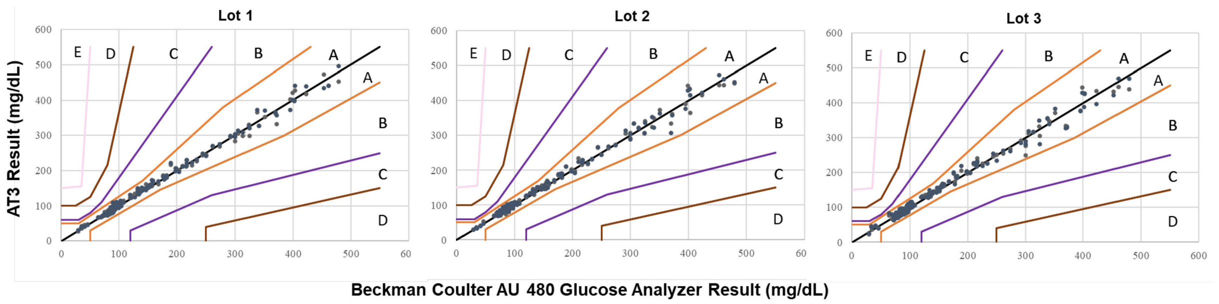

FIGURE 4
Consensus error grid (CEG) analysis of the AT3 measurements measured in the field study.

performance in various species (6, 9, 10, 15, 17, 23). Previous studies have reported variations in results for different lots of test strips in the same glucometer and even when different glucometers of the same model using test strips from the same lot are compared (24, 25). In our study, analysis of each BG sample using multiple TS lots and AT3 glucometers helped negate the effects that any variations in testing system components may have had on assay results. No significant TS lot-to-lot variability were observed (unpublished data). In clinical practice, hematocrit values outside the normal range can also affect the accuracy of glucometer results (10, 26). While the high degree of diagnostic accuracy of the AT3 device in horses is encouraging, it is still good practice to confirm POC glucometer results that are aberrant, inconsistent with clinical findings, or highly variable for the same patient from one test to the next by reassessing the outcomes by means of laboratory analysis (18).

Collectively, the accuracy bias and CEG results from our study affirm that the AT3 system has a high degree of accuracy for BG measurements in horses. While laboratory assays remain the gold standard for BG measurement, this approach is impractical for serial monitoring of BG levels in-clinic or on-farm settings. Ease of use, a wide BG dynamic range, rapid determination of assay results, the need for only ≤0.3 μL blood sample, relatively low cost, and online connectivity represent advantages of the AT3 device versus laboratory assays.

The AT3 glucometer with an equine-specific algorithm and connectivity capability is an improvement over its predecessor AT2 system and represents a reliable and cost-effective method for accurately monitoring BG levels in horses on-farm, in-clinic, or laboratory settings to better address patient needs in a timely manner.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by the Institution Animal Care and Use Committee at William and Woods University. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

SV: Writing – original draft, Writing – review & editing. PS: Data curation, Investigation, Resources, Writing – review & editing. K-HC: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing, Project administration. Y-MP: Writing – review & editing, Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization. BC: Writing – original draft, Writing – review & editing.

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Conflict of interest

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Inertial measurement unit technology for gait detection: a comprehensive evaluation of gait traits in two Italian horse breeds

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Introduction: The shift of the horse breeding sector from agricultural to leisure and sports purposes led to a decrease in local breeds' population size due to the loss of their original breeding purposes. Most of the Italian breeds must adapt to modern market demands, and gait traits are suitable phenotypes to help this process. Inertial measurement unit (IMU) technology can be used to objectively assess them. This work aims to investigate on IMU recorded data (i) the influence of environmental factors and biometric measurements, (ii) their repeatability, (iii) the correlation with judge evaluations, and (iv) their predictive value.

Material and methods: The Equisense Motion S® was used to collect phenotypes on 135 horses, Bardigiano (101) and Murgese (34) and the data analysis was conducted using R (v.4.1.2). Analysis of variance (ANOVA) was employed to assess the effects of biometric measurements and environmental and animal factors on the traits.

Results and discussion: Variations in several traits depending on the breed were identified, highlighting different abilities among Bardigiano and Murgese horses. Repeatability of horse performance was assessed on a subset of horses, with regularity and elevation at walk being the traits with the highest repeatability (0.63 and 0.72). The positive correlation between judge evaluations and sensor data indicates judges' ability to evaluate overall gait quality. Three different algorithms were employed to predict the judges score from the IMU measurements: Support Vector Machine (SVM), Gradient Boosting Machine (GBM), and K-Nearest Neighbors (KNN). A high variability was observed in the accuracy of the SVM model, ranging from 55 to 100% while the other two models showed higher consistency, with accuracy ranging from 74 to 100% for the GBM and from 64 to 88% for the KNN. Overall, the GBM model exhibits the highest accuracy and the lowest error. In conclusion, integrating IMU technology into horse performance evaluation offers valuable insights, with implications for breeding and training.

KEYWORDS

equids, Equisense, IMU, genetics, machine learning

1 Introduction

In the last decades, there has been a significant loss in biodiversity, although is key for maintaining a sustainable environment. Biodiversity enhances animals' resistance and resilience to stress including those caused by climate change. Biodiversity at genetic, species and ecosystem levels helps the challenges posed by distinct and changing environmental conditions and socio-economic factors. According to the Food and Agriculture Organization of the United Nations (FAO), the current rate of biodiversity loss is unprecedented in the past century, with 26% of local breeds at risk of extinction and 67% with unknown risk status (1). This is true in the equine sector as well, where most of the local breeds are considered endangered or at risk of extinction. Italian horse heritage comprises breeds reared and adapted to different regional climates, cultures, and traditions. Over the last century, the horse breeding sector has experienced significant transformations. Indeed, horses that once were bred for meat production, warfare, or agricultural purposes, are now bred for sport or leisure activities (2). Thus, Italian local breeds, historically used for meat or draft work, are facing a decrease in population size, due to the change in the market demand and a consequent loss of their original breeding purpose. For this reason, most of them are now considered endangered by FAO, which in its report declared that, in 2022, horses are among the species with the largest proportion of breeds at risk of extinction (3). Currently, Italy counts 22 distinct breeds (4), 17 of which are classified as endangered. Horses belonging to these breeds are managed by four associations: ANACAITPR (National Association of Breeders of the Italian Heavy Draft Horse), ANAMF (National Association of Breeders of the Murgese Horse and the Martina Franca Donkey), ANACRHAI (National Association of Breeders of Haflinger Horses in Italy) and ANAREAI (National Breeding Association for Equine and Asinine Breeds in Italy), which aim to maintain genetic diversity and monitor population size. However, the transition from agricultural to sport horses presents a challenge for breed conservation since it must face the need to modernize the breeds while preserving genetic diversity. As an example, in the Bardigiano horse breed, which is part of the Italian equine heritage and it is facing the need to modernize towards current market demand, genetic diversity preservation is also pivotal (5). Indeed, it has been shown that both at pedigree and genomic level inbreeding has increased in the latest generations. Therefore, breeding strategies for optimizing the contribution of breeding animals are key to ensure long-term survival of breed (6).

In a few Italian breeds, tools are available to evaluate horses based on traditional and linear scores for conformation, which are used to estimate breeding values (7). These evaluations can be used as indirect measures of movement-related traits, which might help the transition from work horses to leisure ones. However, since the modern market seeks especially sport and leisure horses (8), a comprehensive evaluation of gaits might be necessary. Currently, none of the Italian equine breeds have developed protocols to evaluate gaits and challenges exist due to the extensive training required for judges and the subjectivity involved in gait assessment. Animal breeding relies on precise phenotyping to be effective and often the phenotype recording is a limiting factor (9). Gaits traits in horses fit in this scenario of difficult traits, as they are influenced by both genetics (10) and environmental factors. In addition, these traits are exposed to change over time, and to human error and subjectivity during data collection. Consequently, the gap between objective recording and the difficulty in defining them must be filled to allow faster improvement in the breeding scheme. Therefore, there is a need to propose novel gaits' traits that can provide objective measurements to meet the current challenges that local breeds are facing in shifting their breeding goals (11).

The integration of new tools such as inertial measurement units (IMU) technology and machine learning algorithms for image analysis offers solutions to this challenge. The IMUs are devices that measure acceleration and angular velocity, providing detailed movement data. Machine learning algorithms can analyze this data to predict and evaluate traits with greater accuracy than traditional methods. These tools are often combined to collect data efficiently and cost-effectively, requiring less time and expertise from the judges (12). Indeed, machine learning models can use data from sensor devices (13). The gold standard for kinematic analysis is the optical motion capture (OMC), which uses multiple cameras and reflective markers to track the movement of horses with high accuracy. Although this technology provides highly reliable and detailed data (14) it requires complex setups and is usually limited to research environments due to its high cost and logistical demands.

While sensors are widely used in the animal production sector for health-related monitoring (15), their application in equine performance remains relatively unexploited. Standalone inertial units are often created for riders and used in horse training. Nevertheless, IMU sensors can also be used as reliable sources for more tough challenges such as lameness determination (16). These phenotyping technologies are growing in importance due to their ability to generate real-time, non-invasive, and accurate animal-level information, enabling phenotyping on a large scale (17). Several IMU tools are available for equine gait analysis, each serving different purposes. One of the most used in research is the Equimoves (18) which measure gaits, detect lameness, and estimate speed by applying seven sensors, placed on the head, withers, sacrum and the four legs (19). Other IMU sensors focus specifically on detecting asymmetries in movement, which are therefore highly useful in clinical and veterinary applications. Nevertheless, most of the studies on the comparison between experienced clinicians and IMU evaluation suggested that IMU can strengthen but not replace subjective lameness assessment since the agreement was not always close to unity (20, 21). Finally, it has been shown the potential of IMUs for the evaluation of the horse–rider interaction during dressage riding, training of horses, or coaching (22). The overall advantage of implementing the use of IMU in horses is the possibility of gathering objective movement data in field conditions, where the use of well-established methods like OMC would be impractical and extremely expensive. In addition, since the technology is rather simple to implement, there is the possibility to collect objective data on a large scale of horses. However, IMUs also have some disadvantages, one of which is represented by their usually high cost, which has, nevertheless, decreased in recent years. In addition, to obtain reliable and reproducible data, care must be taken in placing the sensors in the right position, therefore, if not correctly implemented, this technology is also not free from human errors (22).

Therefore, in this article we focus on addressing the above-mentioned gap in knowledge by studying horses' gaits via IMU sensor data. We aim to investigate on IMU recorded data (i) the influence of environmental factors and biometric measurements, (ii) their repeatability, (iii) the correlation with judge evaluations, and (iv) their predictive value. To achieve this, we focused on two Italian horse breeds: the Bardigiano and the Murgese. The Bardigiano, bred in North Italy, was used in the past for meat production and is considered a meso-brachymorphic horse (Figure 1A). The term meso-brachymorphic describes a body type that is medium-sized, with relatively short limbs and a robust, muscular structure. In horses, a meso-brachymorphic horse like the Bardigiano typically has a robust, muscular body with a wide chest, strong limbs, and a more compact appearance. The angles of the joints are very closed. This type of conformation is associated with strength and endurance rather than

FIGURE 1
Example of Bardigiano **(A)** and Murgese **(B)** horse.

speed, making such horses well-suited for tasks like trekking, carrying loads, or agricultural work. The Murgese is bred in South Italy historically for agricultural work, its morphology is substantially different and considered as a meso-dolichomorphic horse (Figure 1B). The term meso-dolichomorphic describes a body type that is medium to tall with a long, lean frame. In horses, a meso-dolichomorphic type, typically has a leaner, taller structure with longer legs and a more refined body shape compared to meso-brachymorphic type (as the Bardigiano horse breed). This conformation is often associated with agility and speed, making these horses well-suited for equestrian sports such as dressage, where longer strides and fluid movements are beneficial. Both breeds are currently facing the conversion from their original purposes to a new breeding objective to match the market demand. Bardigiano is evolving to fit working equitation and trekking activities, while the Murgese is aiming to fit equestrian purposes, such as dressage performance. The two analyzed breeds symbolize Italy's equestrian tradition, revealing adaptability, tight connection with their respective landscapes, and potential to find a place in the current market demands. Through our study, we aim to provide novel tools to enhance the promotion of these equine breeds, ensuring their role in the modern equestrian landscape.

2 Materials and methods

2.1 Sampling

A total of 135, including 101 Bardigiano and 34 Murgese, born between 2000 and 2019, were tested. Those horses were born between 2000 and 2019 which ensures a range of ages that reflects the current genetic diversity and management practices of the breeds. Among these, 72 young horses aged 3 or 4 years old were tested during a n a 70-day performance test. During the 70-day performance test, horses were subjected to a controlled environment that consisted in the same feeding and same trainer as well as a standardized training protocol that allowed theminimization of external variables affecting horse performance. The horses were evaluated three times:

1) First evaluation, where a committee comprising a veterinarian, and two judges evaluated the overall health status of the horse and took biometric measurements.
2) A second trial at 30 days involved a session of free jumping, and an under-saddle session with a standardized trail to reduce environmental influence. Only one rider was allowed to ride all the horses in the designed riding center, conducting a 10-min session comprising two gaits: walk and trot.
3) At the end of the performance test period (70 days), all the horses underwent a second trial repeating the free jumping and under-saddle (ridden) tests, now including canter, and a draft trial was added only for the Bardigiano horses. The same rider of the first test was involved in the second trial. A panel of at least three judges and riders were asked to evaluate the horses based on the criteria outlined in Table 1 for each trial.

The performance test was conducted over 3 years: 2020, 2021, and 2022 with testing periods in June, July, and August for the Bardigiano in two different riding centers and November, December, and January for the Murgese, in three different riding centers. Horses experiencing veterinary issues before or during the test period were excluded from the study. For the 63 horses included in the study which were not sampled during a performance test, the same protocol was used for a total of 10 min trial (Supplementary Figure S1). In addition, a survey to collect animal and environmental factors was developed (Supplementary Figure S2). This protocol included information on sex, birth date, rider's skills, biometric measurements (e.g., height at withers, thoracic circumference, cannon bone circumference, shoulder length), management practices, rider details, arena conditions, and health traits (Supplementary Figure S1). All gait measurements were conducted by the same operator using Equisense Motion S®, [Micromegas, Headquarters: 231 Allée Faust d'Elhuyard 64,210 Bidart, France] a 9-axis inertial unit equipped with an accelerometer (3 axes), a gyrometer (3 axes), and a magnetometer (3 axes); placement is shown in Supplementary Figure S3. The inertial system acquires 100 measurements per second, enabling precise analysis of the horse's locomotion (Figure 2). Furthermore, by adding an electrode, the sensor measures horses' heart rate during the session. The parameters collected by the IMU sensor and electrode included stride frequency, regularity, and elevation for walk, trot, and canter, as well as symmetry and weak diagonal at trot, heart rate, speed, and distance. A specific definition for each trait is reported below:

TABLE 1 Criteria evaluated by judges and riders during the performance test.

Trait	Judges' evaluation	Riders' evaluation
Daily management in the stable		✓
Acceptance of harnessing, mounting, docility in approaching the rider		✓
Technique on jumping – front passage	✓	
Technique on jumping – back passage	✓	
Rideability, response to rider commands, and attitude towards work	✓	
Trot – rhythm, impulsion, amplitude, elasticity, and regularity	✓	
Canter – rhythm, impulsion, amplitude, elasticity, and regularity	✓	
Obedience, and trust towards the rider during the exercise	✓	
Attention to requests	✓	
Free jumping	✓	
Flatwork	✓	
Draft test	✓	
Elevation at a trot		✓
Frequency at trot		✓
Symmetry		✓
Elevation at canter		✓
Frequency at canter		✓
Recovery time		✓

- Stride frequency refers to the number of complete strides a horse takes per minute, where a stride is the sequence of hoof lifts and placements of the same limb;
- Regularity measures the consistency of gait rhythm and is scored on a scale from 0 to 10, where 10 indicates perfect consistency;
- Elevation is the vertical displacement of the horse's body during each stride, measured in centimeters;
- Symmetry is evaluated while the horse trots in a straight line, comparing the lengths of paired strides. It is scored on a scale of 0 to 10;
- Heart rate: the rhythm, in beats per minute (BPM), that beats the heart of the horse.

Distance and speed were not considered in this study since they rely on data collected via GPS which is not included in the Equisense Motion S® but only provided as additional data if during the riding session the rider wears a phone.

2.2 Statistical analysis

2.2.1 Analysis of variance

The effect of environmental factors and biometric measurements was assessed through an analysis of variance (ANOVA), performed using R (v.4.1.2), with the following model:

$$Y_{ijklmn} = \mu + Breed_i + Sex_j + Age_k + Shoeing_l + Rider_m + Training\ level_n + Heigh\ at\ withers\ within\ breed_i + Cannon\ bone\ within\ breed_i + Shoulder\ lenght\ within\ breed_i + \varepsilon_{\mathrm{ijklmn}}$$

Where:

- $Y_{ijklmno}$ is the observed gait trait via the IMU sensor;
- μ represents the intercept of the model;
- $Breed_i$ is the effect of the breed (Murgese or Bardigiano);
- Sex_j represents the effect of the horse's sex (male or female);
- Age_k represents the effect of the horse's age (young ≤4; adult >5);
- $Shoeing_l$ is the effect of the shoeing (shod, forelimb shod or not shod);
- $Rider_m$ indicates the effect of the rider's level (beginner, intermediate or expert) based on the rider's license;
- $Training\ level_n$ is the effect of the horse's training (defined in hours per week: 0–2, 3–4, >4);
- Height at wither within $\mathbf{breed}_i$, Cannon bone within $\mathbf{breed}_i$, Shoulder length within $\mathbf{breed}_i$ effect of the interaction between breed and biometric measurements (divided into quartile classes).
- $\varepsilon_{\mathrm{ijklmn}}$ is the error term.

A total of 134 horses were evaluated for this analysis. Post-hoc Tukey contrast tests were conducted to identify pairwise differences between group means using the base R Tukey HSD function.

2.2.2 Repeatability

The repeatability of the horse's performance was assessed using the rptR package in R (v.4.1.2), on a subset of data comprising 47 Bardigiano horses participating in the performance test. All horses involved in repeatability calculation were female, aged 3 or 4 years, untamed when the performance test started. The evaluation took place after 30 days and 70 days of training, with assessments conducted using both judges' evaluations and Equisense Motion S® data. The repeatability was assessed only on the gaits shared by the two trials: walk and trot, using the following formula (23):

$$R = \frac{V_G}{V_G + V_R}$$

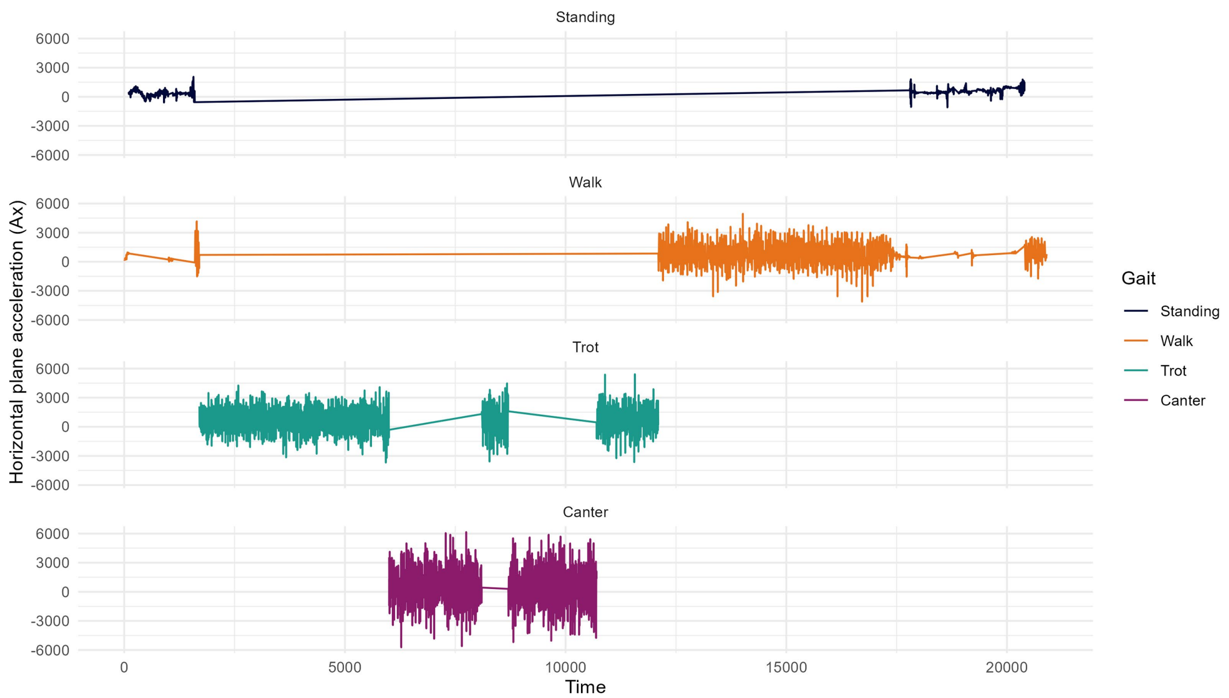

FIGURE 2
Example of data collected by x axis of the Equisense Motion S accelerometer. Data collected during the trial performed by the horses has been divided by gait.

Where R is the repeatability, V_G is the variance among group means while V_R is the residual variance at data level.

2.2.3 Correlation

A subset of 111 trials was selected to evaluate the correlation between judge evaluations and IMU sensor data, focusing on trials with both types of data available. This analysis was restricted to horses participating in the performance test due to the reliability of the judges' and riders' scores. Both the 30 and the 70-day trials were considered. Pearson's correlation coefficient was used to assess the strength and direction of linear relationships between IMU sensor measurements and judges' scores as well as within them.

2.2.4 Predictive models

To determine the feasibility of predicting judge evaluations via Equisense Motion S® objective traits, the judges' evaluations were categorized into binary classes: 'negative' for scores below the mean and 'positive' for scores at or above the mean. The study employed the same dataset used for correlation analysis. Three different algorithms were employed to assess the prediction study: Support Vector Machine (SVM), Gradient Boosting Machine (GBM), and K-Nearest Neighbors (KNN). These algorithms were chosen due to their different classification approaches, aiming to identify the best fit for the data. The SVM works by finding a hyperplane that best separates data points belonging to different classes (24) GBM is a tree-based model where trees are built to correct errors from previous ones (25), and KNN is a non-parametric model classifying data points based on the majority of neighbor labels in the training data (26). The models were evaluated using a 10-fold cross-validation to increase the accuracy and reliability of the analysis. Only judge evaluations concerning behavior, walk, and trot traits were studied due to the completeness of the dataset. The three models were implemented in R (version 4.1.2), SVM via the e1070 package, KNN via the class package, and GBM via the caret package. Several metrics were considered to assess model performance (27), including accuracy (the ratio of correctly predicted instances to the total instances), sensitivity (the proportion of actual positive cases that were correctly identified), specificity (the proportion of actual negative cases that were correctly identified), and F1 score (the harmonic mean of sensitivity and specificity), calculated as follows:

$$F1 = \frac{2\ x\ Precision\ x\ Recall}{Precision + Recall}$$

For the SVM model, the tune function in the e1070 package in R was utilized to tune the gamma, which represents the complexity of the decision boundary, and the cost parameters which represent the balance between margins and misclassification. In the GBM model, the number of trees, shrinkage value, interaction depth, and the minimum number of nodes were tuned for each trial. Specifically, the number of trees represents the total number of boosting stages, the shrinkage value controls the contribution of each tree, the interaction depth determines the maximum depth of the individual trees, and the minimum number of nodes specifies the minimum number of samples required to split a node. For the KNN model, the number of neighbors was tuned using the appropriate function in the class package. All parameters were tuned individually for each trial, and the details of the parameters used for tuning are provided in Table 2.

3 Results and discussion

3.1 Analysis of variance

Table 3 presents the descriptive statistics for gait traits observed in horses participating in this study. Data on symmetry at trot and regularity were missing for some horses. This limitation likely arose from an insufficient training level for cantering and therefore a limited regularity or an inadequate duration of straight-line movement to capture symmetry data. Significant variability was observed in stride frequencies and elevations, reflecting both individual differences and breed-specific characteristics. As phenotypic variation results from the interaction between environment and genotype, the primary aim of this study was to identify environmental factors affecting gait traits (Supplementary Table S1).

Among the factors investigated in the ANOVA, breed showed a significant influence on most of the gait traits measured by the sensor ($p < 0.05$) including elevation at trot and canter, stride frequency at walk, trot, and canter, as well as stride regularity at trot and canter. The significant effect of the breed highlights the different predispositions towards sporting activities and the distinct abilities between Bardigiano and Murgese. Indeed, the Murgese horses displayed on average greater elevation (+2.81 cm at trot, +3.60 cm at canter) (Figure 3A) and lower stride frequency (−2.83 stride/min at walk, −4.27 stride/min at trot) (Figure 3B). Based on those differences we can hypothesize the enhanced potential for sporting activities of Murgese horses, likely due to their physical attributes and selection towards dressage performance. As an example, a higher elevation is particularly valued in dressage competitions, thus, it is not surprising that it is higher in the Murgese breed. On the other hand, Bardigiano horses displayed lower elevation and higher frequency at walk and trot, which are traits favorable for endurance activities such as trekking or working equitation, where energy preservation is essential. The opposite trend was shown at canter, where the Bardigiano horses showed lower frequency (−1.46 stride/min). However, this observation may be influenced by the higher proportion of adult and well-trained Bardigiano horses (50% compared to 26% in Murgese) and should be interpreted with caution. Furthermore, it was observed that un-shod horses showed a greater stride regularity at walk (+1.50) and trot (+0.37) (Figure 3C), along with reduced gait frequency at trot (−3.07 stride/min) (Figure 3D) compared to forelimbs shod horses. This latter result suggested the potential benefits of natural balance and enhanced gait expression in un-shod horses especially compared to front limbs shoed horses. This finding aligns with existing knowledge that shoes can alter gaits, since joint angles of the pastern move differently between shod and un-shod horses (28) as well as that shoes' mass can influence gait (29). Horses only shod in the forelimbs showed lower values of regularity (−0.85 compared with shod and − 1.5 compared with un-shod; p-value <0.0001) (Figure 3C), possibly due to the increased difficulty in balancing and maintaining stable gait during the session, as the center of gravity shifts unnaturally towards the hind legs. Despite shoes being applied to protect against the wear of the hoof wall, to improve performance and to provide additional support on slippery surfaces, they may restrict the hoof mechanism and add additional weight on the distal limb. This increases its inertia, demanding a higher energy expenditure in protracting and retracting the limbs. Thus, the weight of shoes is likely affecting gaits, altering both energy and kinematics of locomotion (30).

The age (Figures 3E,F) had a significant effect on the frequency of walk, by indicating that adult horses (−2.91 stride/min) are commonly better-trained and exhibit lower gait frequency during the session. Additionally, canter heart rate was impacted by age, with younger horses having higher heart rates (+32.02 bits/min), which suggests that training level affects parameters such as the cardiovascular response of horses. The sex had a significant effect on heart rates both at trot and canter (Figure 3G), with males showing a lower heart rate at trot (−9.03 bits/min) and canter (−16.10 bits/min) compared to female. This result may be attributed to pre-selection and increased attention given to training of male horses. Typically, in these breeds, only a few stallions undergo training under the saddle, leading to a pre-selection process to identify the most valuable ones.

Surprisingly, rider experience and horse training level, along with cannon bone circumference and shoulder length, did not yield

TABLE 2 Tuning parameters used for each trait and model.

Model	SVM		GBM				KNN
	Gamma	Cost	Number of trees	Interaction depth	Shrinkage	Min observations in node	*k*
Daily management	0.01	100	100	5	0.1	5	11
Acceptance of harnessing	0.000001	0.1	100	5	0.01	5	11
Rideability	1	1	100	1	0.1	5	11
Trot	0.000001	0.1	150	1	0.2	15	11
Obedience	0.001	100	150	1	0.01	10	11
Attention to requests	1	10	100	1	0.1	5	11
Flatwork	0.01	100	100	3	0.2	5	11
Elevation at a trot	0.1	1	50	1	0.2	5	11
Frequency at trot	0.01	100	50	1	0.2	5	11
Symmetry	0.01	100	50	3	0.2	15	11
Recovery time	0.1	100	50	3	0.2	5	11

TABLE 3 Descriptive statistics of gait traits collected by Equisense Motion S®.

Variable	*n*	Min	Max	Mean	SD
Frequency walk	135	39.33	57.31	51.66	3.32
Regularity walk	134	0.0	7.92	3.46	2.06
Frequency trot	134	78.32	109.33	90.27	6.04
Regularity trot	134	0.24	7.88	5.66	1.11
Frequency canter	132	79.63	112.15	99.73	9.24
Regularity canter	82	0.10	9.02	4.71	2.38
Elevation walk	135	1.13	6.46	2.85	0.84
Elevation trot	134	4.21	12.39	7.53	1.69
Elevation canter	132	7.40	22.33	15.92	2.54
Symmetry	96	1.75	8.70	6.88	1.17
Heart rate walk	108	34.4	121.92	93.28	14.21
Heart rate trot	104	89.84	157.69	126.86	13.14
Heart rate canter	100	108.73	196.67	156.83	18.95

significant effects on gait traits, likely due to the limited variability in these factors in our samples. Indeed, most of the horses were trained by only two professional riders following the same training routine. Regarding biometrical measurements, they did not provide any significant effects, probably because their variability is already included in the breeds' variability.

3.2 Repeatability

The study's second aim was to assess which traits change or stay consistent during the horse's life. This information can provide a better understanding of the effect of training on traits improvements and the identification of traits bound to horse's natural attitude. Stride regularity at walk (0.635) and elevation at walk (0.717) demonstrated the highest repeatability between the two trials, indicating that walk is less influenced by training and remains relatively consistent throughout the horse's life. Conversely, all the other measurements had lower repeatability, suggesting greater susceptibility to environmental influences and the potential for improvement through training (Supplementary Table S2).

3.3 Correlation

Another key aspect when using IMU sensor data collection is to assess how those new traits correlate with traditional evaluation. Correlation analyses within sensor data and between judges' evaluations and Equisense Motion S® performance revealed interesting patterns. Hereafter and in Figure 4, only significant correlations are reported and further discussed. Within sensor data, elevations showed positive correlations among gaits, ranging from high for trot-canter (0.618) to moderate for walk-canter (0.250); this can be due to the horse's training or the rider's attitude to collect the gait. However, since the ANOVA did not highlight any significant difference between riders for the elevation, the differences might be bound to the natural predisposition of the horse. Further studies are needed to investigate this aspect; one potential solution is to study elevation without the rider to truly understand the cause of this correlation. Similarly, heart rate exhibited strong positive correlations among gaits, ranging from trot-canter (0.795) to walk-trot (0.720), indicating that horses' fitness level affects heart rate across all gaits. Stride frequency at trot negatively correlated with elevation at trot (−0.448) and canter (−0.482), suggesting that horses with higher frequencies may expend energy on increasing frequency rather than increasing elevation which is a proxy of gait quality. This may be perceived from a rider's perspective as the tendency to hurry the trot, which is usually considered a negative aspect since it does not create momentum and energy usable for sports activities like jumping or dressage. This trend may be influenced by breed traditional use as those historically used for draft work prioritize forward movement over vertical collection. Conversely, stride frequency at canter is positively correlated with elevation at trot and canter (0.426, 0.303), indicating that increasing stride frequency likely leads to increased elevation and overall gait activity and quality. In small breeds like the Bardigiano horse, the activity of the gait is considered positive and usually is described by the rider as a movement of the body weight on the back limbs, which can lead to an improved propulsion forward and upward.

The correlations between judge or rider scores and sensor data reveal interesting results for both general traits such as rideability and obedience, as well as specific ones like flatwork, trot, or elevation at trot and canter. Rideability correlates positively with stride frequency at walk (0.255) indicating that judges perceive a better work attitude in horses with good activity at walk. A negative correlation was found between rideability and heart rate at canter (−0.318); this suggests that horses with lower heart rates during canter tend to be more rideable and easier for the rider to manage. Similarly, obedience and trust towards the rider during exercises moderately correlate with stride frequency at walking (0.275) and negatively with heart rate at canter (−0.304), suggesting an overlap between the evaluation of rideability and obedience. Elevation at canter, measured by the sensor, exhibited positive correlations (from 0.374 to 0.471) with the evaluation provided by the rider regarding impulsion at canter, frequence at canter and effort recovery time. This suggests riders' capability to discriminate overall gait quality, providing a positive score for horses that are engaging the back limbs in several canter related evaluation. The positive correlation of elevation at canter expressed by the sensor with recovery time expressed by the judges (0.374) can be explained through the association between higher stride and increased energy expenditure with a consequent increase in heart rate, resulting in a longer recovery time. Conversely, the correlation between the elevation measured by the sensor and the evaluation provided by the judges regarding elevation (0.461) and frequency (0.471) indicates a lack of differentiation by riders' scores between the two measurements. Judges tended to unify the two results, considering the overall quality of the canter gait and giving a positive score in both elevation and frequency if the horse reveals a high elevation at canter, recorded by the sensor. Regarding elevation at trot from sensor data, it only correlates significantly with recovery time (0.263), suggesting that higher elevation requires more effort for the horse, resulting in a

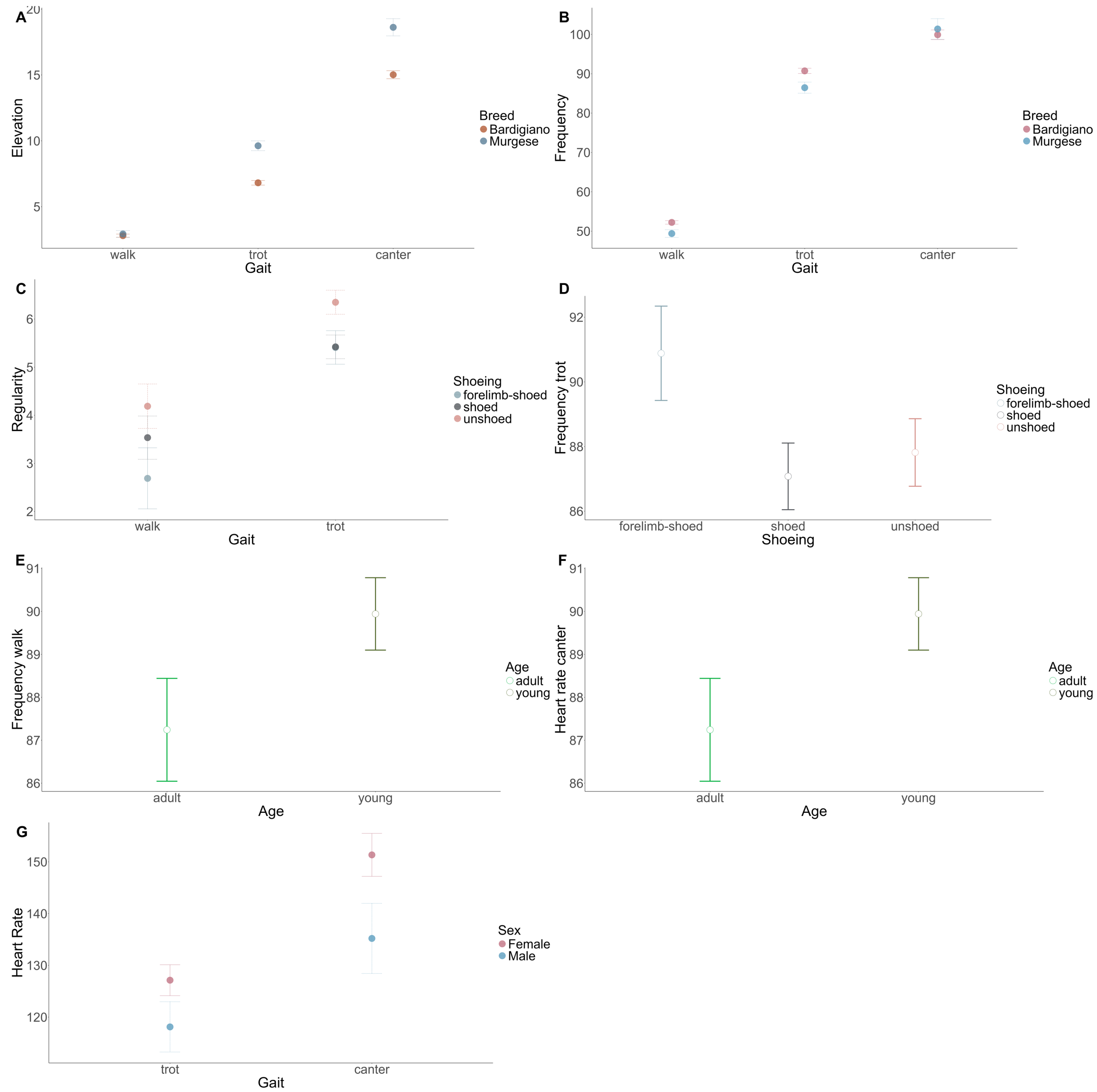

FIGURE 3
LSM (Least Squares Means) results show the effect of breed on elevation and frequency at the three gaits **(A,B)**, the effect of shoeing on regularity at trot and walk **(C)** and frequency at trot **(D)**, the effect of age on frequency at walk and heart rate at canter **(E,F)**, and the effect of sex on and heart rate at trot and canter **(G)**.

longer time needed to recover the energy perceived by the rider. Heart rate during canter displayed negative correlations (ranging between −0.284 to −0.352) with the evaluation of rideability, trot, canter, obedience, and flatwork. This suggests that an increased heart rate during canter results in reduced rideability, obedience, and overall gait quality assessed by judges' scores. Conversely, stride frequency at walk showed moderate positive correlation (ranging from 0.235 to 0.280) with management of the horse, rideability, obedience, and flatwork, reflecting the perception of an active, obedient, and responsive horse engaging its hindlimbs and ready to respond to the riders' requests.

Regarding the correlation within judges' and riders' evaluation, a highly positive correlation between obedience and rideability (0.914) implies that these evaluations may measure the same aspect of the horse's behavior. Therefore, it may be helpful to unify these scores into a single evaluation assessing the overall attitude of the horse towards collaboration with the rider, simplifying the assessment process for judges and ensuring consistency in evaluations. The strong positive correlation between elevation and frequency at canter (0.983) provided by the rider indicates riders' difficulty in objectively discriminating those two traits. Riders often evaluate positively a horse exhibiting an active gait, characterized by both good elevation and frequency. This preference aligns with the improvement that is sought for breeding purposes, such as for the Bardigiano horse, that originally was bred for agricultural purposes; thus, an active canter is not common and at the same time appreciated by the judges.

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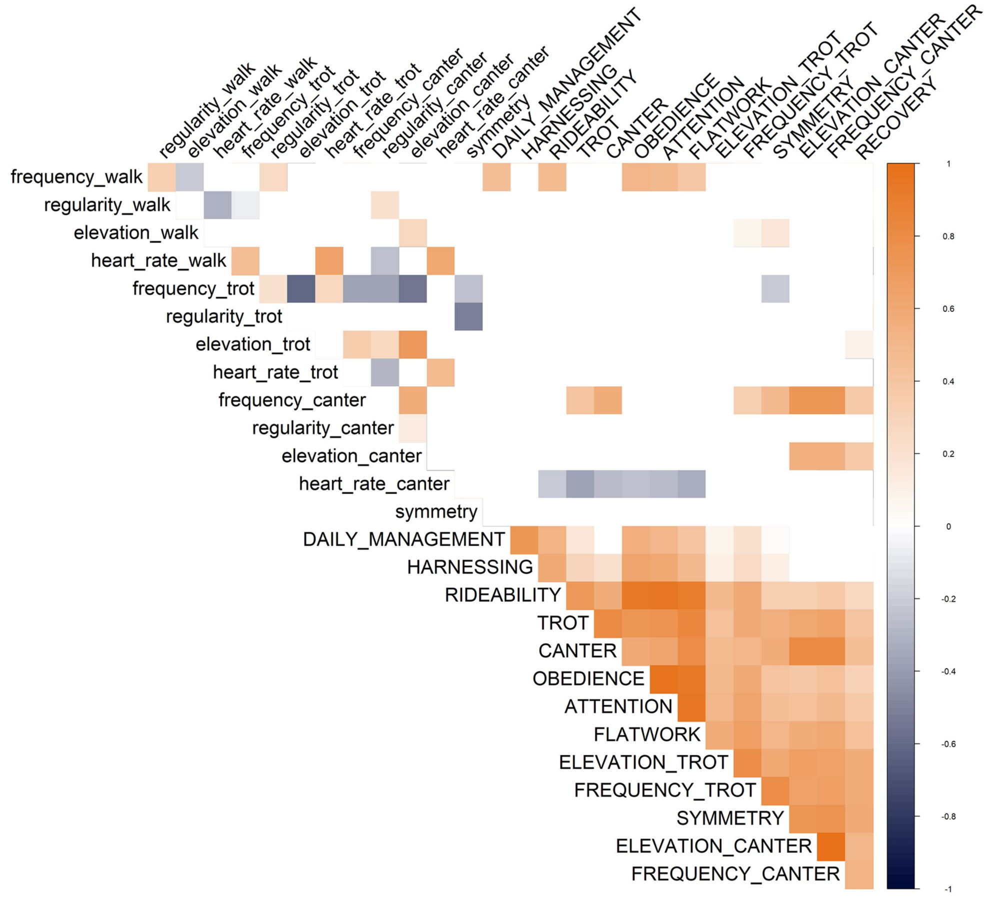

FIGURE 4
Correlation plot. Displays significative correlations between judges' evaluations (uppercase) and sensor data (lowercase). Positive correlations are in orange, while negative correlations are in blue with transparency reflecting correlation's strength.

3.4 Predictive models

To explore the predictive potential of sensor data for evaluation outcomes, three different models were tuned, used and their performance measured within a 10-fold cross-validation. Among the three models tested, the GBM model achieved the highest accuracy and lowest error, with its F1 score consistently surpassing those of the other models (Figure 5).

The SVM model has consistently shown the widest range of all classification performance metrics. Accuracy for the SVM model ranged from 55 to 100%, while GBM and KNN models demonstrated higher consistency, with accuracies from 74 to 100% for GBM and 64 to 88% for KNN (Supplementary Table S3). Despite reaching higher accuracy for some traits, the F1 scores of the SVM models were generally lower, due to a lower specificity. This suggests a tendency to classify all cases as positive; this possibly is due to its susceptibility to unbalanced classes (31), also indicating that our classes may lack clear separation and seem to overlap. Indeed, this model is better suited for classification tasks with distinct class boundaries. The KNN model showed consistency and tended to achieve a high level of specificity but had the lowest sensitivity, which indicates difficulty in detecting positive cases while correctly identifying negative ones. These results also suggest sensitivity to irrelevant features, highlighting the need for careful feature selection and training samples to improve performance, as already proved by several studies (26, 32, 33). In our data this is highlighted by the predictive trials that exhibited lowest sensitivity scores, such as those assessing daily management, rideability, obedience and attention to requests. These traits lack clear sensor-collected values and are objectively more challenging to detect solely through sensor data.

The evaluations predicted with the highest overall accuracy were rideability, attention to the requests, and recovery time, with respective accuracies of 83% (F1 = 0.74), 85% (F1 = 0.75), and 90% (F1 = 0.90). This indicates that we can correctly predict over 80% of the judges' scores using IMU measurements. The closeness between the accuracy in % and the F1 Score suggests that the misclassified results will be equally distributed between False Positive and False Negative, leading to a balanced model.

Focusing on the best model (GBM), the highest accuracies (100%) were observed for daily management, flatwork, and recovery time, followed by an accuracy of 88% for attention to requests and trot trial. Although 100% accuracy must be interpreted with caution, as it may indicate overfitting, it could still be a realistic prediction for very small datasets and easily predictable trials. Indeed, a precise collection of heart rate data makes recovery time easy to predict, similarly the flatwork score should be straightforward to predict since the evaluation is based on frequency, elevation, and regularity. All F1 scores were above 0.85. Some results such as those for trot or flatwork and recovery time were expected due to the direct link of IMU data and judges' evaluations since they are evaluating the same aspects.

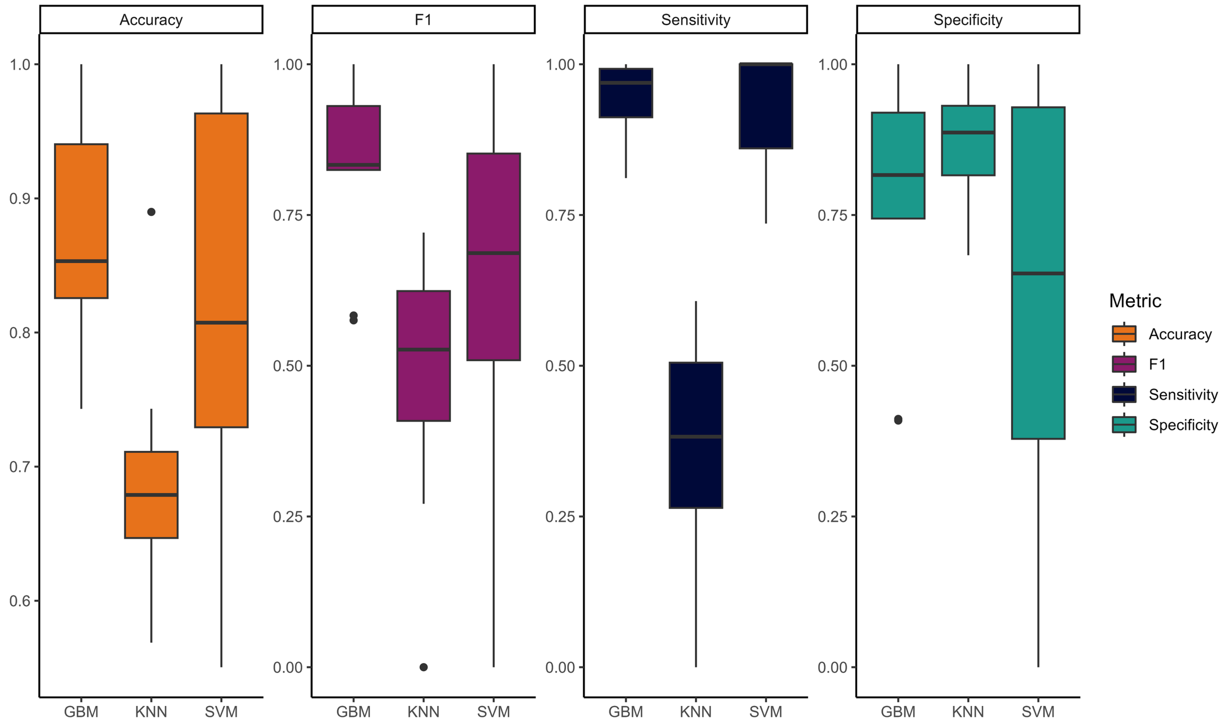

FIGURE 5
Overall model performance comparison through different metrics.

Oppositely, the impact of daily management and attention to requests on the traits measured by the sensor is not straightforward and may lead to poor classification results. However, it appears that gait parameters somehow predict aspects related to the horse's behavior and collaboration with humans. A possible explanation would be that the rider's corrective actions after an unexecuted request may influence the overall balance of the horse and its natural gait, interfering with the sensor-measured traits.

Although these results are preliminary and judges' evaluations were divided into only positive or negative scores, there seems to be the possibility to predict judges' scores from sensor data. With more data collected in the future, it might be possible to predict judges' scores through portable and easy to use sensor data, potentially reducing human error and providing owners or buyers with a more precise way to evaluate animals. This cost-effective method could allow for the evaluation of more animals, aiding in selecting horses that better meet the desires of future owners regarding behavior, dressage performance, or recovery capability, aspects which are increasingly important.

4 Conclusion

In conclusion, this research aimed to study gait traits using sensor data collected via Equisense Motion S®. Differences in several gait traits were identified, highlighting the different predispositions between the Bardigiano and Murgese. These differences emphasize the importance of preserving local breeds, as they possess unique gait traits that are essential for maintaining their genetic and functional diversity. Factors such as shoeing and age showed an effect on most of the gait traits collected via Equisense Motion S®. In contrast, riders' skills and horses' training levels did not significantly influence gait traits, possibly due to the homogeneity of our samples. It was also observed that only the walk gait trait remained consistent across the two trials, suggesting that other gait traits may be more susceptible to variations due to training. Most of the judges' evaluations are correlated to sensor data although some of them were strongly related to each other. This suggests that judges often assess overall gait quality rather than focusing on specific traits. This reinforces the value of sensor data for detailed analysis of gait traits, which are challenging to assess accurately with the human vision alone. Sensor data allowed for accurate prediction of judges' evaluations, demonstrating the potential of this technology for reliable performance assessment. In conclusion, the integration of sensor technology provides valuable insights into horse's performance evaluation, with implications for breeding, training, and competitive sports. This technological advancement can help the breeders in identifying horses with higher rideability potential, thereby accelerating the selection process.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

Ethical approval was not required for the studies involving animals in accordance with the local legislation and institutional requirements because non invasive techniques were employed. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

VA: Conceptualization, Data curation, Formal analysis, Methodology, Visualization, Writing – original draft. MA: Conceptualization, Supervision, Writing – review & editing. AM: Methodology, Writing – review & editing. AZ: Validation, Writing – review & editing. MV: Resources, Writing – review & editing. AS: Project administration, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2024.1459553/full#supplementary-material

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Autonomic regulation in athletic horses repetitively participating in two novice jumping classes on consecutive days

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Introduction: Animal welfare is of great concern in equestrian sports and has been evaluated in athletic horses competing at different levels. However, the impact of consecutive days of jumping competition and the extent of resultant stress responses remains unclear. To address this point, the present study compared the changes in stress response via heart rate variability (HRV) in horses participating in two national jumping events on consecutive days.

Methods: The study involved six experienced horses equipped with heart rate monitoring devices. HRV variables were measured before, during, and after jumping at 10-min intervals for 60 min on each competition day.

Results: Multiple HRV variables decreased to varying degrees on both days from warm-up until 30 min post-jumping. Meanwhile, the mean heart rate increased during jumping and returned to normal levels at 50 min post-jumping on the first day (for all intervals, $p < 0.05–0.001$), while it remained elevated beyond 60 min post-jumping on the second day (for all intervals, $p < 0.01–0.001$). Additionally, maximum heart rate and respiratory rate were higher on the second day than in the first round during the warm-up phase ($p < 0.05$ for both variables). The proportion of the HRV low-frequency band was higher during riding on the second day ($p < 0.05$), while the proportion of the high-frequency band was reduced during warm-up on the first day ($p < 0.05$) and during course riding on the second ($p < 0.01$). Meanwhile, the sympathetic nervous system index took longer to return to baseline on the second day than on the first.

Discussion: These results suggest that autonomic regulation differed in horses between jumping rounds on two consecutive days, with horses experiencing higher sympathetic activity and potentially increased stress in the second round. This information is important for riders, highlighting the need to be mindful of potential stress that could, at least in part, impact the welfare of horses participating in the same jumping competition on consecutive days.

KEYWORDS

heart rate variability, horse, jumping, repetitive competition, stress response, welfare

1 Introduction

Equestrian sports such as dressage, eventing, endurance, and jumping are increasingly popular worldwide. International equestrian events are governed by the Fédération Equestre Internationale (FEI) to ensure fairness during the competition and to preserve horse welfare throughout the competition period (1, 2). The FEI Database contains 272,000 horses and nearly 60,000 are registered annually by national federations (3). Athletic horses frequently encounter stressful conditions during training and competition (4). Although horses are well able to perform a variety of exercises of different intensities, diverse factors may compromise performance and welfare, including insufficient or inhumane training (5), mismatching between rider and horse (6) and insufficient rest before repeated competition (7).

Recently, horse welfare in equestrian sports has become a concern to the general public and stakeholders (8). Researchers have proposed various parameters that may reflect welfare in horses, including hormonal release (9, 10), behaviour changes (11, 12), and heart rate variability (HRV) modulation (13, 14). HRV is a short-term fluctuation in the interval between adjacent heartbeats during the cardiac cycle (15, 16). These variations are governed by the autonomic nervous system (ANS), specifically the interplay between the sympathetic nervous system (SNS) and parasympathetic nervous system (PNS) or vagal components (15, 17, 18), known as sympathovagal interaction. An increased HRV reflects the flexibility and adaptability of the body's biological system to cope with external challenges and maintain its physiological function (15, 19). Modulation in HRV has been used as an indicator of stress responses in horses with cardiac diseases (20, 21), during road transportation (10, 22, 23), and exercise (24–26). HRV has also been used to monitor ANS responses in athletic horses participating in equestrian events such as dressage (9, 27), eventing (28), and jumping (29, 30).

Several HRV variables are applied to indicate sympathetic and vagal activities. Beat-to-beat (RR) intervals and the standard deviation of normal-to-normal intervals (SDNN) reflect the long-term variability of heartbeat, which is influenced by both sympathetic and vagal components (15). In contrast, measurements of the square root of the mean squared differences between successive RR intervals (RMSSD), the number of successive RR interval pairs that differ by more than 50 msec (NN50) and its relative value (pNN50) mirror the short-term variation of heartbeats under the influence of vagal activity (16, 17). A decrease in these time domain variables indicates reduced vagal activity (15, 31, 32). The low-frequency (LF) band modulates under sympathetic and vagal contribution and mirrors the cardiac cycle's long-term variation. An increased high-frequency (HF) band reflects vagal dominance, contributing to short-term heartbeat variation (15–17). The LF/HF ratio is an indicator of sympathovagal balance (17, 33, 34), with a decreased LF/HF ratio reflecting vagal dominance (16, 35). The standard deviation of the Poincaré plot perpendicular to the line of identity (SD1) is used to determine short-term heart rate variation reflecting vagal activity (17), while the standard deviation of the Poincaré plot along the line of identity (SD2) is an indicator of long-term heart rate variability under sympathetic and vagal influences (16, 17). The SD2/SD1 ratio closely relates to the LF/HF ratio for the determination of ANS balance (16, 36).

Show jumping is growing in popularity, and is organised in international and local competitions worldwide (2). The jumping horse has to clear various obstacles of different heights and difficulties within a limited time to obtain a clear round (2, 37). It has been reported that HRV differs between horses competing in jumping courses with different fence heights, with reduced HRV during the course indicating greater stress (29). However, HRV variables have not been examined in horses participating in jumping courses on two consecutive days. Therefore, this study aimed to compare the HRV modification in horses competing over 2 days at a national jumping event. It was hypothesised that the extent of HRV modulation differs between horses participating in the first and second jumping courses taking place on consecutive days.

Abbreviations: HR, heart rate; HRV, heart rate variability; RR, beat-to-beat; SDNN, standard deviation of normal-to-normal intervals; RMSSD, square root of the mean squared differences between successive RR interval; pNN50, relative number of successive RR interval pairs that differ by more than 50 msec; TINN, triangular interpolation of normal-to-normal intervals; VLF, very low frequency; LF, low frequency; HF, high frequency; RESP, respiratory rate; SD1, standard deviation of the Poincaré plot perpendicular to the line of identity; SD2, standard deviation of the Poincaré plot along the line of identity; ANS, autonomic nervous system; PNS, parasympathetic nervous system; SNS, sympathetic nervous system.

2 Materials and methods

2.1 Horses

Nine healthy warm-blooded horses (three geldings, six mares, aged 10–14 years, weighing 400–500 kg), all trained and experienced in national jumping competitions, were enrolled. The study took place during a national jumping competition at the Thai Polo and Equestrian Club in Pattaya, Thailand. Before the study, all horses underwent physical examination by an equine practitioner to assess vital signs and gait. A normal haemogram was also observed following haematological examination. During the competition, horses were housed in a 4x4x5 m designated stable at the venue. Two to three kilogrammes of commercial pellets were fed three times daily. The horses had free access to hay and water in their stables. The inclusion criteria for horses in this study were: (1) the horse had participated in at least one jumping event during the year before the study; (2) the horse had passed the first inspection according to national jumping regulations; (3) the horse had not received medical or surgical treatment for at least 2 weeks before the study. This study did not cause horses to suffer injury or experience circumstances that compromised their welfare before or during the competition. The horses' owners signed consent forms before the start of the study. Riders or the person responsible for the horses' care were able to withdraw the horse at any stage of the study.

2.2 Experimental protocol

This study was conducted as a case–control study, requiring the horses to compete in two jumping courses on two consecutive days. Riders were also designated to ride the same horse on both days. The jumping events were run on a 55 × 90 metres silica sand competition arena, containing 10–11 jumping fences with specific heights for each course. The relative humidity and air temperature during the two-day experiment were 85–90% and 32–34°C, respectively. During the experiment, a heart rate monitoring (HRM) device was installed on horses to record RR interval data for HRV determination. The HRM set included an equine belt for riding (Polar Electro Oy, Kempele, Finland), a heart rate sensor (Polar H10) and a sports watch for receiving RR intervals data (Polar Vantage 2). Briefly, horses were equipped with a heart rate sensor attached to the equine belt on the left side of the chest. The sensor was wirelessly connected to the sports watch. The HRM device was installed at least 30 min before the warm-up session and removed 60 min after jumping. Three horses were withdrawn from the study by their riders after obtaining a clear round at the first jumping course. RR interval data from only the six horses who participated in both event days were used for HRV determination in this study.

2.3 Data acquisition

RR interval data were recovered from the sports watch using the Polar Flow program.[1] Kubios premium software (Kubios HRV Scientific)[2] was then utilised to compute HRV variables from the RR interval data. The premium version of the Kubios program provides automatic artefact correction algorithms, which are validated and produce more accurate time series evaluations than the standard version (38). The additional automatic noise detection function removes unpredictable noise in long-term RR interval recording where the signal may not be high quality throughout.[3] Trend components were modified using smoothness priors at 500 ms. The autonomic noise correction was set at a medium level. The following HRV variables were computed:

Time domain analysis: heart rate (**HR**), beat-to-beat (**RR**) intervals, standard deviation of the normal-to-normal intervals (**SDNN**), square root of the mean squared differences between successive RR intervals (**RMSSD**), relative number of successive RR interval pairs that differ by more than 50 msec (**pNN50**), triangular interpolation of normal-to-normal intervals (**TINN**) and RR triangular index.

Frequency domain analysis: very-low-frequency (**VLF**) (by default 0–0.04 Hz), low-frequency (**LF**) (by default 0.04–0.15 Hz), high-frequency (**HF**) (by default 0.15–0.4 Hz), **LF/HF ratio** and respiratory rate (**RESP**).

Nonlinear analysis: standard deviation of the Poincaré plot perpendicular to the line of identity (**SD1**), standard deviation of the Poincaré plot along the line of identity (**SD2**) and SD2/SD1 ratio.

Autonomic nervous system (ANS) index: stress index, parasympathetic nervous system (**PNS**) index and sympathetic nervous system (**SNS**) index.

The HRV variables were reported as 15 min at rest (control), warm-up period, course jumping period, and 10-min periods at 10, 20, 30, 40, 50, and 60 min post-jumping.

1 https://flow.polar.com/

2 https://www.kubios.com/hrv-premium/

3 https://www.kubios.com/downloads/Kubios_HRV_Users_Guide.pdf

2.4 Data analysis

Data were analysed using GraphPad Prism version 10.2.3 (GraphPad Software Inc., San Diego, USA). Due to missing data during the measurement, a mixed-effects model (restricted maximum likelihood; REML) was applied to evaluate the effects of group, time, and group-by-time on changes in HRV variables in horses on both jumping days. Dunnett's multiple comparisons test was used to estimate the differences in HRV variables within the group compared to those before the competition (control) and between groups at given time points. Due to the normal distribution of the data confirmed by the Shapiro–Wilk test, a paired *t*-test was used to calculate the differences in competition speed, warm-up and course jumping periods between groups. Data were expressed as mean ± SEM. The statistical significance was set at $p < 0.05$.

3 Results

3.1 Warm-up period, riding speed, and riding duration during jumping on both days

The parameters and competition results from the first and second jumping days are shown in Table 1. The horses participated in jumping courses with a fence height of 70–120 cm in the first and 70–130 cm in the second courses, respectively. Horses 1 and 6 jumped an increased fence height in the second course, while the other horses (horses 2–5) jumped a similar height in both jumping courses. On both days, a similar warm-up period (63.13 ± 8.10 vs. 56.96 ± 8.70 min, $p = 0.1827$), riding speed (3.72 ± 0.24 vs. 3.53 ± 0.29 m/s, $p = 0.6098$) and riding duration (71.63 ± 6.95 vs. 74.91 ± 7.68 s, $p = 0.3307$) were observed. Only horse 1 achieved two clear rounds, while horses 2 and 4 were eliminated due to refusal in both courses. Likewise, the performance regarding total penalties of horses 3, 5 and 6 was similar on both days.

3.2 Heart rate variability

3.2.1 Time domain analysis

Time, group and group-by-time affected the modulation of peak HR ($p < 0.0001$, $p = 0.0364$ and $p = 0.0049$, respectively), while group-by-time and time affected changes in minimum HR ($p < 0.0001$ and $p = 0.0084$), mean HR ($p < 0.0001$ for both effects) and mean RR intervals ($p < 0.0001$ and $p = 0.0178$). SDNN, RMSSD, pNN50, TINN, and RR triangular index were associated with time ($p < 0.0001$ for all variables; Tables 2, 3).

A significant increase in mean HR was detected during warm-up in horses in the first jumping course. During the jumping period, the increase in mean HR was not significant ($p = 0.2379$), but an increased mean HR was again detected at 10–50 min post-jumping (for all intervals, $p < 0.05–0.001$) in horses on the first day. On the second jumping day, mean HR increased during the warm-up period and the increase over baseline lasted until 60 min post-jumping (for all intervals, $p < 0.05–0.001$). Mean HR was higher in horses in the second than the first jumping course, but this difference was insignificant ($p = 0.0762$). Minimum HR rose at 10–60 min post-jumping in horses on the first day ($p < 0.05–0.01$), but remained increased from the warm-up period until 60 min post-jumping on the second ($p < 0.05–0.001$). Peak HR increased

TABLE 1 Parameters and competition results of horses participating in 2 days of a jumping competition.

Horses	Fence height (cm)	Average speed (m/s)	Jump faults (points)	Time taken (sec)	Time faults (points)	Total penalty
First jumping day						
No. 1	70	4.02	0	52.83	0	0
No. 2	100	3.45	–	–	–	E
No. 3	70	3.47	8	84.25	18	26
No. 4	100	4.76	–	–	–	E
No. 5	70	3.08	4	69.84	0	4
No. 6	120	3.56	16	79.60	0	16
Second jumping day						
No. 1	90	3.16	0	58.92	0	0
No. 2	100	3.94	–	–	–	E
No. 3	70	4.65	4	93.91	24	28
No. 4	100	3.71	–	–	–	E
No. 5	70	2.86	0	66.84	0	0
No. 6	130	2.86	16	79.98	0	16

TABLE 2 Time-domain analysis of HRV in horses participating in 2 days of a national jumping competition.

Periods	Classes	Time domain results			
		Mean HR (beats/min)	Min HR (beats/min)	Max HR (beats/min)	Mean RR (ms)
Control	1st	35.50 ± 2.38	31.00 ± 1.95	51.43 ± 4.21	1818.17 ± 107.10
	2nd	33.17 ± 1.58	29.17 ± 1.25	52.17 ± 5.38	1837.83 ± 91.12
Warm-up	1st	66.83 ± 7.44^{a}	37.67 ± 5.40	125.67 ± 11.31^{b,x}	963.50 ± 121.47^{b}
	2nd	87.50 ± 7.34^{b}	34.17 ± 2.27^{a}	166.17 ± 8.58^{d,y}	716.17 ± 73.49^{c}
Course riding	1st	114.00 ± 22.00	100.00 ± 10.00	124.00 ± 30.00	545.00 ± 105.00
	2nd	162.50 ± 10.71^{c}	132.67 ± 10.76^{c}	178.50 ± 11.51^{c}	378.17 ± 26.25^{d}
10 min post-jumping	1st	74.60 ± 6.06^{b}	64.40 ± 6.27^{b}	91.00 ± 6.80^{c}	826.20 ± 69.81^{b}
	2nd	76.00 ± 4.32^{c}	60.67 ± 3.14^{c}	120.67 ± 17.08	802.83 ± 45.21^{c}
20 min post-jumping	1st	55.40 ± 3.64^{c}	45.40 ± 1.94^{b}	82.00 ± 9.06	1099.80 ± 66.81^{b}
	2nd	57.33 ± 2.74^{d}	44.50 ± 2.78^{b}	77.67 ± 2.32^{a}	1056.83 ± 46.59^{c}
30 min post-jumping	1st	48.25 ± 3.90^{b}	40.50 ± 2.06^{a}	68.75 ± 8.22	1271.00 ± 107.54^{b}
	2nd	49.33 ± 3.48^{b}	41.83 ± 2.81^{b}	65.83 ± 4.35	1246.50 ± 83.29^{c}
40 min post-jumping	1st	45.50 ± 3.07^{a}	37.50 ± 2.25^{a}	66.50 ± 5.95	1337.00 ± 88.59
	2nd	47.60 ± 4.26^{a}	41.20 ± 2.54^{b}	71.00 ± 3.85	1297.20 ± 105.33^{b}
50 min post-jumping	1st	43.80 ± 1.91^{a}	36.80 ± 1.69^{a}	58.40 ± 5.60	1384.20 ± 65.07
	2nd	46.00 ± 3.56^{a}	40.20 ± 2.69^{a}	62.80 ± 6.30	1330.80 ± 95.15^{b}
60 min post-jumping	1st	42.40 ± 2.48	36.60 ± 1.47^{a}	62.80 ± 8.67	1443.20 ± 91.47
	2nd	48.20 ± 2.33^{b}	40.00 ± 2.37^{a}	70.80 ± 7.97	1264.20 ± 59.53^{b}
Effects (*p*-value)	Time	<0.0001*	<0.0001*	<0.0001*	<0.0001*
	Group	0.1075	0.3645	0.0364*	0.4770
	Interaction	<0.0001*	0.0084*	0.0049*	0.0178*

*Indicate significant effects of time, group, and group-by-time (interaction).
a, b, c and d indicate statistical significance at $p < 0.05$, 0.01, 0.001, and 0.0001 within the group compared to the control value.
x and y indicate statistical differences of a pair of comparisons between groups at given time points. HRV, heart rate variability; RR, beat-to-beat interval; HR, heart rate; SDNN, standard deviation of normal-to-normal RR intervals; RMSSD, root mean square of successive RR interval differences. Recovery post-jumping.

TABLE 3 Time-domain analysis of HRV in horses participating in 2 days of a national jumping competition.

Periods	Classes	Time domain results				
		SDNN (ms)	RMSSD (ms)	pNN50 (%)	TINN (ms)	RR triangular index
Control	1st	66.40 ± 11.41	67.07 ± 13.09	30.99.5.89	379.83 ± 64.68	13.26 ± 1.91
	2nd	61.18 ± 6.80	54.12 ± 4.00	25.87 ± 3.60	339.33 ± 34.15	11.26 ± 1.15
Warm-up	1st	40.55 ± 5.22[c]	25.15 ± 3.98[b]	6.96 ± 2.47[c]	301.33 ± 30.83	7.91 ± 1.44[a]
	2nd	31.95 ± 4.79[c]	17.73 ± 4.00[b]	3.28 ± 1.65[c]	284.83 ± 34.25	5.89 ± 0.76[a]
Course riding	1st	6.85 ± 1.25[d]	4.40 ± 1.60[b]	0.00 ± 0.00[b]	30.00 ± 15.00[c]	2.09 ± 0.59[b]
	2nd	10.07 ± 1.50[d]	3.92 ± 0.15[b]	0.00 ± 0.00[b]	43.33 ± 5.77[c]	3.25 ± 0.37[b]
10 min post-jumping	1st	26.98 ± 9.98[a]	19.20 ± 8.60[b]	2.24 ± 1.46[c]	131.20 ± 39.34[b]	4.63 ± 0.88[b]
	2nd	22.30 ± 6.21[a]	16.30 ± 5.06[b]	3.53 ± 2.48[c]	147.33 ± 39.18[b]	4.46 ± 0.97[b]
20 min post-jumping	1st	40.48 ± 4.47[a]	33.72 ± 4.28[a]	13.50 ± 4.13[b]	193.20 ± 27.15[a]	8.41 ± 1.22
	2nd	44.77 ± 7.68[a]	34.58 ± 5.18[a]	13.34 ± 3.54[b]	247.17 ± 41.33[a]	10.60 ± 1.95
30 min post-jumping	1st	56.35 ± 7.76	46.03 ± 5.43	22.40 ± 4.93	311.00 ± 39.74	12.67 ± 1.85
	2nd	48.60 ± 6.48	40.85 ± 5.95	16.75 ± 4.29	268.67 ± 39.52	10.20 ± 1.27
40 min post-jumping	1st	73.05 ± 12.44	62.45 ± 14.01	30.54 ± 6.95	346.50 ± 58.38	14.15 ± 1.74
	2nd	57.38 ± 11.49	47.30 ± 10.70	19.32 ± 6.07	313.20 ± 50.17	10.52 ± 2.16
50 min post-jumping	1st	71.82 ± 7.11	61.88 ± 6.54	35.29 ± 4.15	326.80 ± 32.72	13.91 ± 1.46
	2nd	61.72 ± 14.40	53.36 ± 11.85	24.69 ± 7.89	312.60 ± 71.56	13.03 ± 2.75
60 min post-jumping	1st	76.36 ± 7.24	77.06 ± 14.61	39.19 ± 7.04	358.40 ± 41.74	13.71 ± 2.65
	2nd	83.48 ± 15.79	67.00 ± 13.25	32.66 ± 7.41	394.60 ± 65.52	17.53 ± 3.07
Effects (*p*-value)	Time	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
	Group	0.7569	0.4132	0.3571	0.8456	0.9401
	Interaction	0.6004	0.9258	0.5899	0.5598	0.1544

*Indicate significant effects of time, group, and group-by-time (interaction).
a, b and c indicate statistical significance at $p < 0.05$, 0.01 and 0.001 within the group compared to the control value.
HRV, heart rate variability; NN50, number of successive RR interval pairs that differ by more than 50 msec; pNN50, relative number of successive RR interval pairs that differ by more than 50 msec; TINN, triangular interpolation of normal-to-normal intervals; RR, beat-to-beat interval.

during the warm-up period (first course: $p < 0.01$, second course: $p < 0.0001$), and values were higher during the second than the first course ($p < 0.05$). Moreover, compared to the control period, an increased peak HR during jumping was detected only during the second course ($p < 0.001$). A significant decrease in mean RR interval was measured during the warm-up period before the first jumping course. Although there was an insignificant decrease in the mean RR interval during jumping ($p = 0.1041$), a considerable reduction in the mean RR interval was subsequently registered at 10–30 min post-jumping following the first course ($p < 0.01$ for all mentioned periods). On the second day, the mean RR interval decreased during the warm-up period and remained decreased until 60 min post-jumping (for all intervals, $p < 0.01$–0.0001). On both jumping days, SDNN and RMSSD decreased during warm-up ($p < 0.001$ and $p < 0.01$, respectively) and then plunged to the lowest value during jumping ($p < 0.0001$ and $p < 0.01$, respectively). They eventually rose to reach values similar to baseline at 30 min post-jumping ($p < 0.05$–0.01 for both variables; Table 2). Although only pNN50 began to decrease in the warm-up period, pNN50 and TINN decreased during jumping and remained lower until 20 min post-jumping (pNN50, $p < 0.01$–0.001; TINN, $p < 0.05$–0.001). The RR triangular index declined during warm-up ($p < 0.05$–0.01), jumping ($p < 0.01$) and 10 min post-jumping ($p < 0.01$; Table 3).

3.3 Frequency domain analysis

There was a group-by-time and time effect on the modification of the proportion of LF ($p = 0.0016$ and $p = 0.0495$, respectively), while the change in HF proportion was only affected by group-by-time ($p = 0.0169$). There was an independent group effect for RESP ($p = 0.0229$). Modulation in VLF % was associated with time ($p = 0.0132$, respectively); meanwhile, the LF/HF ratio was unaffected by the effects of group-by-time, group or time (Table 4).

The VLF proportion decreased at 20–50 min post-jumping (for all intervals, $p < 0.05$–0.01). Even though the LF percentage did not change throughout the study over both jumping days, it was higher in horses during jumping in the second than in the first course ($p < 0.05$). The HF percentage decreased during the warm-up period in horses before the first course ($p < 0.05$) and during jumping in the second course ($p < 0.01$), while the LF/HF ratio rose only during warm-up ($p < 0.05$). There was no change in RESP throughout the first day, while the RESP increased during jumping ($p < 0.05$) and 10 min post-jumping ($p < 0.01$) on the second day. Moreover, RESP during warm-up and 40 min post-jumping was higher on the second than the first day ($p < 0.05$ for both periods; Table 4).

TABLE 4 Frequency domain analysis of HRV in horses participating in 2 days of a national jumping competition.

Periods	Classes	Frequency domain results				
		VLF (%)	LF (%)	HF (%)	LF/HF ratio	RESP (Hz)
Control	1st	0.25 ± 0.03	0.56 ± 0.03	0.19 ± 0.02	3.13 ± 0.48	0.195 ± 0.015
	2nd	0.34 ± 0.03	0.53 ± 0.03	0.12 ± 0.02	4.94 ± 0.77	0.188 ± 0.014
Warm-up	1st	0.32 ± 0.05	0.60 ± 0.05	0.08 ± 0.0[a]	7.88 ± 1.55[a]	0.165 ± 0.027[x]
	2nd	0.28 ± 0.02	0.65 ± 0.02	0.07 ± 0.02	10.62 ± 1.91[a]	0.248 ± 0.012[y]
Course riding	1st	0.33 ± 0.29	0.32 ± 0.02[x]	0.37 ± 0.34	4.36 ± 3.96	0.245 ± 0.165
	2nd	0.40 ± 0.07	0.56 ± 0.08[y]	0.04 ± 0.0[b]	33.22 ± 14.23	0.312 ± 0.040[a]
10 min post-jumping	1st	0.28 ± 0.04	0.56 ± 0.06	0.16 ± 0.04	6.58 ± 3.91	0.180 ± 0.047
	2nd	0.27 ± 0.06	0.57 ± 0.05	0.16 ± 0.03	4.19 ± 0.93	0.302 ± 0.014[b]
20 min post-jumping	1st	0.12 ± 0.05[b]	0.66 ± 0.05	0.22 ± 0.06	3.92 ± 0.75	0.184 ± 0.028
	2nd	0.14 ± 0.03[b]	0.67 ± 0.05	0.20 ± 0.04	4.30 ± 1.01	0.235 ± 0.020
30 min post-jumping	1st	0.14 ± 0.02[b]	0.69 ± 0.03	0.17 ± 0.03	4.48 ± 0.94	0.225 ± 0.010
	2nd	0.15 ± 0.03[b]	0.64 ± 0.06	0.21 ± 0.06	4.81 ± 1.46	0.223 ± 0.010
40 min post-jumping	1st	0.18 ± 0.05[a]	0.67 ± 0.04	0.16 ± 0.04	5.30 ± 1.62	0.200 ± 0.007[x]
	2nd	0.24 ± 0.06[a]	0.57 ± 0.04	0.19 ± 0.05	3.95 ± 0.87	0.232 ± 0.006[y]
50 min post-jumping	1st	0.18 ± 0.02[a]	0.68 ± 0.02	0.14 ± 0.03	6.78 ± 2.43	0.178 ± 0.036
	2nd	0.14 ± 0.02[a]	0.66 ± 0.05	0.19 ± 0.05	4.28 ± 0.98	0.230 ± 0.008
60 min post-jumping	1st	0.18 ± 0.07	0.62 ± 0.05	0.20 ± 0.05	4.02 ± 1.10	0.196 ± 0.033
	2nd	0.18 ± 0.07	0.68 ± 0.06	0.13 ± 0.02	5.60 ± 1.09	0.234 ± 0.007
Effects (*p*-value)	Time	0.0132*	0.0016*	0.2201	0.2196	0.2848
	Group	0.5371	0.6248	0.1448	0.2108	0.0229*
	Interaction	0.8679	0.0495*	0.0358*	0.1085	0.1721

*Indicate significant effects of time, group, and group-by-time (interaction).
a and b indicate statistical significance at $p < 0.05$, 0.01 within the group compared to the control value.
x and y indicate statistical differences of a pair of comparisons between groups at given time points. HRV, heart rate variability; VLF, very-low-frequency band, by default 0–0.04 Hz; LF: low-frequency band, by default 0.04–0.15 Hz; HF, high-frequency band, by default 0.15–0.4 Hz; RESP, respiration rate.

3.4 Nonlinear analysis and ANS index

The SD1, SD2, SD2/SD1 ratio, stress index and PNS index changes were associated with time ($p < 0.0001$ for all variables). In contrast, the SNS index change was associated with group-by-time ($p = 0.0313$) and time ($p < 0.0001$; Table 5).

There was a decrease in SD1 during the warm-up period ($p < 0.01$), and a decrease in both SD1 and SD2 was observed during jumping and up to 20 min post-jumping (SD1, $p < 0.05$–0.01; SD2, $p < 0.05$–0.001). The SD2/SD1 ratio increased during warm-up ($p < 0.001$) and jumping ($p < 0.05$). The stress index increased during warm-up ($p < 0.01$) and reached the highest value during jumping ($p < 0.0001$). Despite a gradual decrease, the stress index remained higher than the control until 30 min post-jumping. The PNS index decreased during warm-up, substantially declined to the lowest value during jumping, and remained below the baseline during 50 min post-jumping (for all intervals, $p < 0.01$–0.0001). The SNS index increased during warm-up and remained higher than the control up to 30 min post-jumping in horses on the first day ($p < 0.05$–0.01), and up to 60 min post-jumping in horses on the second ($p < 0.05$–0.001). The SNS index during jumping was numerically higher in horses during the second course compared to the first, but this difference was not significant ($p = 0.0628$; Table 5).

4 Discussion

In this study, the modulation of HRV variables in horses competing in national jumping events on consecutive days was compared. The study yielded several significant findings: (1) there was an interaction between event day and time for changes in mean HR, minimum HR, peak HR, and HRV variables such as mean RR intervals, LF percentage, HF percentage, and SNS index; (2) compared to baseline values, the mean, minimum, and peak HR values during jumping remained unchanged on the first day but increased on the second; (3) HRV decreased during the warm-up period and reached the lowest values during jumping; (4) mean RR intervals and the SNS index returned to baseline earlier in horses on the first day compared to the second; (5) RESP increased during jumping and 10 min post-jumping only on the second day; and (6) the higher peak HR and RESP during warm-up and the LF percentage during jumping were only detected in horses on the second day. These results indicate that horses exhibited a higher sympathetic tone while participating on the second jumping day compared to the first.

It is interesting to note that the competition results for the horse-rider combinations were quite consistent across both rounds. It is possible that the overall performance of the individual

TABLE 5 Nonlinear analysis and autonomic nervous system (ANS) index of HRV in horses participating in 2 days of a national jumping competition.

Periods	Classes	Nonlinear results			ANS index		
		SD1 (ms)	SD2 (ms)	SD2/SD1 ratio	Stress index	PNS index	SNS index
Control	1st	47.50 ± 9.26	80.52 ± 13.84	1.72 ± 0.16	4.97 ± 0.78	4.29 ± 0.68	−2.64 ± 0.22
	2nd	38.30 ± 3.64	77.08 ± 9.83	2.04 ± 0.22	4.90 ± 0.37	4.40 ± 0.51	−2.76 ± 0.15
Warm-up	1st	17.80 ± 2.82[b]	54.28 ± 6.84	3.19 ± 0.25[c]	9.20 ± 1.43[b]	−0.38 ± 0.65[d]	0.17 ± 0.70[a]
	2nd	12.57 ± 2.84[b]	43.32 ± 6.22	3.74 ± 0.29[c]	11.22 ± 1.50[b]	−1.75 ± 0.46[d]	1.85 ± 0.68[b]
Course riding	1st	3.20 ± 1.20[b]	8.65 ± 2.65[c]	3.47 ± 2.11[a]	54.55 ± 10.85[d]	−2.98 ± 0.87[d]	11.03 ± 0.60[a]
	2nd	2.77 ± 0.10[b]	13.90 ± 2.17[c]	5.06 ± 0.84[a]	53.15 ± 5.87[d]	−4.25 ± 0.19[d]	15.82 ± 1.99[b]
10 min post-jumping	1st	13.68 ± 6.17[b]	35.68 ± 12.99[a]	3.00 ± 0.55	19.96 ± 4.08[b]	−1.28 ± 0.55[d]	2.30 ± 0.94[a]
	2nd	11.50 ± 3.59[b]	29.30 ± 8.06[a]	2.65 ± 0.28	17.90 ± 3.84[b]	−1.29 ± 0.34[d]	2.05 ± 0.76[b]
20 min post-jumping	1st	23.88 ± 3.03[a]	51.92 ± 5.70[a]	2.21 ± 0.12	9.82 ± 1.04[b]	0.54 ± 0.31[d]	−0.55 ± 0.32[b]
	2nd	24.50 ± 3.67[a]	58.37 ± 10.30[a]	2.31 ± 0.15	8.98 ± 1.66[b]	0.37 ± 0.30[d]	−0.55 ± 0.39[b]
30 min post-jumping	1st	32.58 ± 3.84	72.65 ± 10.35	2.21 ± 0.11	6.25 ± 0.58[a]	1.63 ± 0.43[d]	−1.56 ± 0.21[b]
	2nd	28.92 ± 4.22	62.00 ± 8.63	2.17 ± 0.24	7.80 ± 1.33[a]	1.40 ± 0.45[d]	−1.27 ± 0.35[a]
40 min post-jumping	1st	44.20 ± 9.94	93.25 ± 15.03	2.19 ± 0.18	5.50 ± 0.71	2.37 ± 0.44[b]	−1.86 ± 0.17
	2nd	33.48 ± 7.58	73.50 ± 14.85	2.22 ± 0.28	7.26 ± 1.64	1.79 ± 0.61[b]	−1.45 ± 0.42[a]
50 min post-jumping	1st	43.86 ± 4.64	91.58 ± 9.62	2.12 ± 0.21	5.52 ± 0.54	2.57 ± 0.36[b]	−1.98 ± 0.17
	2nd	37.78 ± 8.39	78.38 ± 18.83	1.99 ± 0.24	7.52 ± 2.30	2.13 ± 0.55[b]	−1.54 ± 0.42[a]
60 min post-jumping	1st	55.14 ± 10.86	88.84 ± 11.94	1.85 ± 0.34	4.98 ± 0.52	3.31 ± 0.83	−2.23 ± 0.24
	2nd	47.46 ± 9.39	107.96 ± 20.56	2.27 ± 0.19	5.40 ± 1.01	2.16 ± 0.42	−1.71 ± 0.21[c]
Effects (*p*-value)	Time	<0.0001*	<0.0001*	0.0058*	<0.0001*	<0.0001*	<0.0001*
	Group	0.4024	0.8962	0.2589	0.9951	0.3499	0.2305
	Interaction	0.9294	0.4158	0.5511	0.9685	0.3158	0.0313*

*Indicate significant effects of time, group, and group-by-time (interaction).
a, b, c and d indicate statistical significance at $p < 0.05$, 0.01, 0.001, and 0.0001 within the group compared to the control value.
SD1, standard deviation of the Poincaré plot perpendicular to the line of identity; SD2, standard deviation of the Poincaré plot along the line of identity; PNS, parasympathetic nervous system; SNS, sympathetic nervous system.

horses played a significant role in the observed results (39, 40). Research has shown that horses engaged in different activities exhibit distinct behaviours and emotional responses (41). For instance, it is believed that horses bred for jumping are genetically less sensitive to frightening stimuli compared to dressage horses (42). By familiarising horses with potentially scary situations through training, they may display minimal or absent responses to such stimuli (43). Unexpectedly, horses 2 and 4 were eliminated in both jumping courses. It is possible that these horses exhibited negative fear-related responses to potential challenges during competition, similar to previously reported findings (43).

Ensuring a thorough warm-up session before exercise is crucial to prepare the horse for equestrian competitions (44). Its primary goals include improving muscle function, minimising the risk of injury, and maximising performance (45). Research has found that in dressage competitions, warm-up periods tend to be longer for more complex events (46). However, when it comes to jumping competitions, the length of the warm-up period remains consistent across different days of the competition (47, 48). Consistent with this finding, our study also observed no difference in the warm-up period between jumping courses on consecutive competition days. Nonetheless, the present results contrast with a study by Tranquille et al., which did not detect differences in average and peak heart rate during warm-up periods between days (48). The current findings revealed a trend towards higher average heart rate and a significantly higher peak HR in horses during the warm-up period for the second course compared to the first. This increase in heart rate and respiration may partly be attributed to the rider exerting more effort to prepare the horse for the second course following an unsuccessful ride on the first day.

Interestingly, during the second jumping day, there was a notable increase in the average, minimum, and peak HR from the warm-up to the course itself, compared to the first day where the increases were insignificant. The heightened HR could indicate excitement (49, 50), increased physical activity (29, 51), or stress (52, 53). This rise in HR during jumping may have implications for multi-day competitions (54). Conversely, when a well-matched horse and rider pair is involved, the horse's HR decreases during the ride (6). Since matching pairs participated on both days, the significant increase in all HR parameters during jumping on the second day likely signifies increased effort by the horse. The delayed decrease in mean HR beyond 60 min post-jumping after the second course, compared to a return to baseline at 50 min post-jumping after the first, suggests a higher exercise intensity on the second day.

The literature widely acknowledges that HRV decreases in horses during exercise (24, 25), particularly in the context of equestrian competitions (9, 29). This decrease is indicative of reduced vagal activity and increased sympathetic tone (15, 17). The present study's findings aligned with this understanding, as decreased HRV parameters such as mean RR, SDNN, RMSSD, pNN50, TINN, RR triangular index, SD1, and SD2 were observed during riding periods and up to 20 min post-jumping, signalling sympathetic dominance in horses during these activities. Time of the day has been reported as a contributing factor, as circadian variation means parasympathetic activity is relatively increased at night (55). Accordingly, this factor should be controlled for to ensure accurate HRV analysis (15). The jumping courses in the present study were officially scheduled for approximately 8.00–11.30 AM to avoid hot and humid weather in the afternoon. Hence, the recorded activity occurred at almost the same time across the two-day experiment and an effect of circadian rhythm can be disregarded. Specific changes in mean RR intervals and contributions of the LF and HF bands were also noted between jumping courses, demonstrating distinct autonomic responses in horses. It has been reported that sympathetic and parasympathetic components contribute to the LF band, while the vagal component, in conjunction with respiration rate, affects the change in the HF band (15–17). The higher sympathetic dominance observed during the second jumping course, as evidenced by the increased LF and decreased HF bands, implies that horses experienced greater stress during the second course than during the first. Furthermore, the sustained increase in the SNS index beyond 60 min post-jumping provides additional evidence for heightened sympathetic activity in horses on the second jumping day. This suggests that horses may experience more stress when participating in two jumping courses on consecutive days, particularly during the second event.

While this study offers valuable insights into the autonomic responses in horses participating in consecutive two-day jumping competitions, there are still important unanswered questions. For example, it is unclear to what extent the autonomic response may vary in horses participating for more than 2 days during the same jumping event. Additionally, further investigation is needed to understand how HRV is modulated in horses repeatedly participating in international events with higher fence heights. Another area that requires more research is the interaction between horses and riders, and how this may lead to distinct modifications of HRV variables when repeatedly competing in jumping courses. This study was limited by the small number of horses jumping at the same fence height. Additionally, the variation in fence heights among the courses caused a significant deviation in the HRV values within the group. It is important to note that the high humidity during the experiment may have influenced the HRV modulation, potentially in conjunction with stress or anxiety in the horses. Therefore, any modifications in HRV observed in this study should be interpreted with caution. Lastly, due to concerns from the riders, we were unable to perform blood sampling during the event, preventing the measurement of haematological parameters and stress hormone modulation for this study. These are important considerations for future research in this area.

5 Conclusion

The autonomic responses of horses participating in jumping competitions show significant differences between two consecutive days. Horses participating in the second day repeatedly exhibit increased sympathetic activity, indicating higher stress levels than in the first round. These findings shed light on the autonomic responses of horses competing on consecutive days, emphasising the importance of recognising potential stress that could impact the welfare of horses participating in such events.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal studies were approved by the Kasetsart University's Institute of Animal Care and Use Committee has approved the use of animals in this study (ACKU65-VET-003). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

TW: Formal analysis, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing, Conceptualization, Data curation. OH: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Supervision, Validation, Visualization, Writing – original draft. KS: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing – original draft. CP: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing – original draft. SC: Formal analysis, Investigation, Validation, Visualization, Writing – original draft. SW: Formal analysis, Investigation, Validation, Visualization, Writing – original draft. WC: Formal analysis, Investigation, Validation, Visualization, Writing – original draft. NR: Formal analysis, Investigation, Validation, Visualization, Writing – original draft. RK: Formal analysis, Investigation, Validation, Visualization, Writing – original draft. MC: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Case report: Incomplete bypass ileocolostomy without partial typhlectomy in five horses with acute, non-reducible cecocolic intussusceptions and review of literature

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Cecocolic intussusceptions are a rare condition of acute colic in horses requiring immediate surgical intervention due to persistent uncontrollable pain and ongoing ischemic cecal necrosis. Particularly in cases where reduction of the intussusception is surgically not feasible surgical interventions such as partial typhlectomy through colotomy (partial cecal amputation) combined with or without cecal bypass techniques are described. Alternatively, surgical interventions can also be performed without partial typhlectomy via incomplete bypass ileocolostomy. Information regarding applicable techniques and outcomes base on sparse literature of single case reports or small case series. Therefore, this case series aims to add more cases treated with incomplete bypass ileocolostomy without typhlectomy to existing literature and to compare the outcome by reviewing medical records from January 2009 to March 2024 in context to literature. Five horses were surgically treated and were followed-up between 1 and 9 years. Minor short-term complications were recorded during hospitalization such as transient mild colic and febrile episodes. Long-term outcome revealed that horses received or exceed their previous level of use. By adding the hereby presented cases to published data horses treated with ileocolostomy without partial typhlectomy had a long-term survival rate of 100%. However, numbers of published cases are still low with 49 horses being included in the literature review whereof 42 recovered from surgery. The overall long-term survival rate was 53%. The added value of this study is based on the comprehensive documentation of a cohort of five horses successfully treated with an incomplete bypass procedure, demonstrating favorable long-term outcomes. Furthermore, the study advances the surgical technique by implementing the closure of mesenteric gap. The evidence for the application of the surgical technique has been strengthened.

KEYWORDS
horse, cecum, cecocolic, Intussusception, abdominal surgery, incomplete bypass, cecal amputation, partial typhlectomy

1 Introduction

Cecocolic intussusception (CCI) is an uncommon cause of colic in horses with a reported prevalence of 0.01–1.9% in the majority of reports (1–6) and 14% in one report from New Zealand (7). Surgical intervention poses significant challenges in situations where the intussusception cannot be manually reduced. Several surgical options are published for non-reducible CCI in horses. Partial typhlectomy (partial cecal amputation) through right ventral colotomy is the most often reported technique aiming at surgical reduction of the intussusception and resection of the necrotic cecal intussusceptum (3–5, 7–13). This technique can be combined with either complete (9–11) or incomplete (10) bypass ileo- or jejunocolostomy. However, single reports propose an alternative option by describing complete (6, 14) or incomplete (15) cecal bypass techniques without surgical reduction of the cecal intussusception leaving the non-reducible intussusceptum *in-situ*.

Information regarding complications and outcome of the respective techniques can only be derived from low case numbers and some reports lack information regarding a detailed description of the surgical technique used, a thorough follow-up or do not mention reasons for the horses‘ early death (1, 2). Interventions involving partial typhlectomy are characterized by their invasiveness and the heightened risk of intraoperative contamination. Additionally, the combination of typhlectomy and bypass may prolong the duration of surgery and is considered one of the most technically demanding procedures in equine surgery.

To evaluate long-term outcomes in regard to the different surgical techniques objectively, we conducted a literature review and included the hereby presented cases which were treated surgically between January 2009 and March 2024 due to non-reducible CCI with incomplete bypass ileocolostomy without partial typhlectomy. This technique has been documented for only three cases so far with convincing survival rates in the long-term (15). However, critical concerns regarding the safety of the surgical technique were also mentioned (16). Therefore, the necessity to add more cases to literature seems justifiable. We included cases where the diagnosis of a non-reducible CCI was confirmed by exploratory laparotomy and when interventional surgery was desired by the owner. Signalment, pre-surgical findings, surgical technique as well as post-operative management and outcome were documented. Follow-up was assessed by telephone or e-mail consultation of owners or referring veterinarians with a minimum of 6 months after discharge and continued on a regularly base until horses were lost for further follow-up. Survival analysis was performed using Kaplan–Meier method. 95% confidence intervals were calculated for short- and long-term survival. Log rank test was used to statistically compare the survival curves and to compute p-values. Level of significance was set at $p < 0.05$.

2 Case description

2.1 Surgical technique

The surgical technique complies with the previously published technique (15). Incomplete bypass ileocolostomy was performed as an approximate 10-cm side-to-side hand-sewed anastomosis of two

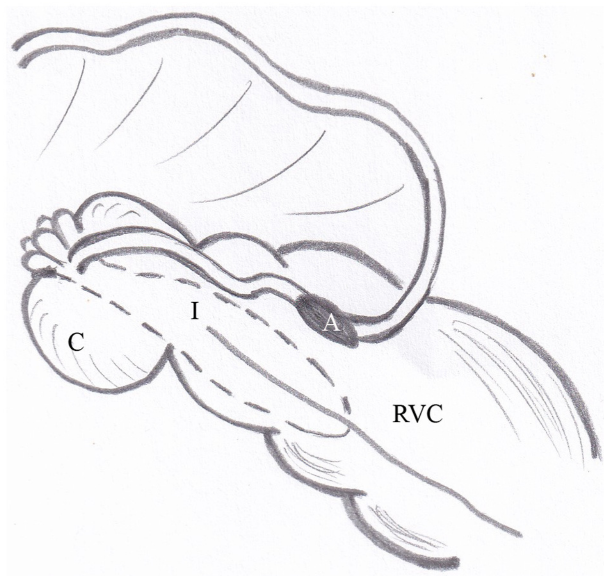

FIGURE 1
Illustration of the surgical technique of incomplete bypass ileocolostomy. The side-to-side anastomosis of ileum und colon is positioned with the ileum in anti-peristaltic orientation between the ventrolateral and ventromedial taenia of the ventral colon at its most orally exteriorized portion. A, anastomosis; C, cecal stump; I, intussusceptum; RVC, right ventral colon.

layers. The first layer performed as a full thickness suture in single continuous pattern followed by a second seromuscular Cushing-pattern using 2-0 polyglactin 910. The ileal-colonic orientation was set anti-peristaltic with the ileum positioned between the two free bands (ventrolateral and ventromedial) at the most orally exteriorized portion of the ventral colon (Figure 1). In addition to the published technique, closure of the gap between the ileocecal fold, cecocolic fold and colon serosa was performed with simple continuous patterns using 2-0 polyglactin 910 in order to reduce the risk of intestinal entrapment. The intussusception was not oversewed nor manipulated further.

2.2 Signalment and pre-surgical findings

Within the defined period five horses met the inclusion criteria (Table 1). Age ranged from 2 to 11 years (median 7). Four mares and one gelding represented the following breeds: 3 Ponies, 1 Quarter Horse, 1 Warmblood. Duration of clinical signs of colic prior to admission to the hospital ranged from 6 to 24 h (median 12). None of the horses had a history of previous colic episodes. All horses had been treated medicinally without appropriate response by the referring veterinarian. Information about anthelmintic medication regimes was available for four cases (3 regularly, 1 none). Horses were used for breeding (1), pleasure riding (2) and two horses were unbroken. All horses had an overall breed and age characteristic body condition score.

At admission three horses demonstrated signs of severe abdominal pain (case 1, 2, 5), one horse showed mild abdominal

TABLE 1 Overview of signalment, clinical signs and outcome five horses treated with ileocolcostomy without cecal amputation due to a non-reducible cecocolic intussusception.

	Signalment				Clinical findings at admission						Selected blood parameter at admission				Follow-up		
Case	Breed	Age (years)	Sex	Intended use	Heart rate (beats/min)	Respiration rate (breaths/min)	Rectal temperature (°C)	Intestinal sounds	Mucous membrane color	Capillary refill time (secs)	PCV (L/L)	TP (g/L)	WBC count (G/l)	Lactate (mmol/L)	Duration	Clinical signs	Level of use or exercise
1	Shetland pony	11	Mare	Breeding	88	30	36.5	Absent	Reddened	>3	0.61	56	3.0	12.6	5 years (euthanized unrelated to colic)	Mild colic, treated medically once	Breeding
2	Sport pony	10	Mare	Pleasure	48	20	37.5	Absent	Pale pink	2	0.28	60	5.5	1.4	9 years (sold)	None	Pleasure riding
3	Pony	7	Gelding	Pleasure	52	24	38.2	Reduced	Reddened	>3	0.45	65	5.6	1.3	1 year (sold)	None	Pleasure riding
4	Quarter horse	2	Mare	Unbroken	60	24	38.0	Reduced			0.44	62	17	1.5	3 years (still under follow-up)	None	International level, western
5	Dutch Warmblood	4	Mare	Broken	90	34	n.r..	n.r.			0.30	46	n.r.	2.1	1 year (still under follow-up)	None	First ridden training level

n.r., not recorded; PVC, packed cell volume; TP, total protein; WBC, white blood cells.

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pain (case 3), and one horse was depressed (case 4). Clinical findings were tachycardia (48–90/min, median 60), tachypnoea (24–34/min, median 24) and a reduced or absent intestinal motility on auscultation. Transrectal temperature was within the physiological range. All horses demonstrated reddened mucous membranes and a prolonged capillary refill time. Gastric reflux could be obtained in one horse (case 1). Transrectal palpation revealed no abnormal findings in two horses (case 2, 4). In three horses, rectal palpation was not performed due to the small size of the horse (case 1), a superficial rectal mucosa laceration (case 3) and severe uncontrollable colic signs (case 5). Transabdominal ultrasonography demonstrated the characteristic target-like large intestinal pattern on the right side of the caudoventral abdomen in two horses (case 3, 4) where a presumptive diagnosis of intussusception was made. One horse (case 1) demonstrated distended and amotile small intestinal loops along the ventral abdomen. One horse was ultrasonographical unremarkable (case 2) and one could not be examined due to the horse‘s agitation (case 5). Blood analysis at admission revealed a wide range of packet cell volume (0.28–0.61 L/L, median 0.44), total protein (46–65 g/L, median 60) and lactate 1.3–12.6 mmol/L (median 1.5). The total white blood cell count ranged from 3.0 to 17 G/l (median 5.5). Abdominocentesis was not performed. Indication for surgery based on the severe grade of pain (case 1, 2, 5), the duration of colic (case 1, 4) and the non-responsiveness to medical treatment (all). Furthermore, abnormal sonographic findings were considered indicative for surgery in three horses (case 1, 3, 4), (Table 1).

2.3 Specific surgical intervention

Surgery was performed as previously described in all horses. In one horse (case 2), evacuation of the small intestine was required due to severe intraluminal fluid accumulation, which was addressed through jejunal enterotomy. In another horse (case 4), the colon was emptied via pelvic flexure enterotomy before suturing the anastomosis. Horses recovered without assistance as is customary in our clinic.

2.4 Post-operative management

All horses recovered uneventful from anesthesia. Post-operatively horses received intravenous lactated Ringer solution and electrolyte supplementation according to hydration status under acid-base and electrolyte control. Antibiotic therapy of sodium penicillin (20,000 UI/kg IV every 8 h) and gentamicin sulfate (6.6 mg/kg IV every 24 h) was maintained for 6 to 14 days (median 9) in all horses. Additionally, one horse received metronidazole (20 mg/kg orally every 12 h) for 7 days and a plasma transfusion (case 5). Further medication included flunixin meglumine in a dose of 0.6 mg/kg IV twice daily for 6 to 14 days (median 8) and 2 % lidocaine hydrochloride in a continuous rate of 0.05 mg/kg/min administered within the first 48 h after surgery. Twenty-four hours after surgery horses were introduced to water. Hay was given in small portions upon the third post-operative day increasing in portion size. All horses received anthelmintic oral medication (praziquantel 1.5 mg/kg and 200 μg/kg ivermectin).

In addition to the standard follow-up examinations of horses that underwent colic surgery, the focus was placed on transcutaneous abdominal ultrasound assessments to evaluate small intestine motility, to document the resolution of the invagination, and to monitor signs of peritonitis, such as increased abdominal fluid. Data of repeated post-operative ultrasonographic examinations was available from two horses (case 1, 5). The first reported case was hospitalized for scientific interest to follow-up the characteristic target-like large intestinal pattern at the right caudoventral abdomen signs to be no longer reproducible up for 34 days whereas in the other case the typical ultrasonographic findings were still present until discharge at day 15.

Initially two of the five horses showed a depressed general behavior for up to 3 days after surgery (case 1, 5). During hospitalization intermittent mild to moderate colic episodes were noticed in three horses at the first post-operative day (case 2, 3) and until day 14 (case 4), These symptoms resolved after hand walking and a single dose of 2.5 mg/kg metamizole IV. A temporarily febrile rectal temperature was noted in one case (case 4) for three post-operative days. Time of hospitalization ranged from 12 to 34 days (median 15). All horses survived to hospital discharge. The abdominal wounds were unremarkable without signs of a surgical site infection at that date.

2.5 Outcome and literature review

Long-term follow-up from 1 to 9 years (median 3) was available for all horses. Owner reported that all horses regained their pre-surgical level of use or were broken and ridden uneventfully (Table 1). For one horse a single episode of mild and unspecific colic was reported 1 year after surgery which was treated with a single analgesic medication by the home veterinarian (case 1).

Derived from literature detailed information on applied surgical procedures for non-reducible CCI was available for 49 horses of which 42 (86%) recovered from surgery. Thirty-five horses were discharged from hospital (71%). A long-term follow-up with a minimum of 6 months was available for 26 horses, calculating an overall long-term survival rate of 53% of all surgically treated horses (Table 2). By adding the present case series to published data, incomplete bypass ileocolostomy without partial typhlectomy demonstrated a long-term 100% survival rate in 8 horses over a follow-up period of 1–9 years whereas complete bypass techniques without partial typhlectomy refer to 7 horses with a 100% discharge rate and a long-term survival rate of 57% (6, 14). Data derived from reports describing partial typhlectomy through colotomy revealed 39 horses of which 25 horses (64%) were discharged and 19 (49%) were reported to be alive for at least 6 months. No information about outcome after discharge was provided for eight horses and two horses died within the follow-up period (3–5, 7–13). Based on this data an incomplete bypass ileocolostomy without partial typhlectomy demonstrates superior survival rates than techniques including a complete bypass or partial typhlectomy. The comparison of outcomes between the surgical technique

TABLE 2 Literature review of horses surgically treated due to non-reducible cecocolic intussusceptions focusing on surgical technique and outcome.

CCI to number celiotomies	Age and breed	Details and number of surgically treated non-reducible CCI	Outcome: number horses and duration	Tapeworm evidence in CCI	Reference
Partial typhlectomy through colotomy (with and w/o bypass)					
2/216 (1.9%)	16 y Hunter	Total sx 1 Partial typhlectomy through colotomy (1/1)	0/1 finished sx	2/2	(3)
n.r.	1 y TB	Total sx 1 Partial typhlectomy through colotomy and ileocolostomy (1/1)	1/1 discharged long-term n.r.	n.r.	(8)
n.r.	1 y FR	Total sx 1 Partial typhlectomy through colotomy and oversew point of invagination (1/1)	1/1 discharged 1/1 follow-up 9 mo	n.r.	(12)
n.r.	2 y TB	Total sx 1 Partial typhlectomy through colotomy and oversew point of invagination (1/1)	1/1 discharged 1/1 follow-up 6 mo	n.r.	(13)
n.r.	2 y SB	Total 1 sx Partial typhlectomy through colotomy and oversew point of invagination and complete bypass ileocolostomy (1/1)	1/1 discharged 1/1 follow-up 1 y	1/1	(11)
4/310 (1.3%)	2–10 y Pony (3), TB-Mix (1)	Total 4 sx Dead during induction (1/4) Partial typhlectomy through colotomy (3/3) and Complete bypass jejunocolostomy (1/3)	1/3 finished sx 0/3 to discharge	1/4	(4)
11/842 (1.3%)	7 mo—8 y 7 SB, 3 TB	Total 4 sx Partial typhlectomy through colotomy (3/4) Ileocolostomy and oversewing cecal base in 2nd sx (1/4)	0/3 discharged 1/1 discharged 1/1 died 3 wks post sx due to colic	8/10	(5)
19/135 (14%)	8 mo—12 y Breed n.r.	Total 7 sx Partial typhlectomy through colotomy (7/7)	4/7 finished sx 3/7 discharge long-term n.r.	n.r.	(7)
n.r.	1–8 y Breed n.r.	Total 8 sx Partial typhlectomy through colotomy (8/8)	8/8 discharged 1/8 died 3 mo post sx colic 7/8 follow-up 6-96 mo	6/8	(9)
n.r.	7 mo—30 y Mainly SB	Total 11 sx Partial typhlectomy through colotomy (9/11) Complete bypass ileocolostomy (2/11)	9/9 discharged 0/2 discharged 9/11 follow-up 12 mo	15/30	(10)
Complete bypass w/o partial typhlectomy					
n.r.	1 y TB	Total 1 sx Complete bypass ileocolostomy w/o partial typhlectomy (1)	1/1 discharged 1/1 follow-up 4 mo	n.r.	(14)
8/541 (1.48%)	6 mo—6 y WB (2), Pony (2), SB (1), L (1)	Total 6 sx Complete bypass ileocolostomy w/o partial typhlectomy (1/6) Complete bypass jejunocolostomy w/o partial typhlectomy (5/6)	6/6 discharged 2/6 survived 2.5 mo 4/6 follow-up 1–7.5 y	n.r.	(6)
Incomplete bypass w/o partial typhlectomy					
n.r.	2 y, 1 y SB (3)	Total 3 sx Incomplete bypass ileocolostomy w/o typhlectomy (3/3)	3/3 discharged 3/3 follow-up 12 mo	n.r.	(15)

mo, months; n.r., not recorded; sx, surgery; w/o, without; wks, weeks; y, years; CCI, cecocolic intussusception; FT, Foxtrotter; FR, Frisian; L, Lipizzaner; SaB, Saddlebred; SB, Standardbred; TB, Thoroughbred.

of partial typhlectomy via colotomy (with or without bypass) and the presented approach of incomplete bypass ileocolostomy without partial typhlectomy yielded a significant result (p=0.045). No significant differences in outcomes were observed between complete bypass ileo/jejunocolostomy without partial typhlectomy and the published cases of incomplete bypass ileocolostomy without partial typhlectomy in comparison to the present case series (Table 3).

TABLE 3 Summary of literature review and presented cases regarding short- and long-term outcome.

	Surgical techniques and number of horses	Short-term outcome (discharged horses)	Long-term outcome (>6 mo follow-up)
Literature review	Partial typhlectomy through colotomy with and w/o bypass: 39	25 (64%) (95% CI [47,2-78,3%])	19 (49%) (95% CI [32,7-65%])
	Complete bypass ileo/jejunocolocstomy w/o partial typhlectomy: 7	7 (100%) (95% CI [56,1-100%])	4 (57%) (95% CI [11,8-79,8%])
	Incomplete bypass ileocolostomy w/o partial typhlectomy: 3	3 (100%) (95% CI [31,0-100%])	3 (100%) (95% CI [31,0-100%])
	Total number sx: 49 **Recovered horses: 42**		
Presented case series	Incomplete bypass ileocolostomy w/o partial typhlectomy: 5	5 (100%) (95% CI [46,3-100%])	5 (100%) (95% CI [46,3-100%])
	Total number sx: 5 **Recovered horses: 5**		

CI, confidence interval; mo, months; sx, surgery; w/o, without.

3 Discussion

The presented case series adds five cases to the veterinary knowledge of a rare colic-related condition in horses. The authors consider publication of this case series as important for equine surgeons because descriptions of surgical interventions vary and in the majority of reports partial typhlectomy through colotomy is included in the procedure. When summarizing all published data, the incomplete bypass technique without partial typhlectomy should be seriously considered as a feasible and straight-forward alternative technique with an excellent outcome though the number of cases is still comparatively low.

In all presented horses, surgery was performed within a short duration after the onset of colic signs. Even then, attempts to reduce the intussusception manually for ∼10 min were unsuccessful. In consequence, the described surgical intervention was applied. It is the authors opinion that leaving the intussusception *in-situ* allows a less invasive surgical procedure, reduces the risk of intra-operative contamination and seems time-saving. However, definite comparison of surgery times would require such data from other techniques and therefore this remains an author‘s statement. A very early report already mentioned the surgical technique that aims on an intraluminal necrotizing procedure by oversewing the cecal intussusception (17). Some authors performed oversewing of the cecal stump after partial thyphlectomy but an improvement of safety seems questionable as the value of the procedure cannot be derived from published data (5, 11–13). None of the presented and reported horses treated without partial typhlectomy developed clinical and ultrasonographic signs of septic peritonitis nor signs of adhesion formation post-operatively (6, 14, 15). It can be assumed that non-manipulation the CCI gradually leads to intraluminal adhesion formation and retraction of the remaining cecal stump. The mentioned intraluminal necrotizing process can be followed by the typical ultrasonographic appearance of the intussusception which was still visible at day 15 in one horse but not at day 34 in another. Also, transient mild colic symptoms and febrile episodes, which were noticed in three cases during hospitalization, might be related to the involution procedure of the intussusception. Systemic antimicrobial coverage for a duration of 6 days up to 2 weeks and anti-inflammatory medication based on clinical signs, laboratory and abdominal ultrasonographic findings and are comparable to other reports not including partial typhlectomy (6, 14, 15).

CCI affected horses demonstrate a rather young horse population in some reports which is in contrast to the presented cases where only one horse had an age of <3 years (5, 8–13). All five horses in this study were admitted in an acute stage of colic with a duration of ≤24 h. Subclinical and chronic courses of the disease are also reported (6, 7, 9, 10). However, although in the disease‘s etiopathology tapeworm (*Anoplocephala perfoliata*) infection demonstrates strong evidence, no clear statement can be drawn whether tapeworm infections correlate to the stage of clinical signs. Summarizing data provided by literature, tapeworm infections were detected in 60% of CCI affected horses (3–5, 9–11). As the cecal intussusception was not manipulated in the presented study no information regarding intraluminal tapeworm occurrence can be provided. A final statement on tapeworm prevalence is therefore not possible since a negative fecal analysis is regarded unreliable (18).

The grade of pain, the non-responsiveness to medication and abnormal sonographic findings were indications for performing surgery. However, setting a presumptive diagnosis of a CCI was possible in two horses in the present study considering that one horse demonstrated amotile intestinal loops and one case was excluded due to severe colic signs, thus concluding one unremarkable or misdiagnosed case. The value of transabdominal ultrasonographic examination for pre-operative diagnosis has been already highlighted by other authors (6, 7, 10, 15). Another indicative finding is a transrectal palpable firm mass at the right dorsal/caudal abdomen (2, 5–7, 9, 12–14). Unremarkable or unspecific rectal findings like documented for the two assessable horses in the present study are also described (1, 2, 5, 6, 9, 11, 15). It is assumed that in subacute and chronic cases the typical rectal palpable mass can be found more frequently (10).

Literature research revealed the difficulty to assess case series with precise descriptions of the surgical technique used as well as the clear definition of CCI being either reducible or not. Therefore, two reports describing 1 and 9 horses respectively were not included in the review although information regarding the incidence and clinical signs of CCI in horses are provided (1, 2). The literature review confirms that CCI has to be considered as a rare condition in horses and illustrates that in non-reducible cases surgical intervention carries an overall guarded prognosis. The comparable low number of horses treated with incomplete bypass ileocolostomy without partial tyhphlectomy is a clear limitation of the study, however a 100% long-term survival rate stated by two independent reports is considered promising to increase application in equine surgery. The publication of even small case numbers appears urgently necessary for such a rare disease in order to further investigate the evidence of the surgical techniques.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

Ethical approval was not required for the studies involving animals in accordance with the local legislation and institutional requirements because horses were clinical patients and were treated according to the scientifically accepted veterinary standards. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

AT: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. DS: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Non-invasive scalp recording of electroencephalograms and evoked potentials in unanesthetized horses using a 12-channel active electrode array

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Despite the long history of the horse-human bond, our understanding of the brain and mind of horses remains limited due to the lack of methods to investigate their brain functions. This study introduces a novel methodology for completely non-invasive, multi-channel recording of electroencephalography (EEG) and evoked potentials in awake horses to examine equine auditory cortical processing. The new approach utilizes specially designed brush-shaped active electrodes that facilitate stable signal acquisition through the hair coat by penetrating electrode pins and integrated pre-amplifiers. A 12-channel electrode array provided greater scalp coverage than prior work. As a proof of concept, clear cortical auditory evoked potentials (CAEPs) were recorded in response to sound onsets and offsets. The equine CAEP waveform morphology resembled the human P1-N1-P2-N2 complex, although the latencies were shorter than typical human values. The CAEP amplitudes were maximal at centroparietal electrodes, contrasting with the frontocentral distribution seen in humans, potentially explained by differences in auditory cortex orientation between species. This non-invasive multi-electrode method enables the evaluation of cognitive abilities, normal and abnormal brain functions, and advances scientific understanding of the equine mind. It offers potential widespread applications for recording EEGs and evoked potentials in awake horses and other medium-to-large mammalian species.

KEYWORDS

equine, EEG, auditory evoked potential (AEP), animal welfare, neuroimaging

1 Introduction

The bond between humans and horses has evolved over millennia, culminating in the current practices of utilizing horses for transportation, recreation, competitive sports, and therapy. To sustain and enrich this human-equine relationship, a scientific understanding of the equine mind and brain is essential. However, limited knowledge exists concerning the higher brain functions of horses and their associated disorders (1, 2), reflecting the inherent challenges of conducting laboratory experiments with these animals.

Scalp-recorded cortical evoked potentials (EPs) and event-related potentials elicited by sensory stimuli, such as auditory, visual, and somatosensory stimuli, serve as valuable tools in human medicine and neuroscience for non-invasively probing cerebral functions and associated disorders. However, the utilization of scalp-recorded cortical EPs in equine subjects has been scarce, despite their potential benefits. This underutilization stems primarily from methodological challenges specific to recording cortical EPs in alert horses, rather than a general difficulty in acquiring electrical signals from the living equine brain. Prior studies have successfully recorded continuous electroencephalograms (EEGs) in conscious horses (3–6), as well as brainstem auditory (7, 8), visual (9, 10), and somatosensory (11, 12) evoked potentials under anesthesia. Nonetheless, equine recordings of cortical EPs without sedation remain sparse, to our knowledge, with only one study reporting the acquisition of visual evoked potentials in non-sedated horses using invasive subcutaneous needle electrodes (13). The lack of non-invasive methods for awake cortical EP is not specific to horses but is a common issue for other medium to large-sized mammals, such as such cattle and swine (14–17), although successful attempts have been made to record non-invasive EEG in smaller animals, such as dogs (18) and monkeys (19, 20).

The primary objective of this study was to develop a methodology for completely non-invasive recording of cortical EPs in unanesthetized equine subjects. This development was an extension of our previous success on recording non-invasive EEG and EP in smaller animals, namely, macaque monkeys and common marmosets (19–21). We focused specifically on the auditory modality to record cortical auditory evoked potentials (CAEPs) in horses, although the methodology developed herein would be widely applicable for acquiring various types of EPs and continuous EEG in horses and other medium to large-sized mammals, such as cattle and swine.

The secondary objective was to characterize the morphology and scalp topography of scalp-recorded CAEPs in equines, thereby identifying the homologous components to those observed in human CAEPs (e.g., P1, N1, and P2). This would facilitate future utilization of equine CAEPs for neurological assessments and scientific investigations into equine auditory processing.

To achieve these objectives, it was necessary to employ a sufficient number of EEG electrodes that provided extensive coverage over the areas of the head surrounding the animal's brain. This necessity arises from the fact that EEG and evoked potential recordings reflect the voltage differential between pairs of scalp electrodes, representing the summation of volume-conducted electrical activity originating from the brain. Volume conduction refers to the phenomenon wherein electrical signals generated by neural activity propagate through the head tissues (including the brain, skull, and skin), resulting in recorded potentials that are spatially smoothed and distorted representations of the underlying neural sources. In humans, for instance, CAEPs exhibit maximal amplitudes over frontocentral scalp regions, as electric currents originating in the left and right auditory cortices on the lower banks of the lateral sulci project upwards to converge around the vertex (22). Conversely, the scalp topography of equine CAEPs remains unknown, as the precise location of the horse auditory cortex has not been determined, and the effects of volume conduction in this species are also uncharacterized.

Therefore, an exploratory investigation utilizing a multi-electrode array to provide broad coverage across the equine scalp was requisite to empirically determine the topography of CAEPs in horses. This posed a technical challenge, as the dense hair coat of the equine head, which has high electrical impedance, hinders reliable multichannel acquisition of EEG signals from the scalp. This is particularly true when the horse is alert and moves its head, destabilizing the skin-electrode contact. To our knowledge, previous non-invasive EEG and EP recordings in conscious horses have been limited to a maximum of four frontal electrodes, although the number of electrodes could be increased to nine when sedated (9), or 11 in a partly invasive awake recording wherein the hair was shaved and the exposed skin was abraded with sandpaper to reduce electric impedance (3).

To address this challenge, we introduced a novel animal EEG recording methodology utilizing specially designed brush-shaped, active electrodes (Figure 1). A brush-shaped electrode facilitates signal acquisition via silver pins that partly penetrate through the hair coat, thereby enabling a more stable electrical interface between the skin and electrode compared to conventional disk electrodes, without necessitating hair removal. Additionally, these electrodes were "active," incorporating small pre-amplifiers to facilitate low-noise recordings even when electrode-skin impedances could not be minimized. Active electrodes are becoming increasingly prevalent in human EEG applications such as brain-computer interfaces and infant recordings, and this study is the first to apply this device to equine EEG recording, to our knowledge. As a proof of concept, we successfully recorded completely non-invasive CAEPs in three awake equine subjects using a 12-channel electrode array providing more extensive scalp coverage than previously achieved.

2 Materials and methods

2.1 Subjects

Three equine subjects (Equus ferus caballus) were utilized, referred to as Horses A, B, and C. Horse A was a 10-year-old Thoroughbred gelding with an estimated weight of 520 kg. Horse B was a 23-year-old Halflinger gelding weighing ∼420 kg. Horse C was a 28-year-old crossbred mare with an estimated weight of 470 kg. Horse A was housed at Niigata University, while Horses B and C were maintained by Teikyo University of Science. All experimental procedures were approved by the Internal Review Board of Niigata University.

2.2 Stimuli

Pure tones with frequencies of 1,200 and 1,500 Hz were presented in an oddball paradigm. We chose these frequencies to ensure they are within the hearing ranges of horses (23), humans, and non-human primates (24, 25), thereby enabling future comparisons of CAEPs across species. Stimulus duration was

300 ms, with sound onset asynchronies randomly varying between 700 and 900 ms. The standard stimulus was presented on 80% of trials, while the deviant stimulus occurred on the remaining 20%. Stimuli were delivered in uninterrupted blocks of 300 trials lasting 4 min, with standard and deviant tones counterbalanced across blocks: the 1,200 Hz tone served as the standard in half the blocks, and 1,500 Hz as the standard in the other half. The number of blocks was determined by confirming CAEP waveforms. Block counts varied between subjects due to inconsistent signal-to-noise ratios across horses and recording sessions. For Horse A, 56 blocks of data (16,800 trials) were collected over 7 days; for Horse B, 38 blocks (11,400 trials) were collected over 3 days; and for Horse C, eight blocks (2,400 trials) were collected in 1 day.

The stimuli sequences were digitally synthesized on-the-fly using LabVIEW (26) on a notebook computer (Latitude 5330, Dell Technologies), converted to analog signal by an analog-to-digital converter (NI-9262, National Instruments), amplified, and delivered via a powered loudspeaker (MS101 II, Yamaha Corporation) positioned ∼1.5 m from the subject's head. Digital triggers informing sound onset timing were sent from LabVIEW via a digital input-output interface (NI-9401, National Instruments) to the EEG amplifier, delayed by 4.4 ms to accommodate the sound traveling time from the loudspeaker to the subject. Stimulus sound pressure level was ∼60 dB SPL measured at the subject's head.

2.3 EEG electrode and montage

A novel brush-shaped active electrode (BA-EEG electrode, TK221-009) was designed and manufactured in collaboration with Unique Medical Co., Ltd. (Tokyo, Japan; Figure 1). The electrode consisted of 12 silver pins (1 mm diameter, 5 mm length) protruding from a cylindrical plastic casing that housed a preamplifier. All pins were electrically interconnected within the casing. The number and size of the pins were optimized to balance the electrode's mechanical durability with the stability of the skin-electrode contact. The preamplifier performed impedance transformation to reduce noise interference along the lead wire connecting to the main EEG amplifier. The plastic casing had a central hole (2 mm diameter) through which EEG gel could be applied after fixing the electrode on the horse's head. Although the electrode could sometimes be used dry when the hair was short, applying electrode gel significantly improved signal quality by ensuring stable electrical contact between the skin and electrode pins. Each electrode was connected via a 0.6 m lead wire to a battery-powered unit (TK219-013, Unique Medical Co., Ltd.) fixed to the horse's headcollar at the nape during recording. This unit supplied power to the preamplifier and relayed the EEG signal from the electrode to the main amplifier. Twelve active electrodes recorded EEGs, with one serving as the reference channel. Additionally, a passive electrode of the same shape but without a preamplifier circuit served as the ground channel. All electrodes were attached to a 1 mm thick vinyl sheet cut to fit the horse's head, constructing an electrode cap (Figure 1).

After extensive exploration using Horse A, a referential (monopolar) montage with the electrode layout shown in Figure 1C was deemed appropriate for recording equine CAEPs. The position of the central electrode (Cz) was defined as the intersection of two diagonals, each connecting the inner corner of one eye to the medial edge of the contralateral earlobe. The positions of the other electrodes were determined relative to Cz, as illustrated in Figure 1. The electrodes were labeled according to the International 10-10 system for electrode placement (27). However, due to the difficulty in identifying key anatomical landmarks required by the 10-10 system (e.g., nasion, inion, and the left and right preauricular points) on the horse head, the electrode names used here only approximated their intended positions based on the 10-10 nomenclature. The position of the reference electrode was slightly below the level of the eyes (Figure 1).

2.4 EEG recording

Horse A was acclimated to the headcollar by wearing it for 1 h per day over 4 days. In contrast, Horses B and C did not require an adaptation procedure, likely because they were accustomed to interacting with unfamiliar humans through activities such as horseback riding events.

The horses remained vigilant yet calm throughout the EEG recordings, which were conducted during daytime in their stables. For preparation, a headcollar was applied, and the electrode cap (Figure 1) was attached to the head by fixating it to the headcollar using Velcro. Light pressure was applied to press the electrodes toward the scalp by subtending rubber bands over and across the electrode cap, with their ends fixed to the cheekpieces of the headcollar on both sides. After positioning the electrode cap, 1 cm^3 of electrode gel (V15, Brain Products, Germany) was applied to each electrode through its central hole (Figure 1) using a syringe with a blunted needle. The electrode leads were connected to the power unit, a plastic case fixed to the crownpiece of the headcollar at the nape. The power unit relayed the EEG signals from the electrodes to the main amplifier (Brain Amp DC, Brain Products, Germany) via a 3 m cable.

The EEGs were acquired with a 0.016–100 Hz bandpass filter and digitized at 5 kHz with 0.1 μV/bit precision for Horse A; the data were resampled to 1 kHz prior to analyses. For Horse C, the sampling rate was 1 kHz with a 0.016–100 Hz bandpass filter and 0.1 μV/bit precision. The amplifier setting for Horse B was the same as Horse C, except half the data were recorded with a 100 Hz lowpass filter without a lowcut for direct current recording.

2.5 CAEP analysis

The data were analyzed utilizing the EEGLAB software (28) with the ERPLAB extension (29). The continuous EEG data were bandpass filtered (2–40 Hz), segmented time-locked to the onset of the stimulus (−100 to 400 ms), corrected for baseline by subtracting the pre-stimulus period average, screened for artifacts, and rejected if they contained an artifact of ±100 μV relative to the baseline. Finally, the artifact-free segments were averaged to obtain cortical auditory evoked potentials (CAEPs). The number of non-rejected data segments was 15,971 (95.1%), 9,871 (86.6%), and 1,953 (81.4%) for Horses A, B, and C, respectively. Since no apparent and reliable differences were observed in the CAEPs between the standard

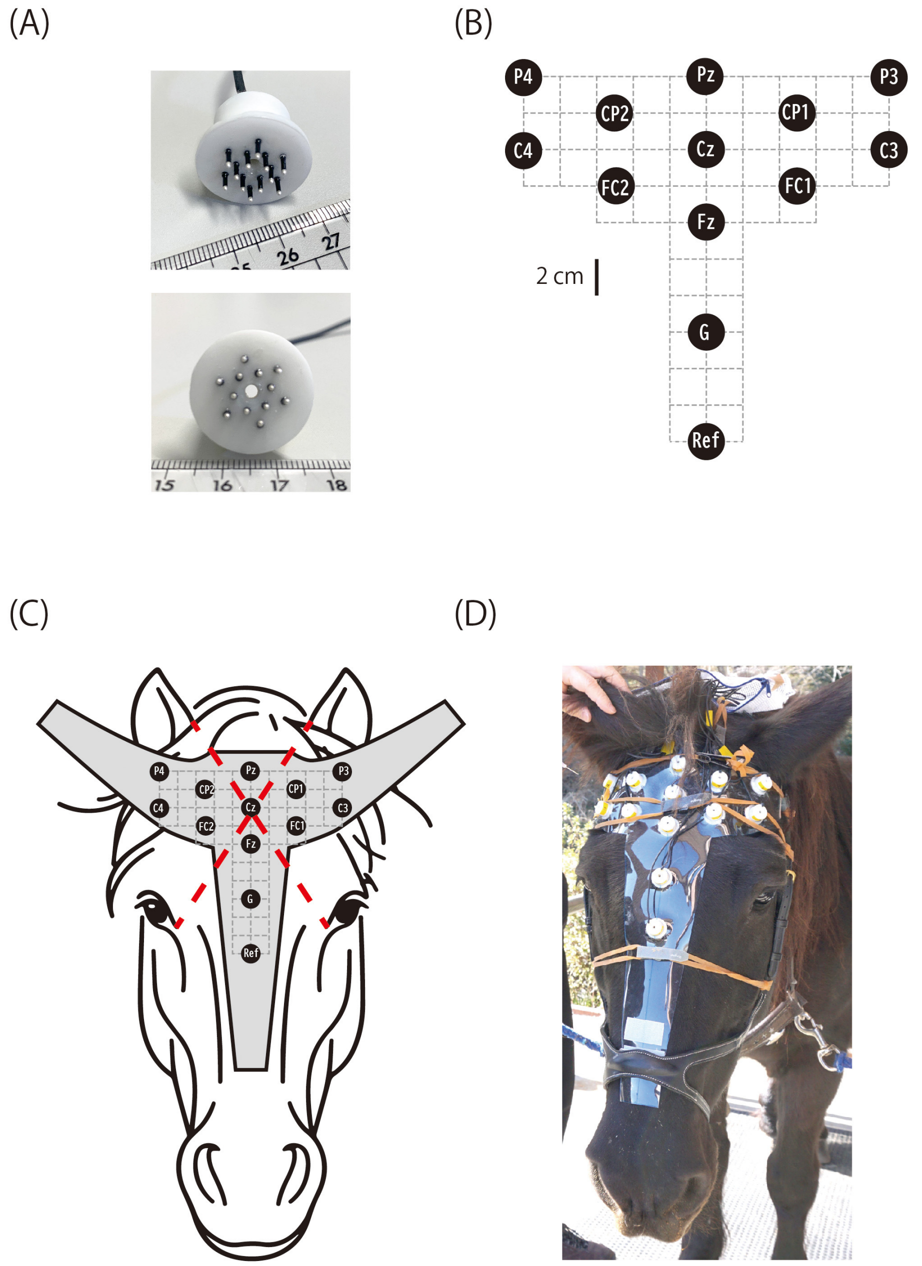

FIGURE 1
Horse EEG electrode and layout. **(A)** Brush-shaped active electrode with pins that penetrate the hair coat. The cylindrical housing contains a pre-amplifier. **(B)** Electrode layout (G, ground; Ref, reference). **(C)** The electrodes were fixed on a vinyl sheet cut to the shape of a horse's head, and the position of Cz was determined as the intersection of two lines, each connecting the inner corner of one eye and the medial rim of the contralateral ear. **(D)** Horse C wearing the electrode cap.

and deviant stimuli of the oddball sequence, all trials were pooled together to obtain a single waveform of CAEP for each horse.

3 Results

3.1 Continuous EEG

Noise-free EEGs were successfully recorded from alert but quiet horses, as shown in Figure 2. Typical artifacts included those caused by eye movements as observed concurrently with the EEG by the experimenter (Figure 2, marked with ovals), which were usually recorded at the FC1 and FC2 electrodes. This was reasonable because these electrode sites were near and tangential to the retinal plane, the major source of electric currents originating from the eye (30). Subtle muscular tensions that did not cause overt movements manifested as electromyograms (EMGs), mostly at posterior electrodes such as Pz, CP1, CP2, P3, and P4 (Figure 2, marked with a rectangle). This spatial feature could be explained by their anatomical positions relative to the underlying temporalis muscle. Gross head and body movements resulted in

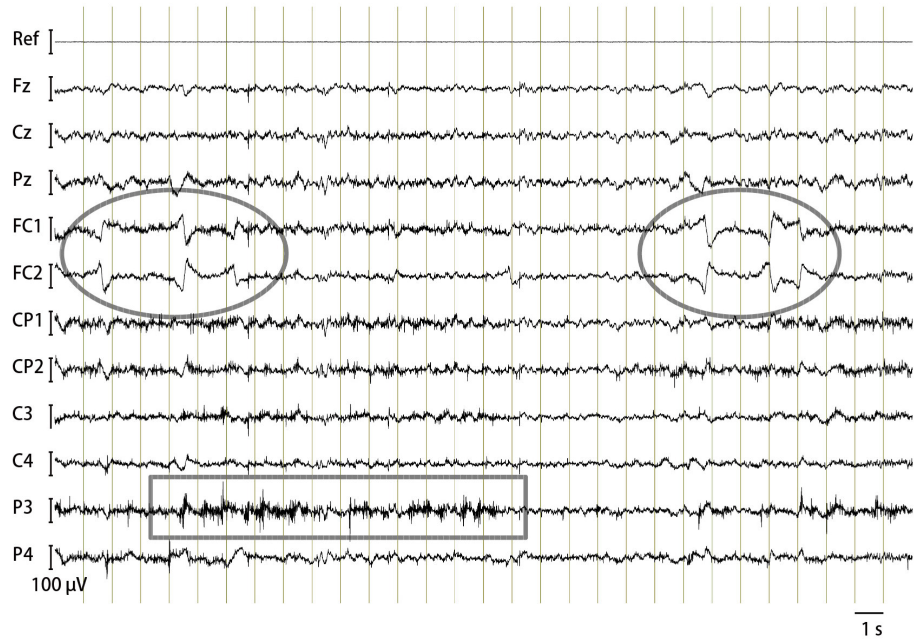

FIGURE 2
Horse EEG. An example trace of bandpass filtered (1–100 Hz) continuous EEG of Horse B containing typical artifacts: oval, eye movements; rectangle, electromyogram.

extensive artifacts over all electrodes, and such EEG segments were automatically discarded from the CAEP analyses. The EEG power spectrum of the horses, reflecting alpha, beta, and other activities, varies significantly among individuals and with the animals' arousal states (4–6). Analyzing the commonalities and variations in the continuous EEG power spectrum was beyond the scope of this study.

3.2 CAEP

Clear CAEPs were elicited by the onset of sounds, referred to as onset-CAEP, and to the offset of sounds, referred to as offset-CAEP, in all horses (Figure 3, Table 1). The overall features of waveform morphology and scalp topography of onset-CAEP and offset-CAEP were largely similar across subjects, although individual differences were observed.

Like the human onset-CAEP that typically has four exogenous components called P1, N1, P2, and N2 (32, 33), the equine onset-CAEP also comprised a series of positive-negative-positive-negative waves, although some components were not clearly identified in some cases (marked as n.a. in Table 1). These components were labeled here as eqP1, eqN1, eqP2, and eqN2, where "eq" stood for equine. The approximate peak latencies for these waves were 30 ms (eqP1), 40 ms (eqN1), 50–80 ms (eqP2), and 70–100 ms (eqN2) as summarized on Table 1. The peak latencies were measured at the electrode displaying the highest amplitude for the CAEP components.

The offset-CAEP was recorded as a wave complex that occurred 30–80 ms after sound termination. Based on polarity and latency, four components similar to those found in the onset-CAEP were identified: eqP1, eqN1, eqP2, and eqN2 (Table 1). The amplitudes of offset-CAEPs were apparently smaller than those of the onset-CAEP, similar to human findings (34).

The CAEP waveforms were broadly distributed over the scalp, consistent with volume conduction. Whereas human CAEPs usually have a frontocentral distribution when ear, mastoid, or nose reference is used, equine CAEPs showed the greatest amplitude over centroparietal electrode sites when the reference electrode was placed on the nose bridge below the eyes. This species difference might be explained by the orientation of the Sylvian fissure (lateral sulcus). In humans, the sulcus is oriented roughly horizontally with a slight downward tilt from the back to the front of the brain, causing the electric dipoles in the auditory cortex to project to the frontocentral scalp. In horses, the sulcus is more vertically oriented (31), causing the dipoles in the auditory cortex to project posteriorly (Figure 4). However, this interpretation assumes that the auditory cortex in horses is located on the lower bank of the Sylvian fissure, a hypothesis that, to our knowledge, has not been confirmed through studies of cortical cytoarchitecture or fiber connections. For instance, in the African wild dog, the cytoarchitectonically defined auditory cortex is situated outside and dorsal to the Sylvian fissure (35), which, like in horses, is oriented vertically. Further research is needed to explain the scalp topography of equine CAEP.

Regarding left-right asymmetry, the amplitudes were largely symmetric in Horse A when comparing the CAEP waveforms between corresponding electrodes across the hemispheres, such as P4 vs. P3 and CP1 vs. CP2 (Figure 3). In contrast, the eqN1 amplitude showed slight right dominance in Horse B when comparing P4 to P3, while in Horse C, it displayed slight left dominance when P4 and CP2 were compared to P3

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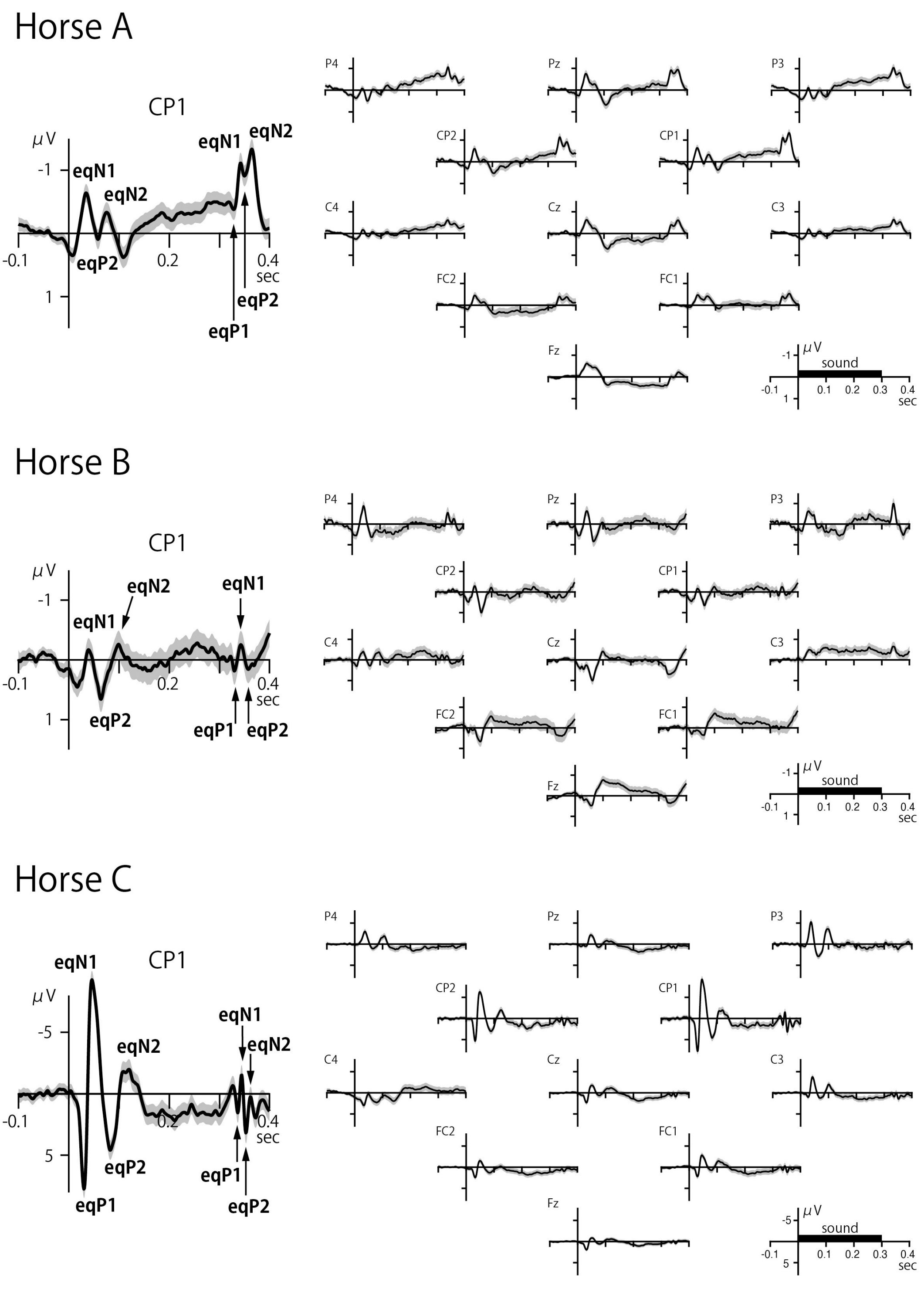

FIGURE 3
Scalp-recorded CAEPs in three horses. The left panel shows the CAEP at electrode CP1, and the right panel shows the waveforms for all electrodes. The onset and offset of sound elicited transient responses within a latency of 100 ms over distributed areas of the scalp. The black bar on the time axis indicates the duration of the sound stimulus (300 ms). The shaded error band represents 2 times the standard error.

and CP1, respectively. Intriguingly, in Horse C, the greatest CAEPs were recorded not at the midline but slightly laterally at CP1 and CP2. Such a topography is rarely, if ever, observed in neurologically normal human subjects. This topography in Horse C could be explained by assuming that the electric dipoles were oriented slightly laterally, reminiscent of the radial components of the human CAEP recorded over the temporal scalp (36). However, given the individual differences in scalp topography among the three horses, this issue warrants further investigation.

Another interesting feature of Horse C was that the CAEP amplitude was ∼10 times greater than in the other two horses. Although the exact reason for this difference remains unclear, potential causes may include variations in skull and hair coat thickness, which can affect impedance.

4 Discussion

The use of innovative brush-shaped active electrodes enabled completely non-invasive multi-channel recording of EEG and cortical CAEPs in unanesthetized horses. The use of active electrodes, which did not require skin preparation to lower skin-electrode impedance, made the electrode application process easier and more time-efficient compared to conventional passive electrodes. This reduction in preparation time allowed for the use of a greater number of electrodes while ensuring sufficient time for EEG recording. Specifically, our protocol required <30 min to prepare a horse for EEG recording, even with a multi-electrode array of 12 electrodes, which provided greater scalp coverage than previously reported. Furthermore, the procedure did not require hair shaving, offering a significant advantage over previous methods that relied on passive electrodes. Our novel method enhances veterinary care, promotes research, and improves animal welfare by providing a means for evaluating brain functions and their disorders in alert horses and, potentially, other medium- to large-sized animals significant to humans, such as pigs and cattle.

To our knowledge, this is the first study to record CAEPs in horses, whether awake or sedated, although brainstem auditory evoked potentials under anesthesia have previously been reported (7, 8). Similar to the human CAEP, evident cerebral cortical responses were recorded in horses to the onset and offset of sounds, and candidate homologs of the human P1, N1, P2, and N2 components could be identified. Although their absolute latencies were shorter than those typical for humans, this finding is not surprising, as humans have the longest latencies for these components compared to other primate species (19, 20, 37). Whether these potential CAEP components in horses represent true functional homologs to human counterparts requires further research using more advanced neurophysiological and experimental techniques. Nevertheless, the existence of cortical auditory potentials analogous to human CAEPs provides an avenue to understand auditory processing and perception in an important domestic animal species, where most previous experiments on equine perception and cognition used the visual modality (1, 2).

The study employed a convenient method for determining the position of the vertex electrode (Cz), namely, the intersection of diagonals connecting the inner corner of one eye to the medial edge of the contralateral earlobe. Although this scalp location is unlikely to match the position of Cz as defined in human EEG, such a mismatch is difficult to avoid due to the challenges in identifying the referential landmarks for the 10-20 system (inion, nasion, and preauricular points) in the horse. Nevertheless, the Cz as determined by the current method apparently approximated the vertex in prior equine EEG/EP experiments (7–9, 13), although most of these studies did not describe the exact method for

TABLE 1 Peak latency and peak amplitude of horse CAEP components.

	Onset CAEP			
Horse	eqP1	eqN1	eqP2	eqN2
A	n.a.	35 ms	51 ms	71 ms
		−0.76 μV	0.50 μV	−0.42 μV
		(Pz)	(P4)	(FC1)
B	n.a.	41 ms	62 ms	100 ms
		−0.89 μV	1.01 μV	−0.77 μV
		(P4)	(Cz)	(Fz)
C	30 ms	46 ms	82 ms	97 ms
	7.77 μV	−9.28 μV	4.60 μV	−3.61 μV
	(CP1)	(CP1)	(CP1)	(P3)

The latencies of the CAEPs were measured at the electrode (indicated in parentheses) where the component displayed the maximum amplitude across the scalp.
n.a., not available.

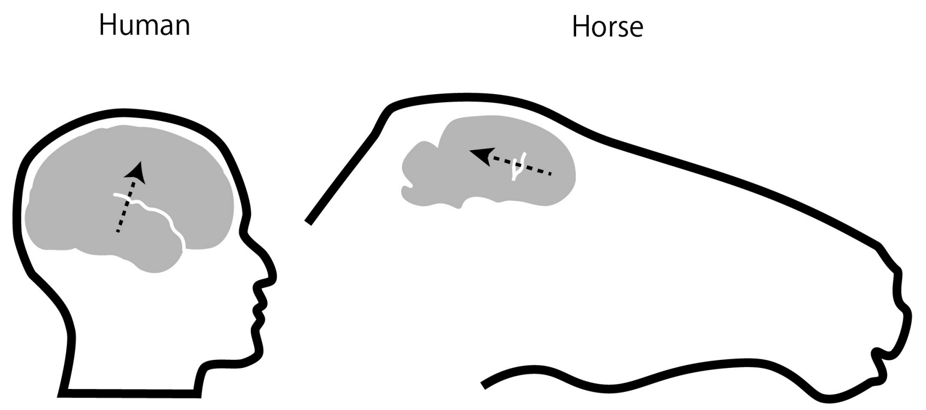

FIGURE 4
Position and orientation of EEG sources in auditory cortex. Assuming that the equine auditory cortex is located on the lower bank of lateral sulcus (also called Sylvian fissure, draw in white) as in humans, electric dipoles of auditory cortical activities in the horse would project posteriorly as opposed to fronto-centrally in humans (arrows). The equine brain and lateral sulcus were drawn based on Figure 6 in Johnson et al. (31).

determining its position, making strict comparison difficult. The current method of determining the vertex position is hoped to establish a common ground for comparing electrode positions among different studies. Determining the locations of the other non-vertex electrodes remains an issue for future research.

5 Conclusion

In conclusion, this study described a novel method for non-invasively recording multi-channel EEG and evoked potentials from awake horses. The successful identification of potential equine homologs to human CAEPs demonstrated the feasibility and validity of this approach for investigating brain functions in horses. This paves the way for further applications, such as examining normal cognitive abilities, brain disorders, and ultimately furthering our understanding of the mind of this important domestic animal species.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by Internal Review Board of Niigata University. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

KI: Conceptualization, Funding acquisition, Investigation, Methodology, Writing – original draft, Writing – review & editing. NK: Investigation, Methodology, Resources, Writing – original draft, Writing – review & editing. TM: Methodology, Writing – original draft, Writing – review & editing. SH: Funding acquisition, Investigation, Resources, Visualization, Writing – original draft, Writing – review & editing. MR: Investigation, Methodology, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Comparison of treatments for equine laryngeal hemiplegia using computational fluid dynamic analysis in an equine head model

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Introduction: Computational fluid dynamics (CFD) is gaining momentum as a useful mechanism for analyzing obstructive disorders and surgeries in humans and warrants further development for application in equine surgery. While advancements in procedures continue, much remains unknown about the specific impact that different surgeries have on obstructive airway disorders. The objective of this study was to apply CFD analysis to an equine head inhalation model replicating recurrent laryngeal neuropathy (RLN) and four surgical procedures. CFD was hypothesized to corroborate the order of the different trials based on impedance and to provide an impedance value numerically similar to the experimental results. In addition, it was hypothesized that CFD would offer insights into the changes in airflow associated with each procedure on a finite scale.

Methods: An equine cadaver head underwent airflow testing and computed tomographic (CT) scans to replicate the disease state (RLN) and four surgical procedures: laryngoplasty, combined laryngoplasty and corniculectomy, corniculectomy, and partial arytenoidectomy. Pressure measurements at the pharynx and trachea were recorded, along with airflow data, for each trial.

Results and discussion: The CFD and experimental models showed that partial arytenoidectomy had the lowest impedance in this case. While this procedure did have the largest rima glottidis area, the remaining procedural order was not dictated by the rima glottidis area. Recurrent laryngeal neuropathy and combined laryngoplasty with corniculectomy models showed negative pressure concentration on the luminal surface of the left arytenoid cartilage, which indicated a greater collapsing force on the tissue in this region. Narrowing within the caudal larynx at the level of the saccule showed increased negative pressure and higher velocity in the procedures with greater impedance, while partial arytenoidectomy exhibited more uniform pressure and velocity. Although this specific experimental head model contradicted previous flow studies, the CFD model reflected the experimental findings for the procedure with the least impedance and provided some insights into why these discrepancies occurred in this particular case.

KEYWORDS

equine, CFD, computational fluid dynamics, laryngeal hemiplegia, recurrent laryngeal neuropathy, laryngoplasty, partial arytenoidectomy, respiratory-mechanics

1 Introduction

Equine upper airway surgery remains a complex challenge due to the nature of the diseases and the procedures used to treat them. Recurrent laryngeal neuropathy (RLN) can lead to significant airway collapse and is often treated with laryngoplasty ("tie-back") or partial arytenoidectomy (PA) (1, 2). Both procedures were shown to improve impedance in an experiment with live horses, but the results are less consistent in *ex vivo* models (1, 3). As an alternative, arytenoid corniculectomy has been proposed, but it has only been explored in a vacuum flow model (4). Although these studies provided new information about these procedures, the understanding of fluid mechanics in the equine airway remains largely superficial and warrants further exploration to better represent the nuances of fluid mechanics in clinical patients.

Computational fluid dynamics (CFD) modeling continues to evolve as a useful diagnostic and decision-making tool in human respiratory surgery. It has been used to explore the relationship between complex human pharyngeal and laryngeal anatomy, vocalization, and obstruction (5–8). It estimates the development of negative pressure along the airway wall, which dictates the collapsing forces acting on the airway (9). The development of human CFD models has involved experimentation with various mesh types and turbulence models to determine the influence of these different techniques in a variety of applications (8, 10). Although airway CFD analysis continues to evolve, it has proven to be a powerful tool for identifying regions of greatest airflow resistance, anatomical variations that potentiate disease, and the ideal corrective surgical procedure on a patient-by-patient basis (7, 11, 12).

The use of CFD to evaluate surgical procedures in humans has provided insights into the interaction between surgical manipulations, airflow, and patient outcomes when addressing problems such as obstructive sleep apnea. CFD has been advocated as a pre-surgical decision-making tool to aid in the manipulation of airway geometry with the goal of decreasing resistance and improving airflow (7, 11). One study compared the effects of palatal stiffening, palatal resection, and mandibular advancement on pharyngeal collapse and demonstrated that all of these procedures improved airway mechanics in the patient geometry that was evaluated (7). This type of analysis has been performed for only one specific procedure in horses, establishing an "optimal" level of arytenoid abduction for laryngoplasty through the analysis of three different abduction levels.

One previously reported equine upper airway CFD model compared the differences between equine and human respiratory mechanics, further highlighting the complexity of equine respiratory mechanics (13). Although airflow during human breathing is primarily laminar/transitional and becomes turbulent only with heavy effort or obstruction, most equine respiration is overwhelmingly turbulent (13). Humans breathe roughly 12 times per minute with a tidal volume of approximately 0.5 L, while horses breathe an average of 10 times per minute with a tidal volume of 5.5 L. (1, 14, 15) Human airway cross-sectional areas range from 50 to 177 mm^2, while the equine airway has been reported to range from 1,127 to 4,516 mm^2 (14, 15). Reynolds numbers on the order of 100,000 have been reported based on flow parameters within equine airways; this indicates high turbulence compared to human flows, which have the Reynolds number ranging from 900 to 1,400 and are considered transitional (15, 16). Due to the complexity of turbulence in computational modeling, further development and validation of an equine CFD model are needed before it can be confidently applied to clinical patients.

A previous study examined the influence of RLN, left laryngoplasty with concurrent left ventriculocordectomy (LLP), laryngoplasty with left corniculectomy (LLPCOR), left arytenoid corniculectomy (COR), and PA on laryngeal impedance in an *ex vivo* model (4). The geometrical changes associated with each surgical procedure on the larynx were also examined (17). CFD was then performed to examine the flow patterns associated with each state within the larynx in the vacuum box model (18). The objective of this study was to investigate the influence of each procedure on flow development within the equine larynx *in situ,* using an experimental vacuum model of the entire equine upper airway, followed by CFD analysis of the model. The hypothesis was that the CFD model would confirm the experimentally derived results regarding the effects of surgical procedures on relative impedance. In addition, it was hypothesized that the changes in shape associated with each surgical procedure would influence the anticipated impedance of the larynx, the change in pressure across the larynx, and the amount of flow separation observed downstream from the larynx as air moves into the trachea. Finally, the development of negative pressure and wall stress within the larynx would be influenced by the upstream pharyngeal geometry, in contrast to the *ex vivo* model.

2 Materials and methods

2.1 Experimental data and image collection

One equine cadaver head from a six-year-old Quarter Horse mare with no history of airway disease was obtained postmortem, with the larynx *in situ* and four tracheal cartilage rings. The horse was humanely euthanized due to persistent septic arthritis and had no history or signs of airway disease. The head was kept on ice for approximately 14 h prior to testing. The horizontal portion of the mandible was removed to allow access to the soft palate by cutting through the lateral buccal cavity and performing bilateral osteotomies through the vertical ramus of the mandible. The soft palate and epiglottis were anchored using a #2 nylon suture (Ethilon, Ethicon US LLC, Bridgewater, NJ), passed through the

Abbreviations: CFD, Computational fluid dynamics; COR, Corniculectomy; CT, Computed tomography; LLP, Left-sided laryngoplasty with ipsilateral ventriculocordectomy; LLPCOR, Left-sided laryngoplasty with ipsilateral ventriculocordectomy combined with corniculectomy; PA, Partial arytenoidectomy; RLN, Recurrent laryngeal neuropathy.

cartilage and soft palate and tied to the hyoid apparatus, which was left intact. The nostrils were sutured in the open/flared position to replicate the muscular dilation that occurs during exertion. The pharyngeal region was observed endoscopically during this process and with airflow to determine whether collapse of the epiglottis or palate would occur during testing. The epiglottis was positioned using a suture through the base to the hyoid to replicate the ventral movement that occurs with head extension during maximal exercise in the live animal. The trachea was sealed over a section of polyvinyl chloride (PVC) pipe attached to a vacuum. The anatomy of the equine head is presented in Figure 1, along with the points of interest. An orifice plate was placed approximately 60 cm downstream from the tracheal outlet to measure airflow, and the vacuum was placed another 30 cm downstream. This was located on the right side of the head, as shown in Figure 1. This allowed air to be drawn through the head to replicate inhalation. Polyethylene tubing ran from a connector within the PVC pipe to a pressure transducer to measure pressure within the trachea relative to the room (Point D, Figure 1). An additional set of tubing and a transducer were used to measure pressure within the pharyngeal region by tunneling the tubing through a hole created with a 14-gauge needle and mosquito hemostats (Point B, Figure 1). Catheter placement in the pharynx was confirmed via endoscopic examination.

Five different scenarios were created in the upper airway at point C, as shown in Figure 1. The first replicated the disease state RLN, where the left arytenoid cartilage collapsed under airflow. Laryngoplasty was performed to replicate intact cricoarytenoideus dorsalis function on the right side of the larynx using a #5 Ethibond (Ethibond Excel, Ethicon US LLC, Bridgewater, NJ) suture, tied to a surgeon's throw followed by four additional throws, while the left arytenoid was left to collapse into the airway. This was tested with

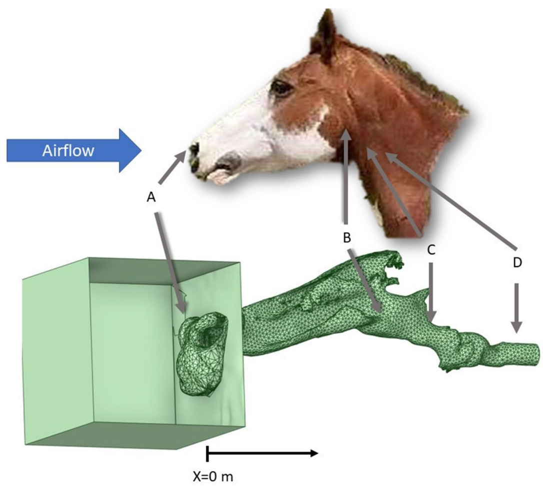

FIGURE 1
Final geometrical model with reference to the external view of a horse head. Point A represents the area just outside the nostrils where pressure is atmospheric and there is minimal velocity. Point B represents the area of the pharyngeal pressure measurement. Point C represents the area of surgical manipulation within the airway. Point D represents the area where the tracheal pressure measurement occurs, upstream from the orifice plate and vacuum.

airflow and induced tracheal pressure to replicate a horse with RLN. Next, left LLP was performed, as recommended as the standard treatment for RLN, using a laryngotomy approach to the ventricle with a roaring burr for exteriorization of the saccule (19). This was also tested with negative pressure generation. Left COR was then performed by removing the corniculate process of the left arytenoid cartilage, as reported previously (4). This was tested with the suture left intact, replicating combined LLPCOR. The left suture was then cut to allow the collapse of the arytenoid cartilage, replicating the plain COR procedure, and was tested. Finally, left PA was performed by excising the left arytenoid body and was tested (20).

Concurrent CT scans were performed to capture the three-dimensional geometry and anatomy of the head and airway under airflow conditions. The Toshiba Aquilon One (Canon Medical Systems, Markham, ON, CA) was used to acquire CT scans with 1 mm slices, employing a helical scanning algorithm. The scanning window could not extend from the nares to the trachea in one full run; however, the nostrils were scanned first, followed by the rest of the head and the nostril geometry. The nostril geometry was unified with the caudal anatomy during the geometry editing stage to create an entire head model.

2.2 Segmentation

The CT scans of the head for each state were segmented using Fiji (ImageJ, National Institutes of Health, Bethesda, MD), an open-source software used for generating three-dimensional views from DICOM images. Using the segmentation editor, the area of effective airflow was outlined slice by slice. The sinuses were not included in the model to reduce computational burden as previous studies have shown no significant airflow in the sinus regions (13). The slices were then compiled into an .stl file using the 3D viewer plugin and smoothed in MeshLab (MeshLab version 2020.12, Visual Computing Lab; CNR-ISTI, Pisa, Italy). Unconnected vertices and facets were removed using the corresponding filters, and the HC Laplacian smoothing and remeshing filters were applied as previously described (21, 22). The geometry was imported into Ansys® SpaceClaim (Ansys 2021 R1, ANSYS, Inc., Canonsburg, PA) for the manipulation and correction of irregularities to create watertight geometry. A box, approximately 200 mm^3, was created in SpaceClaim to provide an inlet boundary away from the immediate nostril opening, and a nose imprint was created in the box to simulate inhalation in a static room of air. It was then imported into Fluent (Ansys 2021 R1 build 10,179, ANSYS, Inc., Canonsburg, PA) for meshing, and a surface mesh was generated. A volumetric mesh was then applied using a hexahedral mesh with approximately 10 million elements.

2.3 Numerical model

For the numerical model, the density, temperature, and humidity of air were held constant. The density of air was taken to be 1.204 kg/m^3 at 20°C (15). Uniformity was assumed. Wall rigidity was also assumed, with the soft tissue in the fully developed flow state. This study aimed to replicate the airway during mid-inhalation to end inhalation, where the airway had accommodated the stress of negative pressure but airflow had not yet begun to decrease. Incompressible flow was also assumed.

The Reynolds-averaged Navier–Stokes (RANS) and continuity equations were solved as follows. Flow was assumed to be incompressible, isothermal, unsteady, and fully turbulent. As flow is primarily horizontal, the effects of gravity were considered negligible. The resulting Equations 1, 2, are presented below, as reported in previous models (15):

$$\frac{\partial u_j}{\partial x_j}=0 \quad (1)$$

$$\frac{\partial u_i}{\partial t}+\frac{\partial u_i u_j}{\partial x_j}=-\frac{1}{\rho}\frac{\partial p}{\partial x_i}+\nu\frac{\partial^2 u}{\partial x_j^2}-\frac{\partial\left\langle u_j^{'}u_i^{'}\right\rangle}{\delta x_j} \quad (2)$$

The κ-ϵ realizable turbulence model was used for the Reynolds stress, with standard wall functions applied in the near-wall region; a no-slip condition was assumed (13).

Ansys Fluent (Ansys 2021 R1 build 10179, ANSYS, Inc., Canonsburg, PA) was used to implement the finite volume method for solving the model equations. Convection was modeled using a second-order upwind differencing scheme. A SIMPLE scheme was then used to solve the pressure and velocity fields (15). The AMD Threadripper Pro 3955WX Processor (4.30 GHz) with 128 Gb of RAM was used to perform the simulations. Using 1,000 iterations resulted in decreased continuity residuals of 10^{-2} or less for each run, and it was observed that the residuals stopped changing. The average computation time for each simulation was approximately 1.5 h.

2.3.1 Boundary conditions

The nostrils were each defined as inlets, the trachea was treated as an outlet, and the airway wall was defined as another boundary. Pressure within the trachea and atmospheric pressure outside the nares were used as the outlet and inlet pressures, respectively (Points D and A, Figure 1). The measured pressure within the trachea from each CT scan was used. As described above, this pressure was measured just downstream from the fourth tracheal ring for each laryngeal procedure, as shown in Figure 1.

2.3.2 Mesh Independence study

A mesh independence study was conducted by generating a surface mesh with a local sizing of 0.5 mm in the region of the larynx and pharynx. A maximum mesh size of 20 mm was allowed, but this size was only reached in the box portion of the model. Five inflation layers were applied at the wall with a smooth transition. The maximum and minimum sizes were adjusted to generate mesh sizes of 1.7, 4.9, 6.9, 10, and 13.8 million cells, respectively, for the LLPCOR model. Planes of interest were generated parallel to the dorsal and axial planes, and the area-averaged pressure for each plane within each mesh size was calculated and plotted. The peak minimum pressure for each plane was also plotted. By examining the area-averaged pressure and peak minimum pressure for each plane, it was found that 10 million cells provided an appropriate balance between the anticipated calculation accuracy and computational demand.

2.4 Data analysis

Each procedural trial geometry was divided into cross-sectional planes, 4 mm apart, starting with the box edge as x = 0 m within the model. For each plane, pressure, velocity, and kinetic energy (as a measure of turbulence) were calculated as an area-weighted average. Within the pharyngeal and laryngeal regions, approximately x = 0.36–0.48 m, planes were added to create 1 mm spacing to better examine the changes specific to each procedure in that region. Trans-laryngeal impedance (I) was calculated by subtracting the measured experimental tracheal pressure from the area-averaged pharyngeal pressure at the catheter site calculated from the corresponding CFD model and divided by the volumetric flow rate, Q calculated at the CFD model outlet, as shown in Equation 3. For the experimentally reported impedance, the measured values for each of these variables was used.

$$\mathrm{I}=\frac{\left|P_{pharynx}-P_{trachea}\right|}{Q} \quad (3)$$

Separate planes were generated to perform the qualitative analysis. Planes parallel to the sagittal plane were generated, with a focus on the characteristics just inside the left and right sides of the larynx. Transverse planes were generated to examine the characteristics of the ventral larynx, mid-larynx, dorsal larynx, mid-pharynx, and mid-nostrils. Cross-sections were also captured at the mid-nostrils, mid-pharynx, laryngeal opening, mid-saccule, narrowest portion of the larynx, caudal dilation corresponding to the cricoid level, and finally a section at the level of the trachea. The larynx was specifically examined in the larger planes, with a focus on the region of the larynx to observe changes in pressure, velocity, and turbulent kinetic energy.

3 Results

The head model successfully replicated airflow in the equine upper airway, from the nares to the trachea.

3.1 Quantitative results

Both the experimental and CFD scenarios identified PA as the procedure with the lowest impedance; however, the reported impedance was very different (83–87%) for all procedures. The measured tracheal pressure, pharyngeal pressure, flow rate, impedance, and rima glottidis area are reported in Table 1. The rima glottidis area was measured using the CT cross-sectional images and the final SpaceClaim® geometry. These areas are reported in Table 2. The LLPCOR procedure showed the largest difference in the rima glottidis area. Plots for the airway cross-sectional areas are shown in Figures 2A,B. Plots of pressure, velocity, and turbulent kinetic energy along the upper airway and specifically in the larynx are shown in Figures 3A–F.

3.2 Qualitative results

There were similar nostril and pharyngeal characteristics across the procedures. The parasagittal section observed just

TABLE 1 Experimental and computational (CFD) results for pharyngeal pressure (kPa), airflow (L/s), and impedance (kPa*s/L) by procedure.

		Experimental			CFD		
Simulated state	TP (Pa)	PP (Pa)	Airflow (L/s)	Impedance (kPa*s/L)	PP (Pa)	Flow rate (L/s)	Impedance (kPa*s/L)
RLN	−8,258	−563.5	10.8	0.712	−1379.5	57.7	0.1193
LLP	−8,409	−243.5	10.5	0.774	−1292.6	55.1	0.1292
LLPCOR	−7,500	−117.8	8.8	0.837	−1357.4	58.7	0.1046
COR	−7,319	−70.2	9.95	0.728	−1010.9	50.5	0.1249
PA	−7,106	−108.4	10.1	**0.695**	−1424.4	61.8	**0.0919**

Tracheal pressure was measured experimentally and used as the boundary condition for the tracheal outlet in the computational model. Note that the procedure with the lowest impedance in both instances was PA, highlighted in bold. CFD, Computational fluid dynamics results; TP, Tracheal air pressure; PP, Pharyngeal air pressure; RLN, Recurrent laryngeal neuropathy; LLP, Left-sided laryngoplasty with ipsilateral ventriculocordectomy; LLPCOR, Left-sided laryngoplasty with ipsilateral ventriculocordectomy combined with corniculectomy; COR, Corniculectomy; PA, Partial arytenoidectomy.

inside the left arytenoid cartilage showed higher negative pressure within the RLN state, LLP, LLPCOR, COR, and PA procedures compared to the right side. It was most pronounced for the RLN state. In the transverse planes, lower pressure and higher velocity were observed in the RLN state and LLP procedures. The LLPCOR, COR, and PA procedures appeared to be more uniform. All procedures showed a narrowed conformation ventrally caudal to the saccule, but this was more exaggerated in some procedures than in others. This is shown in Figure 4, where the top two rows of images show changes in pressure, while the bottom two show changes in velocity. Each of the (A) rows demonstrates the “lateral” view of the larynx, looking at a parasagittal plane captured just inside the left arytenoid cartilage, while the (B) rows demonstrate an orthogonal plane taken through the middle of the laryngeal ventricles dorsoventrally.

The cross-sectional planes at the laryngeal opening showed narrowing for the RLN state, coinciding with more negative pressure and high velocity. At the mid-saccule region, greater negative pressure was observed inside the left wall of the RLN state and LLPCOR procedure models, denoted by black arrows in Figure 5. The top two rows display the pressure distributions within the larynx, while the bottom two display the velocity distributions. The removal of the arytenoid body in the PA procedure expanded the region on the left side; and therefore, decreased air velocity and less negative pressure were observed. Even caudal to the saccule, there was a similar trend of less negative pressure and reduced velocity in the narrowest portion of the PA procedure. These are shown in Figure 5, where row A depicts the laryngeal opening and row B shows the opening of the ventricles.

All of the planes captured and examined can be found in the Supplementary Information. Pressure, velocity, and turbulent kinetic energy were all examined in cross-sectional, sagittal, and transverse planes. All were adjusted to the same color scale for each of the planes, as reported by the procedure. The differences in the shape of the planes were due to the position of the head within the CT.

TABLE 2 Experimentally and computational (CFD) model rima glottidis area by procedure.

	Rima Glottidis Area (mm^2)		
Simulated state	Experimental	CFD	% difference
RLN	1,218	1,169	4.1
LLP	1,122	1,159	3.3
LLPCOR	1,482	1,314	11.4
COR	1,247	1,172	6.0
PA	1,671	1,593	4.6

The experimental rima glottidis area was measured from the corresponding CT section, while the CFD model rima glottidis area was measured using Space Claim needs a trademark. Percent difference refers to the difference between the experimentally measured and CFD model as compared to the experimental values. CFD, Computational fluid dynamics results; RLN, Recurrent laryngeal neuropathy; LLP, Left-sided laryngoplasty with ipsilateral ventriculocordectomy; LLPCOR, Left-sided laryngoplasty with ipsilateral ventriculocordectomy combined with corniculectomy; COR, Corniculectomy; PA, Partial arytenoidectomy.

4 Discussion

The experimental and CFD models were in agreement regarding the procedure with the lowest impedance for this horse model but had slight differences regarding the overall procedure order. The numerical comparison showed a larger disparity between the impedance values for the laryngeal region in the experimental and computational values than what was expected based on previously reported equine and human models (13, 15, 23). The flow rates reported from the experimental portion of the study were lower than expected. This discrepancy may have resulted from a measurement error due to small-scale oscillations in the flow, caused by passage through the tissue, which could have disrupted the orifice plate measurements. Alternatively, it could reflect an accurate representation of collapse within the cadaver. The CFD model reported values that were much closer to the anticipated values for the flow rate and therefore might have been more accurate in this case.

High impedance was reported between the pharynx and the tracheal outlet, both numerically and relative to the impedance at different portions of the airway. Although multiple studies have reported that 50% of the upper airway resistance should be attributable to the nasal passage, this was not the case in the present study. A number of factors may explain this discrepancy (13, 24, 25). Throughout each of these studies, different breeds were used. In this study, a Quarter horse was used as the model, while a previous study used a Thoroughbred (13). It is also important to consider the

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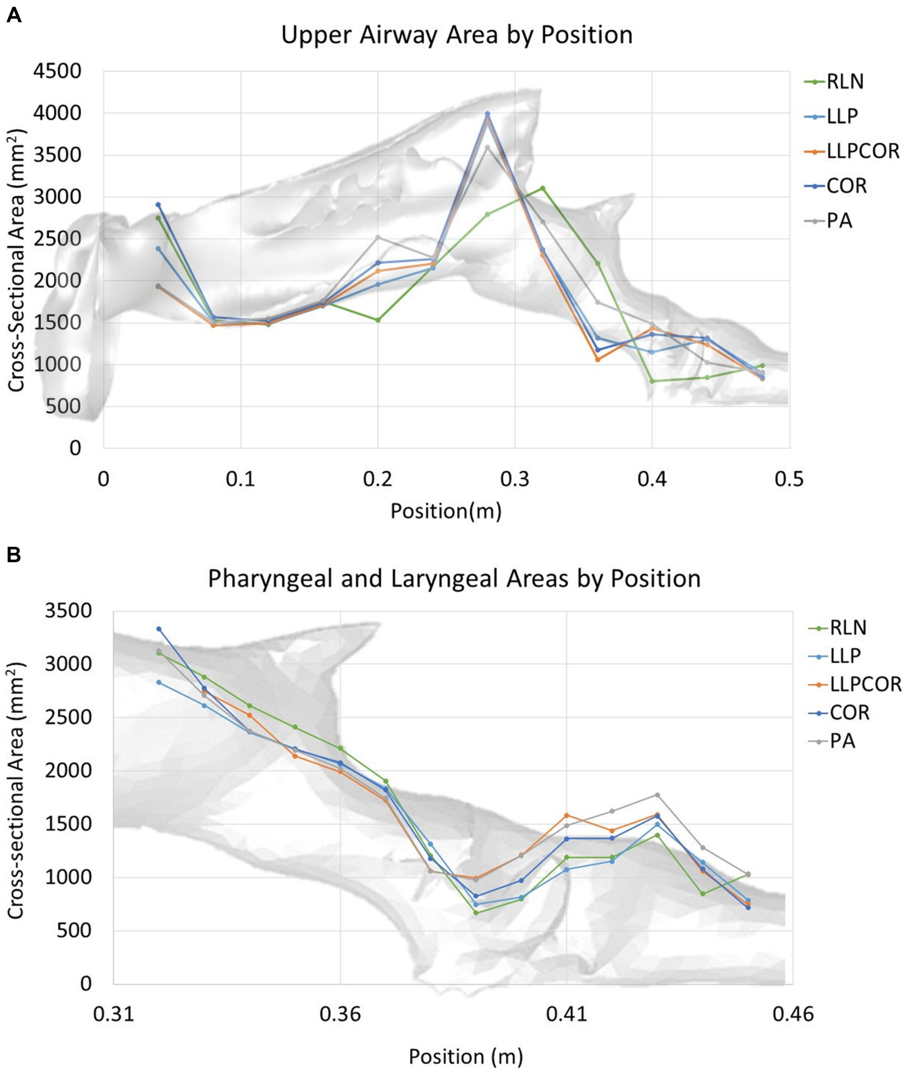

FIGURE 2
(A) Cross-sectional area along the upper airway by procedure. The RLN state exhibited the lowest cross-sectional area in the laryngeal region and showed the most variability across the cross-sectional areas in general. **(B)** Cross-sectional area along the larynx by procedure. The RLN state exhibited the lowest cross-sectional area at the region of the rima glottidis, while PA showed a larger area throughout the length of the laryngeal region. The pharyngeal region was similar between the procedures.

application as the current study sought to establish a truly inhalation-based model by subjecting the head to negative airflow after stabilizing the collapsible portions of the airway. This approach might have resulted in higher reported impedance in the regions of the collapsible tissue. Rakesh et al. (13) also found a constriction in the caudal nasal region where air dropped in the pharynx, which resulted in high negative pressure and turbulence. The horse used in this study did not have the same narrowed region, and even the original CT images showed significant overlap between the caudal nasal passage and the pharynx in the current study model. Some of the earlier studies reporting higher nasal resistance also incorporated transducer catheter placement in the pharynx but did not specify the exact location within that region. Pharyngeal resistance varies significantly from rostral to caudal, which could influence the reported resistance and explain the disparities between studies (24, 26). The current study correlated more closely with a previous fiberglass model, which found the nares, pharynx, and larynx each contributed approximately 30% of the airway resistance (25). In addition, the negative tracheal pressure was much higher than in some reports. However, it has been demonstrated that horses compensate for upper airway resistance by generating more negative pressure in the lower airways, within the ranges reported here for tracheal pressure (27).

This study followed a similar approach to a previous study, wherein the equine larynx was modeled in isolation (18). A similar jet effect from the caudal nostrils into the larynx was observed, as seen before. However, the anatomical change in direction ventrally from the ethmoid region, dropping into the pharynx and then subsequently into the larynx, was much more abrupt than in the straight box model. The funneling effect of the pharynx was observed, as documented previously (18). There appeared to be less abrupt changes in the airway, which might have been a function of the geometry construction. In this study, airflow regions were traced manually to establish the geometry, while in the previous study, the tissues were isolated. Manual segmentation was necessary in this case as the shape/contour of the sinuses and conchal bullae were expected to confound the process of semi-automated segmentation. Across both studies, the RLN state and COR procedure were more obstructive and characterized by higher negative pressure and velocity. The saccule also influenced the flow, as shown in Figure 4. There was a pattern of sudden changes in pressure and velocity over a short distance with the higher impedance procedures, as noted in the other study. Unique to this study, the LLP and LLPCOR procedures did not reduce impedance as effectively and the PA procedure exhibited the lowest impedance.

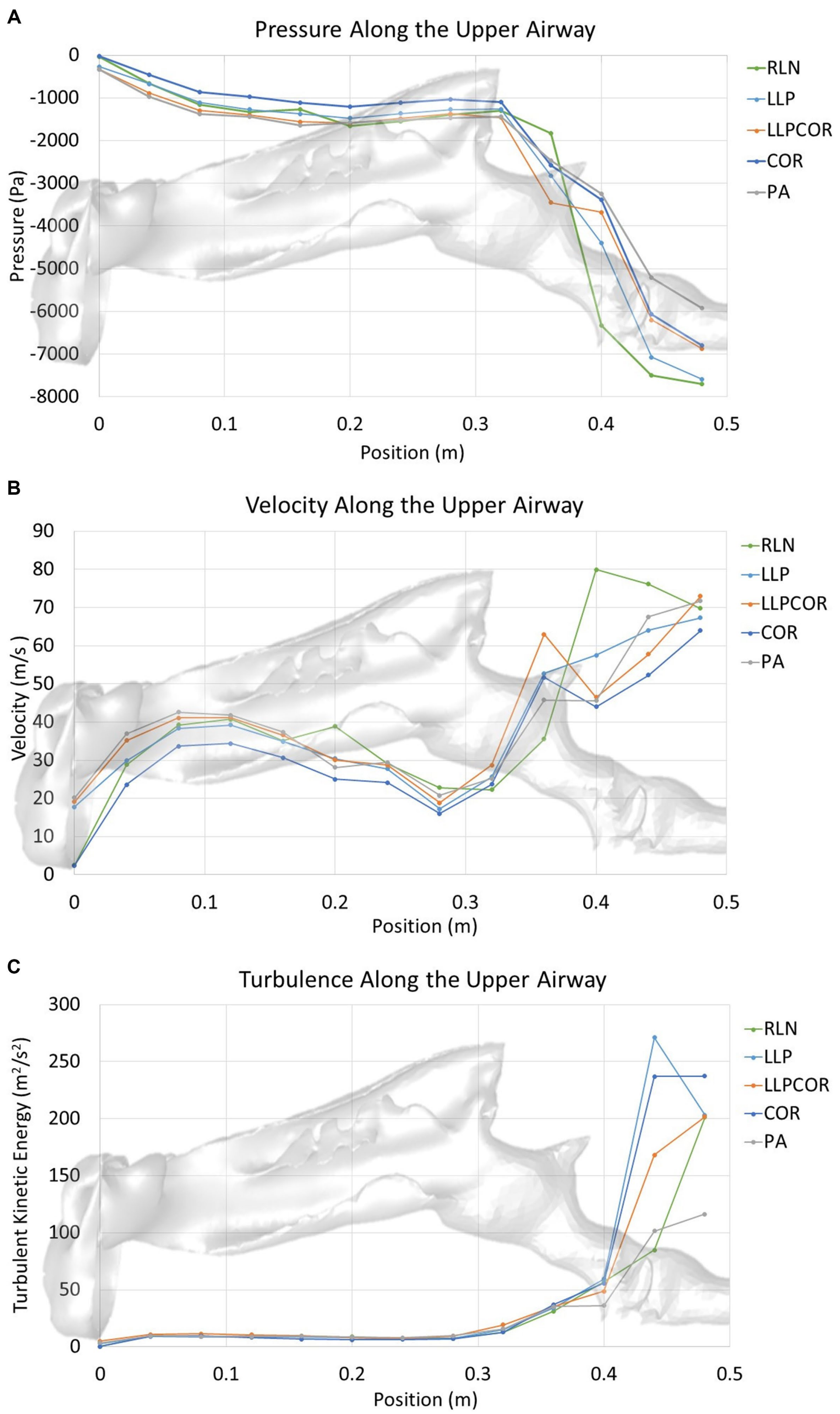

FIGURE 3 (Continued)

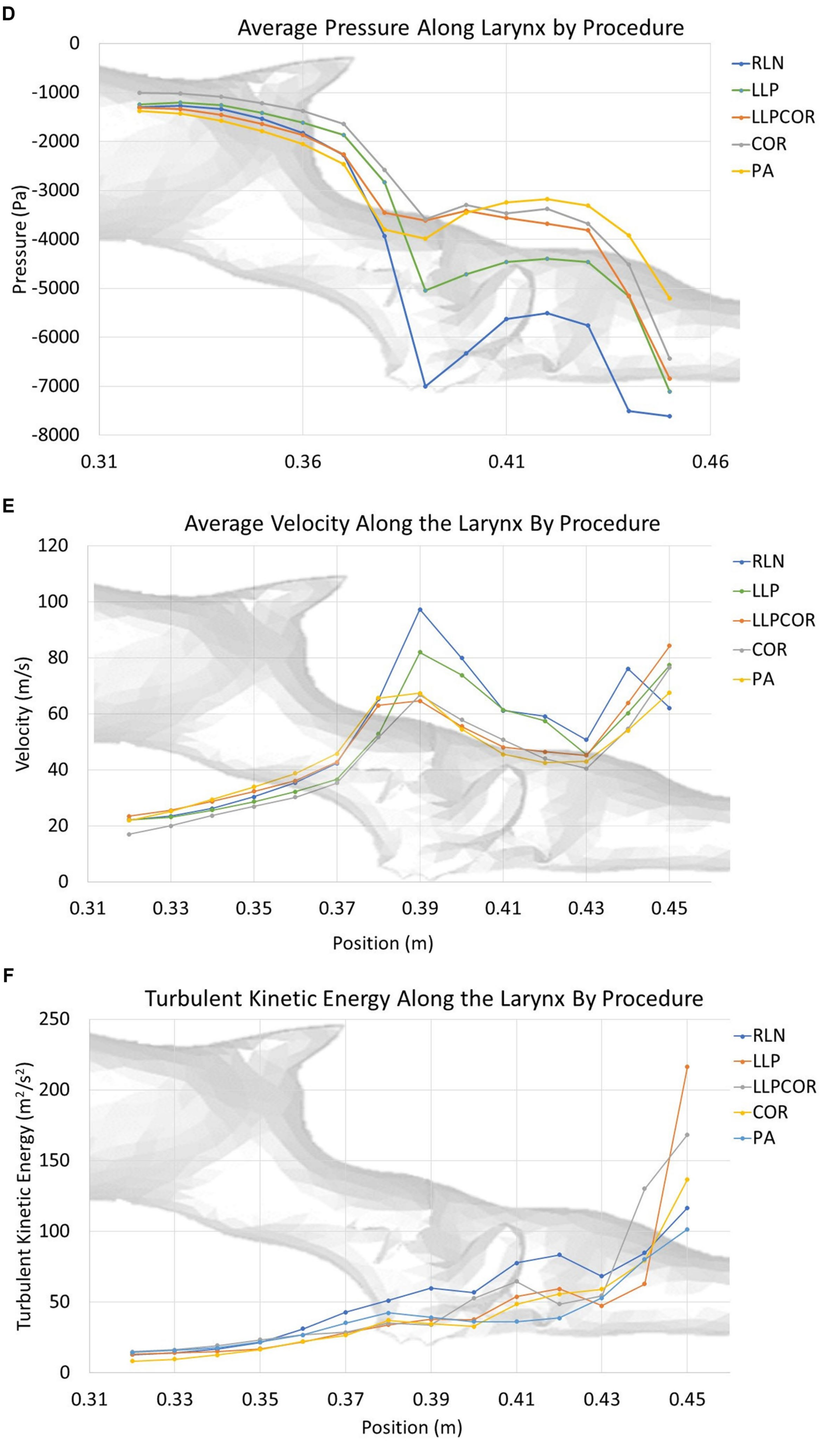

FIGURE 3
(A) Area-averaged pressure along the upper airway by procedure. The RLN state exhibited the lowest pressure in the region of the laryngeal opening, with LLP showing the second lowest pressure. LLPCOR, COR, and PA were more similar in their relative distribution. The LLPCOR procedure showed increased negative pressure in the caudal portion of the larynx as it transitioned into the trachea. **(B)** Area-averaged velocity along the upper airway by procedure. The RLN state showed the highest velocity in the region of the laryngeal opening, with LLP showing the second highest velocity. The remaining three procedures were more similar in their relative distribution. **(C)** Area-averaged turbulent kinetic energy distribution along the upper airway by procedure. Turbulent kinetic energy was largely similar across the procedures, except in the caudal laryngeal/tracheal region, where the cadaver model showed compression of the caudal airway. In general, the pharyngeal and laryngeal regions are the areas of increased turbulent kinetic energy compared to the rest of the airway. **(D)** Area-averaged pressure along the larynx by procedure. As demonstrated in the larger-scale plot, the RLN state exhibited the lowest pressure at the rima glottidis, best characterized as one significant pressure drop across that region. LLP showed the second-lowest pressure. LLPCOR, COR, and PA were more similar in their relative distribution. **(E)** Area-averaged velocity along the larynx by procedure. The RLN state exhibited the highest velocity along the larynx, followed by the LLP procedure. The remaining three procedures were more similar in their relative distribution. **(F)** Area-averaged turbulent kinetic energy distribution along the larynx by procedure. Turbulent kinetic energy specifically within the larynx was highest for the RLN state, with the LLPCOR procedure showing the second-highest level. All of the procedures showed increased turbulent kinetic energy at the laryngeal-tracheal junction.

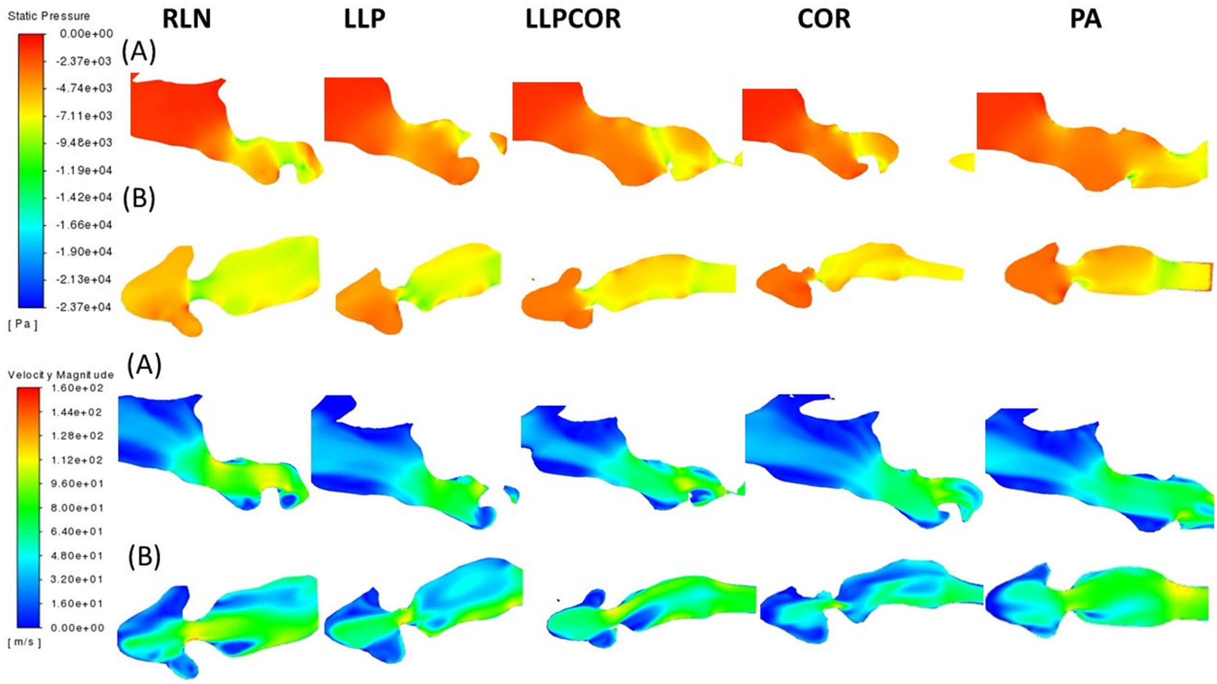

FIGURE 4
Pressure and velocity distribution through the left parasagittal and ventral laryngeal planes for all procedures. Row (A) shows the parasagittal left slice taken for each procedure, while row (B) shows the ventral laryngeal transverse section. Pressure is shown at the top, with velocity at the bottom. Air flows from left to right in the image, with the horse's left side up in the transverse sections, the nostrils on the left, and the trachea on the right.

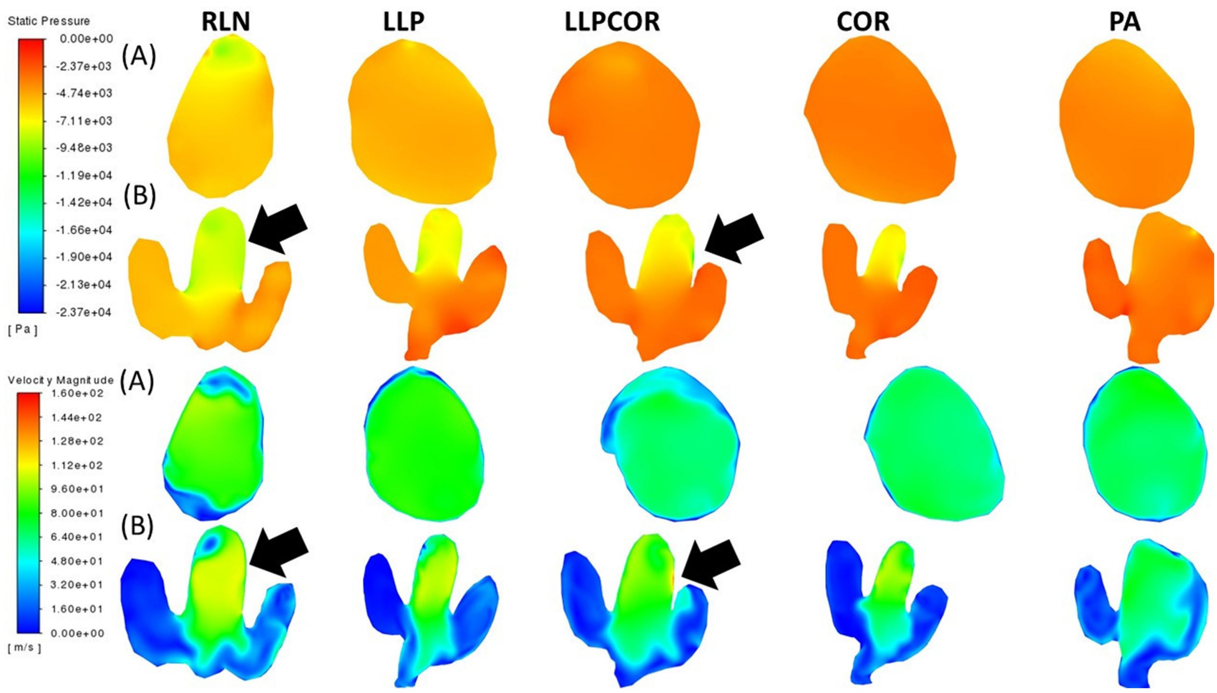

FIGURE 5
Laryngeal opening and mid-saccule cross-sectional planes by procedure. Row (A) shows the laryngeal opening slice taken for each procedure, while row (B) shows the mid-saccule section. Pressure is shown at the top, with velocity at the bottom. These sections are perpendicular to airflow and captured straight on so that the horse's left side is on the right side of the image. The black arrows represent the regions of particularly low pressure or high velocity, observed with the RLN state and LLPCOR procedure.

One explanation for this is that the head was oriented in the upright position, which might have resulted in the compression of the ventral soft tissue structures and reduced airway space. The PA procedure might have resulted in a greater cross-sectional area in this region through simple tissue excision alone. The use of a cadaver head resulted in unexpected impedance findings, with the LLP procedure showing higher impedance than the RLN state. Narrowing of the airways at the level of the nostrils and in other regions might have contributed to these unexpected results. The use of live patient geometry would be beneficial for future studies. In addition, the inclusion of more study subjects is warranted.

One important outcome of this study, which is supported by other studies, is that the PA procedure did not have the lowest velocity or the highest negative pressure, despite having the lowest impedance. Looking at the rima glottidis region alone, the negative pressure for the PA procedure was not the highest. However, upon proceeding caudally, the PA procedure compensated in the more caudal regions of the larynx, especially as the left ventricle region was incorporated into the cross-sectional flow area. This aligns with the modified PA report, which indicated that the changes in the rima glottidis area did not reflect the changes in impedance observed with the LLP and PA procedures (1). Looking at one specific portion of the airway is not entirely predictive of impedance, and the use of CFD allows for the incorporation of the entire airway (18). This finding is also supported by multiple sleep apnea studies in humans, where airway volume was correlated with impedance (5, 28).

One significant limitation of this study is the use of a single subject in the creation of the CFD models for the various surgeries, meaning that the relative outcomes regarding the different surgical procedures may only apply to this horse. However, one of the goals of this preliminary investigation was to determine whether a CFD model could corroborate the experimental "fitness" of a particular surgery within an individual "patient," which was successfully achieved. The findings regarding the differences between the surgical procedures and flow characteristics should be interpreted with caution as this model is one of only four equine-based CFD models reported in the literature to date (13, 15, 18, 22). Another limitation is the use of cadaver tissue instead of live tissue. The use of a cadaver was considered more ethical and potentially representative as the alternative— using a horse under anesthesia— would still have presented similar problems with airway soft tissue collapse, as observed in this study. It was decided to use fresh tissue preserved on ice due to concerns about the consistent freezing and thawing of a cadaver head, with special consideration for the respiratory mucosa and cartilages.

Although the experimental portion of the study reported the same findings for airflow and impedance as reported in other studies, the CFD results were much closer to the expected findings based on previous literature and offered insights into why the experimental discrepancy potentially occurred. The differences in nasopharyngeal geometry between this equine patient and a previous study, along with the consistent expansion over distance provided by the PA procedure and the consistent pressure and velocity throughout the volume, were all more apparent in the CFD analysis and help explain the disparities. Capturing an accurate representation of the three-dimensional upper respiratory anatomy in the equine patient is a worthwhile pursuit, given the potential insights that can be gained from the advanced analysis provided by CFD. This study is unique to both the veterinary and human fields in seeking the validation of a CFD model based on experimental data obtained from a cadaveric model. Further calibration and refinement of CFD application in an equine head model are necessary before it can be applied to clinical patients. However, the potential benefits are significant, given the ongoing advancements in computational and mechanical knowledge.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The requirement of ethical approval was waived by Western College of Veterinary Medicine ACUC for the studies involving animals because this study used cadaveric tissues not live animals. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

MT: Conceptualization, Funding acquisition, Methodology, Writing – original draft, Writing – review & editing. DW: Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Writing – review & editing. DB: Data curation, Methodology, Software, Supervision, Writing – review & editing. JC: Conceptualization, Formal analysis, Funding acquisition, Methodology, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2024.1478511/full#supplementary-material.

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Gait kinematics at trot before and after repeated ridden exercise tests in young Friesian stallions during a fatiguing 10-week training program

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Background: Appropriate training is essential for equine athletes to improve fitness and ensure welfare. Young Friesian stallions must complete a 10-week training program for acceptance as breeding stallions. Earlier, this training program was demonstrated to induce overtraining.

Objectives: To evaluate how this training program affects stallions' trot locomotion variables in relation to fatigue.

Study design: Prospective analytical study.

Methods: 3 or 4 years-old (n = 16) Friesian stallions performed three ridden indoor standardized exercise tests (SETs) in week-1 (SET-I; n = 15), week-6 (SET-II; n = 11) and week-10 (SET-III; n = 4), measuring heart rate (bpm) and lactate concentration (LA, mmol/L). Before and after each SET, stallions' locomotion was measured with seven inertial sensors (EquiMoves, 200 Hz) during in-hand trot on a straight line. Stride characteristics, limb angular changes, and upper body kinematics were calculated. The within-measurement coefficient of variation (CV) was calculated for all parameters. Linear mixed models were used to analyze gait variables related to SET, pre-or post-SET and a peak LA ≥4 mmol/L during SETs.

Results: Horses showed individual responses in gait kinematics to moderate fatigue. The range of motion of the withers ($ROM_{withers}$) increased post SET-II and SET-III compared to post SET-I. In horses reaching LA ≥ 4 mmol/L, CV increased post SETs for several stride characteristics and upper body asymmetry. Upper body vertical movement asymmetry was above the described reference ranges in 69% of the horses.

Main limitations: Number of horses used and only four horses managed to complete the 10-week training program as breeding stallions.

Conclusion: The young Friesian stallions showed individually different responses in absolute gait kinematics after exercise and during an intense training program. The increased $ROM_{withers}$ and CV of stride characteristics after SETs suggest an acute effect of fatigue on the locomotion pattern. Further investigation is warranted for the pronounced upper body movement asymmetry related to published asymmetry reference values.

KEYWORDS
horse, training, workload, gait analysis, fatigue, welfare, Friesian horse, breeding selection

1 Introduction

In the last few years, societal attitudes toward horses have changed. Equestrians should minimize negative welfare effects and avoid unnecessary harm to the horse (1, 2). Equine stakeholders named training management as one of the five essential domains in managing of sport horse health and welfare (3). An appropriate training program can contribute to improving performance and is also valuable for the welfare of equine athletes (4–6). Physical exercise can lead to fatigue. The term fatigue, however abstract, has many different classifications (7, 8). Exercise-induced fatigue can be acute or chronic and can refer to a (neuro) muscular deficit (peripheral) or decline in mental function (central) (7–11). Muscle fatigue can be defined as "a decrease in maximal force or power production in response to contractile activity" (7, 10) or as a "reversible loss of muscle force during work over time" (9). On neuromuscular level, the nervous system fails to recruit muscle motor units adequately when being in a fatigued state. This mechanism leads to a decrease in proprioception, motor control and movement coordination (11) and thus increases the risk of injuries (8, 10–12). Chronic fatigue can lead to underperformance or overtraining (10, 12). In athletes, delaying the onset of fatigue is an essential feature of an effective training program (12, 13). However, it is difficult to quantify fatigue reliably, and early fatigue detection is challenging as currently there is no single conclusive marker of fatigue (11). Recovery is seen as the time to restore physiological and mental triggers from (intense) exercise. Appropriate recovery time reduces injury risk and improves the quality of the subsequent training (4, 10, 12).

Quantitative gait analysis systems have become more affordable and practical for daily use (14, 15). Kinematic gait analysis using inertial measurement units (IMU's) is a reliable and practical manner to objectively measure and document horses' gait in clinical or research settings (14, 16) with good repeatability and reproducibility (17–19), especially when sensors are placed both on the upper body and on the limbs (20). Gait symmetry measurements are an important feature of objective gait analysis techniques and are applicable to symmetrical gaits (e.g., walk, trot, and tölt) on a straight line or lunge, and can be used in lameness assessment of horses (14). Gait parameters can also be compared using repeated measurements of the same horses over time (14) and it has been shown that fatigue can influence movement patterns in horses (21–24). However, this influence is different when measuring on a treadmill or in field conditions (25). In field conditions, reduced stride frequency, as well as the protraction of forelimbs and retraction of hind limbs, was shown in fatigued horses (25, 26).

Yearly, the Royal Friesian Horse Studbook (KFPS) selects young Friesian stallions as breeding stallions. Part of this selection is a studbook approval test consisting of a 10-week training program. Horses are brought together at a training facility to assess athletic capability for dressage and driving and evaluate character and health (27).

This study aimed to evaluate gait parameters before and after repeated ridden standardized exercise tests (SET) in relation to exercise-induced fatigue and medium to long-term fatigue in young Friesian stallions during a 10-week training program. We hypothesized that exercise-induced fatigue would lead to altered gait kinematics and a higher step-by-step variability. Additionally, we hypothesized that medium to long-term fatigue would result in a less expressive gait as shown by reduced stride length and reduced range of motion of limbs and upper body kinematics.

2 Materials and methods

The Animal Ethics Committee of Utrecht University approved all research procedures (reference number 5204-1-04, approval date 24 June 2020). Written owners' informed consent was also obtained. The physiology and training load data of this study has been published elsewhere (5). It was demonstrated that a state of chronic fatigue due to an intense training load, was induced. During the 10 weeks, horses did not get enough recovery time between training sessions and showed signs of overreaching or overtraining indicated by higher heart rates (HR) and plasma lactate concentrations (LA) during SETs at the end of the training program compared to the start of the training program (5).

2.1 Horses

Data were collected from privately owned young Friesian stallions. Horses ($n = 16$), age 3 ($n = 13$) or 4 ($n = 3$) years, were selected by the KFPS to participate to the 10-week stallion training program as part of the selection process for studbook approval. Horses were assessed by studbook judges in the months before the training program repeatedly on multiple selection days. Selection criteria consisted of exterior traits, gait characteristics, ridden performance and character as well as genetic information and health assessments such as sperm quality, left laryngeal hemiplegia and osteochondrosis dissecans (27). Horses selected by the studbook were included in the study after owner consent. Horses were kept in individual stables at the same training facility and were fed an individual diet consisting of roughage and concentrates during the entire study. Water was provided ad libitum. Horses had daily turnout in a sand paddock or a horse walker. Horses underwent dressage and driving training for 10 weeks. The

Abbreviations: HR, Heart rate; LA, Plasma lactate concentration; SET, Standardized Exercise Test; CV, Coefficient of Variation; KFPS, Royal Friesian Horse Studbook; IMU, Inertial measurement unit; StanceF, Stance duration forelimbs; StanceH, Stance duration hind limbs; ROM_F, Range of motion forelimbs; ROM_H, Range of motion hind limbs; ROM_{head}, Range of vertical motion of the head; $ROM_{withers}$, Range of vertical motion of the withers; ROM_{pelvis}, Range of vertical motion of the pelvis; HD_{min}, vertical displacement minimum difference of the head; HD_{max}, vertical displacement maximum difference of the head; WD_{min}, vertical displacement minimum difference of the withers; WD_{max}, vertical displacement maximum difference of the withers; PD_{min}, vertical displacement minimum difference of the pelvis; PD_{max}, vertical displacement maximum difference of the pelvis.

TABLE 1 Protocol of a submaximal ridden standardized exercise tests carried out in an indoor arena (20×60 m) for young Friesian horses, measuring heart rate and plasma lactate concentration.

Time (min)	Exercise	Measurement	Speed
SET	Indoor Arena under saddle		
00:00–01.00	Trot		
01:00–05:00	Walk		~ 2 m/s
05:00–07:00	Left trot		~ 3.5 m/s
07:00–09:00	Right trot		~ 3.5 m/s
09:00–10:00		LA	
10:00–12:00	Canter-1		~ 5.0 m/s
12:00–13:00		LA	
13:00–15:00	Canter-2		~ 5.0 m/s
15:00–16:00		LA	
16:00–26:00	Walk		Recovery ~2 m/s
26:00–27:00		LA	

Speeds (m/s) are estimates. SET, standardized exercise test; LA, plasma lactate concentration.

training program was determined by the head trainer of the studbook (KFPS) and details are described in Siegers et al. (5). Horses were trained by 4 experienced riders and 2 experienced drivers. The stallion's behavior, stable manners and occurrence of veterinary problems were documented.

2.2 Study design

During the 10-week training program, three submaximal ridden standardized exercise tests (SETs, Table 1) were performed in week 1 (SET-I), week 6 (SET-II) and week 10 (SET-III), measuring HR and LA. Gait analysis was performed before and after each SET. Gait analysis data was collected on a 40 m straight line on a hard surface (indoor) in trot (twice up and down per session) to collect a minimum of 10–20 strides per measurement session for analysis. One person (ES) handled the horses for all measurements. Studbook judges assessed the stallions in week 6 and week 10. Stallions could be eliminated from the stallion test by failing to obtain a positive evaluation by the judges, or as a result of an injury or other illness assessed by a veterinarian assigned by the studbook.

2.3 Equipment

During SETs, HR measurements were performed using a HR sensor (beats per minute (bpm), Polar V800 ® Polar Electro Oy, Kempele, Finland). Blood samples were collected by venepuncture of the left or right jugular vein using a sterile 2 mL syringe and a 23-gage needle to determine LA [mmol/L; Lactate Pro 2® (Arkray Inc. Kyoto, Japan)]. Ambient temperature (°C) and relative humidity (RH, %) were measured during the SETs using a heat stress wet bulb globe temperature (WBGT) device (Extech instruments HT30, Nashua, U.S.A.). Gait data collection was done using the wireless IMU-based EquiMoves software system (15). Seven IMU sensors (Inertia Technology B.V. Enschede, The Netherlands) with a sampling frequency of 200 Hz were placed as described by Bosch et al. on the following locations: the poll, the withers (using a girth), between the tubera sacrale of the pelvis and on the lateral aspect of the mid metacarpus/metatarsus of each limb. The sensors on the limbs were placed on a standard location (lateral aspect of the cannon bone) on dedicated brushing boots (Figure 1). Horses were accustomed to wearing brushing boots before the study. The IMU sensors were calibrated for 5 s according to the manufacturer's recommendation.

2.4 Data collection

The SETs consisted of three incremental steps, HR was measured during the entire SET. Blood samples to determine LA were taken 30–60 s after each step, and after 10 min of recovery at walk (see Table 1). Gait analysis was performed in all horses within 2 h before each SET, and within 5 min after each SET.

2.5 Data processing

2.5.1 Gait analysis data

Hoof events were automatically detected (28) and the signals were processed following the procedures described by Bosch et al. (15). The extracted parameters are described in Table 2. From the data collected from the IMU sensors, stride duration (sec), stance duration (sec), range of motion of the protraction of the distal limbs (ROM degrees), maximal protraction and retraction angles of the distal fore and hind limbs (degrees), and vertical displacement of the head (ROM_{head}, mm), withers ($ROM_{withers}$, mm), and pelvis ($ROM_{pelvis,}$ mm) were calculated for each individual stride with custom MATLAB scripts (R2022b, MathWorks Inc., Natick, United States). Swing duration was calculated by the following equition:

$$\textit{swing duration} = \textit{stride duration} - \textit{stance duration}$$

From the gait analysis data, we created fore (F) and hind (H) parameters by averaging the individual left and right fore and hind

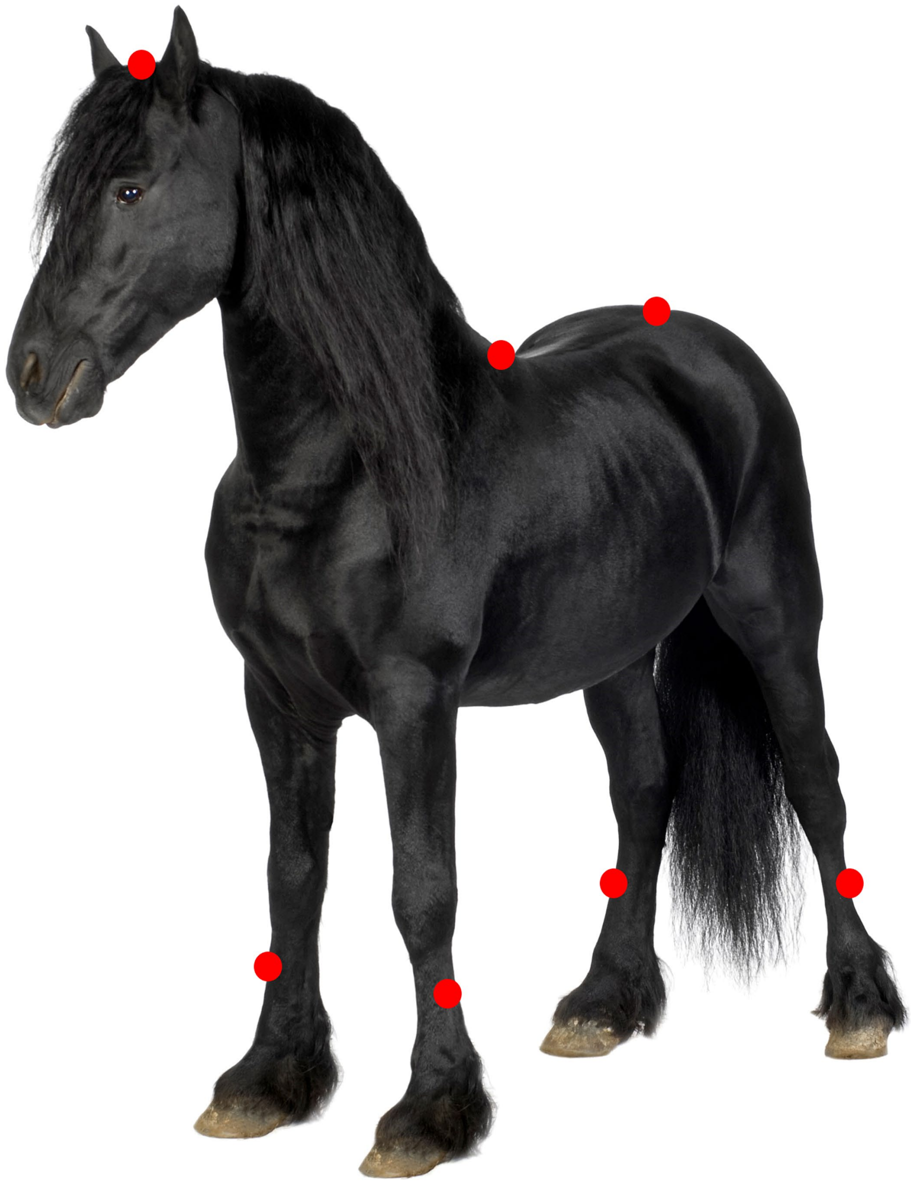

FIGURE 1
Locations of IMU sensor placements indicated on a Friesian horse. IMU placement sides are indicated with a red circle on the poll, withers, pelvis and on the mid-lateral aspect of the metacarpus/metatarsus on all 4 limbs.

limb values, respectively. Vertical movement asymmetry parameters were also obtained with the EquiMoves software, namely the differences in local minima and differences in local maxima reached by the head, withers and pelvis (HDmin/max, WDmin/max and PDmin/max respectively). In order to analyze changes in the amplitude of asymmetry only and not the side of the asymmetry, we used the absolute values of these parameters for the statistical analysis.

2.5.2 Fatigue classification

Based on maximum LA values during the SET, horses were classified as more or less fatigued using LA concentration of ≥4 mmol/L as cut-off value. This value is generally considered as the anaerobic threshold (29, 30). Horses with a LA result ≥4 mmol/L were classified as more fatigued compared to horses with LA values <4 mmol/L. Horses completing the entire study were referred to as '*finishers*'.

2.5.3 Heart rate data

Before processing, all HR data were visually checked for artifacts. If many artifacts were present (>5% of the measurement), data was not used for analysis. For HR analysis in the different gaits, the mean HR of the last 60 s in each gait and after 5 and 10 min of recovery were used.

2.6 Sample size estimates

Power calculations to determine the required number horses in the study were not possible, since this was a field study and the authors

TABLE 2 Detailed description of the calculated gait parameters used in this study.

Name	Abbreviation	Description
Stride duration (sec)		Time between hoof-on and subsequent hoof-on of the left hind limb
Stance duration (sec)		Time between hoof-on and hoof-off, mean of both fore/hind limbs
Fore	StanceF	
Hind	StanceH	
Swing duration (sec)		Stride duration minus stance duration, mean of both fore/hind limbs
Fore	SwingF	
Hind	SwingH	
Range of motion on the sagittal plane of the distal limbs (degree)		Range of motion of the sagittal limb angle as the angle range between maximal protraction and maximal retraction of both fore/hind limbs
Fore	ROM_F	
Hind	ROM_H	
Protraction distal limbs (degree)		Maximal forward protraction of the distal limb measured at the metacarpus/– tarsus in the sagittal plane
Fore		
Hind		
Retraction distal limbs (degree)		Maximal backward retraction of the distal limb measured at the metacarpus/– tarsus in the sagittal plane
Fore		
Hind		
Range of motion (mm)		Range of vertical displacement of the sensor
Head	ROM_{head}	
Withers	$ROM_{withers}$	
Pelvis	ROM_{pelvis}	
Vertical displacement minimum difference (mm)		Measure of asymmetry, difference in minimum dorsoventral displacement of the sensor between left and right hoof-on/hoof-off in one stride
Head	HD_{min}	
Withers	WD_{min}	
Pelvis	PD_{min}	
Vertical displacement maximum difference (mm)		Measure of asymmetry, difference in maximum dorsoventral displacement of the sensor between left and right hoof-on/hoof-off in one stride
Head	HD_{max}	
Withers	WD_{max}	
Pelvis	PD_{max}	

were restricted to use the number of horses included in the KFPS stallion selection process.

2.7 Statistical methods

2.7.1 Outlier detection

All gait analysis data was checked for artifacts before analysis. All strides with a duration above or below 1.95 x standard deviation (s.d.) from the mean per individual horse per SET and per timepoint were considered outliers and were removed from the dataset.

2.7.2 Data preparation

For the asymmetry parameters HD_{min}, HD_{max}, WD_{min}, WD_{max}, PD_{min} and PD_{max}, absolute values were used for analysis to account for the amplitude of asymmetry rather than the side of the assymetry. The within measurement coefficient of variation (CV, %) was calculated for each parameter by the following equation:

$$CV = \left(\frac{s.d.}{mean}\right) \times 100$$

2.8 Statistical analysis

Statistical analysis was performed using R-studio cloud 2024® (Boston, MA, United States), using the packages libraries dplyr (version 1.0.10), tidyr (version 1.2.0), ggplot2 (version 3.4.0), lme4 (version 1.1–35.1) and glmmTMB (version 1.1.5). Descriptive statistics are presented as mean ± s.d.

Linear mixed effect models were used for gait analysis and SET (HR, LA) data. Horse was used as a random effect. Whether horses finished the entire study (finishers) or not, SET number (I, II, III), pre/post SET and interaction effects between SET and pre/post SET measurement, and pre/post and LA ≥ 4 mmol/L were included as fixed factors in the linear mixed effect model. Deviations of normality and homogeneity of variances of the residuals were visually checked by examining the QQ plot and residual plot. Akaike's Information Criterion (AIC) was used for model reduction. For important effects in the final model, 95% confidence intervals (95% CI) were calculated and presented as estimates and 95% CI.

Asymmetry results in our population compared with the reference values for Warmblood horses as described by Hardeman et al. (31) 2019 (12 mm for HD_{min}, 9 mm for HD_{max}, 3 mm for WD_{min} and WD_{max}, 4 mm for PD_{min} and PD_{max}) because they used the same gait analysis system as was used to validate the Equimoves® system and horses were also measured in-hand in trot on a hard surface.

To evaluate whether horses with more asymmetry before SET-I were related to elimination due to lameness or injuries, a logistic regression analysis was performed for elimination reason "lameness" (Y/N) using the factors HD_{min}, HD_{max}, WD_{min}, WD_{max}, PD_{min}, and PD_{max}. Odds ratios (OR) for relevant factors from the regression model were calculated.

3 Results

3.1 Descriptives

A total of 16 horses were included in the study. Twelve horses were withdrawn from the studbook stallion selection process prematurely: seven due to judge decisions and five due to injury/lameness. One stallion entered the training program 2 weeks after the start of the study because of an injury, and did not participate in SET-I. This horse completed the rest of the study. Resulting in 15 participating horses in SET-I. Of these horses, 10 participated in SET-II and the horse entering the program 2 weeks later was also participating in SET-II (total of 11 horses). From the horses participating in SET-II, four were also participating in SET-III. An overview of the number of horses participating to each SET, and available gait analysis and physiology data is presented in Table 3. A total of 1,332 strides were collected, of which 84 strides were outliers (6%) and were removed before data analysis. For each horse a mean of 20 ± 6.2 strides (min 4, max 34 strides) were available for analysis per sampling moment (SET-I/-II/III, before and after SET).

3.2 Heart rate and plasma lactate concentration

Detailed descriptions of training data and heart rate and lactate results have been published elsewhere (5). HR and LA increased in SET-II and SET-III compared to SET-I, indicating that the training program led to a decreased fitness after 6 and 10 weeks (see Table 4).

3.2.1 Gait analysis

Results of the linear mixed effect models of gait parameters are presented in Tables 5–8.

For all gait parameters, horses showed different individual responses to the training program and the effect of fatigue (after SET and LA ≥ 4 mmol/L). As examples, the individual responses for $ROM_{withers}$, forelimb forward protraction and StanceH are shown for each horse in Supplementary Figure S1.

3.2.2 Coefficient of variation

For stride duration parameters there were important increases in CV in more fatigued horses (Figure 2). The variation in stride duration (intercept 6.2; 95% CI 4.6, 7.7) and StanceH (intercept 7.8; 95% CI 6.1,

TABLE 3 Number of horses of which data is available for analysis per standardized exercise test in young Friesian stallions during a 10-week training program, and number of horses with a plasma lactate concentration ≥ 4 mmol/L during the standardized exercise test (percentage of participating horses).

	Total	Gait analysis		Physiology data	
		Before	After	HR and LA	LA ≥ 4 mmol/L
SET-I	15	15	15	15	5 (33%)
SET-II	11	11	9	8	5 (45%)
SET-III	4	3	4	4	4 (100%)

SET, standardized exercise test; LA, plasma lactate concentration (mmol/L); HR, heart rate (bpm).

TABLE 4 Mean ± s.d. results for heart rate and plasma lactate concentration in young Friesian horses during repeated ridden-submaximal standardized exercise tests after the first (Canter-1) and second (Canter-2) canter bouts.

	SET-I (*n* = 15)	SET-II (*n* = 8)	SET-III (*n* = 4)
HR			
Canter-1	141 ± 15	153 ± 17*	169 ± 7*
Canter-2	148 ± 17	164 ± 23*	175 ± 13*
LA			
Canter-1	3.1 ± 1.2	2.9 ± 1.0	5.4 ± 1.5*
Canter-2	3.2 ± 1.4	3.8 ± 1.7*	6.2 ± 1.6*

SET, standardized exercise test; LA, plasma lactate concentration (mmol/L); HR, heart rate (bpm). *Indicating a significant increase compared to SET-I, determined by statistical analysis using a linear mixed effect model and Akaike's Information Criterion for model reduction.

TABLE 5 Results of stride characteristics in young Friesian stallions before and after repeated submaximal ridden standardized exercise test in trot (in-hand), related to high (≥4 mmol/L) or low plasma lactate concentration.

		Main effects				Interaction effects		
Parameter	Intercept	SET-II	SET-III	After SET	Finishers	SET-II x after SET	SET-III x after SET	After SET x LA high
Stride duration (s)	0.77	−0.02*	0.00	−0.01	0.03*	0.01	−0.01	−0.02*
95% CI	0.75; 0.79	−0.03; −0.01	−0.01; 0.01	−0.02; 0.00	0.00; 0.07	−0.0; 0.02	−0.03; 0.01	−0.04; −0.01
% Change		−2.7	0.13	−1.1	4.4	1.2	−1.1	−3.1
Stance duration fore (s)	0.31	−0.01*	0.00	0.00	0.02*	−0.00	−0.02*	n.s.
95% CI	0.30; 0.32	−0.01; −0.00	−0.00; 0.01	−0.00; 0.01	0.01; 0.04	−0.01; 0.01	−0.03; −0.01	
% Change		−3.0	0.9	0.7	6.9	−0.09	−7.3	
Stance duration hind (s)	0.31	−0.01*	−0.02*	0.01*	n.s.	n.s.	n.s.	n.s.
95% CI	0.29; 0.33	−0.01; −0.01	−0.02; −0.01	0.00; 0.01				
% Change		−3.1	−4.8	2.0				
Swing duration fore (s)	0.46	−0.01*	0.00	−0.01	n.s.	n.s.	n.s.	−0.02*
95% CI	0.45; 0.48	−0.01; −0.00	−0.01; 0.01	−0.01; −0.00				−0.03; −0.00
% Change		−1.6	0.8	−1.6				−3.2
Swing duration hind (s)	0.48	−0.01*	0.02	−0.01	n.s.	0.01	−0.02*	−0.02*
95% CI	0.45; 0.50	−0.02; −0.00	0.01; 0.03	−0.02; −0.01		−0.00; 0.02	−0.04; −0.01	−0.03; −0.01
% Change		−2.2	4.5	−2.9		1.5	−4.3	−4.3

SET, standardized exercise test; LA, plasma lactate concentration (mmol/L). *Indicating important difference from SET-I/before SET or LA low. n.s. indicating that this factor was excluded from the statistical model. Number of horses: SET-I *n* = 15, SET-II *n* = 11, SET-III *n* = 4.

TABLE 6 Results of range of motion of head, withers and pelvis in trot (in-hand) in young Friesian stallions before and after repeated submaximal ridden standardized exercise test, related to high (≥4 mmol/L) or low plasma lactate concentration.

		Main effects				Interaction effects		
Parameter	Intercept	SET-II	SET-III	After SET	Finishers	SET-II x after SET	SET-III x after SET	After SET x LA high
ROM head (mm)	84.25	−5.40*	5.34	n.s.	11.99*	n.s.	n.s.	n.s.
95% CI	77.51; 91.15	−8.86; −1.94	−0.34; 11.03		2.58; 21.27			
% Change		−6.4	6.3		14.2			
ROM withers (mm)	121.20	−4.52	4.62	−6.6	n.s.	6.34*	6.41*	−6.59*
95% CI	115.63; 126.95	−8.82; −4.24	1.20; 8.04	−8.82; −4.24		3.43; 9.34	2.06; 10.76	−9.89; −3.20
% Change		−3.7	3.8	−5.4		5.3	5.3	−5.4
ROM pelvis (mm)	91.87	−1.50*	−7.23*	−1.34	9.18*	n.s.	n.s.	−3.46*
95% CI	85.44; 98.04	−2.85; −0.16	−9.53; −4.94	−3.01; 0.32	0.04; 18.34			−6.33; −0.59
% Change		−1.6	−7.9	−1.5	10.0			−3.8

SET, standardized exercise test; LA, plasma lactate concentration (mmol/L); ROM, range of motion. *Indicating important difference from SET-I/before SET or LA low. n.s. indicating that this factor was excluded from the statistical model. Number of horses: SET-I *n* = 15, SET-II *n* = 11, SET-III *n* = 4.

9.3) was higher in more fatigued horses shown by higher CV after SETs in horses with LA ≥ 4 mmol/L (2.5, 95% CI 0.8, 4.1 and 4.3; 95% CI 2.4, 6.1 respectively) compared to before SET. Variation in SwingH (intercept 7.3; 95% CI 5.6, 8.9) increased after SET-III compared to before SET-III (7.6; 95% CI 3.9, 11.2).

The CV of the asymmetry parameters HD_{max}, WD_{min} and PD_{max} also changed in more fatigued conditions, but were not different for the other asymmetry parameters. The CV of WD_{min} (intercept 5.4; 95% CI 3.4, 7.3) increased after SET-III (29.2; 95% CI 4.6, 53.8) compared to before SET-III, but was overall lower after the SETs in horses with LA ≥ 4 mmol/L (−15.7; 95% CI −28.2, −3.2). The CV of HD_{max} (intercept 119.0; 95% CI 97.0, 141.1) was lower in SET-III (−34.1; 95% CI 65.3, 2.9) compared to SET-I. The CV of PD_{max} (estimate 64.3; 95% CI 51.0, 77.7) increased after SET-II (23.2; 95% CI

TABLE 7 Results of protraction and retraction angles of the limbs in trot (in-hand) in young Friesian stallions before and after repeated submaximal ridden standardized exercise test, related to high (≥4 mmol/L) or low plasma lactate concentration.

		Main effects				Interaction effects		
Parameter	Intercept	SET-II	SET-III	After SET	Finishers	SET-II x after SET	SET-III x after SET	After SET x LA high
ROM forelimbs (°)	95.63	1.33	3.55	−0.67	−6.50*	1.68*	−2.70*	−0.74
95% CI	93.63; 97.65	0.58; 2.08	2.35; 4.76	−1.48; 0.14	−9.44; −3.51	0.63; 2.73	−4.24; −1.16	−1.93; 0.44
% Change		1.4	3.7	−0.7	−6.8	1.8	−2.8	−0.8
ROM hind limbs (°)	61.57	−0.45	−2.17	−0.95	n.s.	1.32*	3.77*	n.s.
95% CI	60.26; 62.99	−1.08; 0.18	−3.15; −1.20	−1.59; −0.31		0.44; 2.21	2.60; 4.95	
% change		−0.7	−3.5	−1.5		2.2	6.2	
Hoof on protraction forelimbs (°)	25.43	0.22	−0.66*	0.27	−2.33*	n.s.	n.s.	n.s.
95% CI	24.63; 26.22	−0.13; 0.58	−1.22; −0.11	−0.045; 0.58	−3.51; −1.19			
% Change		0.9	−2.6	1.1	−9.2			
Hoof on protraction hind limbs (°)	26.49	−0.69	−2.92	1.13	n.s.	1.57*	1.63*	n.s.
95% CI	24.12; 28.85	−1.47; 0.9	−4.06; −1.79	0.34; 1.93		0.47; 2.66	0.17; 3.09	
% Change		−2.6	−11.0	4.3		5.9	6.2	
Hoof off retraction forelimbs (°)	−41.56	−0.85	−0.27	0.94	n.s.	−1.58*	−0.33	n.s.
95% CI	−43.14; −40.05	−1.32; −0.37	−1.02; 0.48	0.45; 1.42		−2.24; −0.90	−1.21; 0.56	
% Change		−2.0	−0.7	2.3		−3.8	−0.8	
Hoof off retraction hind limbs (°)	−23.60	−1.19*	−2.05*	−0.32	n.s.	n.s.	n.s.	1.96*
95% CI	−26.42; −21.14	−1.66; −0.73	−2.85; −1.26	−0.87; 0.23				0.67; 3.26
% Change		−5.1	−8.7	−1.3				8.3

SET, standardized exercise test; LA, plasma lactate concentration (mmol/L); ROM, range of motion. *Indicating important difference from SET-I/before SET or LA low. n.s. indicating that this factor was excluded from the statistical model. Number of horses: SET-I $n = 15$, SET-II $n = 11$, SET-III $n = 4$.

51.0, 77.7) compared to before SET-II, but was overall lower in SET-III compared to SET-I.

The CV of the ROM_{head}, $ROM_{withers}$, ROM_{pelvis}, ROM_{protF} and ROM_{protH} did not change significantly.

3.2.3 Asymmetry parameters

The asymmetry parameters HD_{min}, HD_{max}, WD_{min}, WD_{max}, PD_{min} and PD_{max} were measured twice per SET per horse, resulting in a total of 342 individual average data points. The mean ± s.d. and percentiles per parameter and per SET are presented in Supplementary Table S1 and are compared to reference ranges for Warmblood horses (31). Eleven out of 16 stallions (69%) had no upper body asymmetry measurements within the reference range, and all horses had at least one measurement above the reference range on all occasions (SET-I, -II and III). From the measurements exceeding the reference value ($n = 334$, 97.6%), only eight were less than 10% above the reference value. A total of 259 out of 334 (75.7%) measurements were more than 50% above reference value. One horse had a PD_{min} of 30.7 mm before SET-II (7.7 times higher than the reference limits). Both before and after SET-I, three measurements for HD_{min} were below the reference range but only two stallions had a HD_{min} within the reference range on both occasions.

In the logistic regression model only WD_{min} was related to elimination from the study due to an injury or lameness. The log Odds ratio was 0.093 (95% CI 0.046; 0.14), the OR was 1.097 ($p < 0.001$).

4 Discussion

In this study, it was demonstrated that individual young Friesian horses have different changes in limb and upper body kinematics in response to a submaximal exercise test and to a longitudinal fatiguing training program. In more fatigued conditions, horses showed a higher coefficient of variation in stride characteristics and asymmetry parameters. However, variation in ROM of limbs and upper body remained constant. Additionally, 97.6% of the upper body asymmetry parameters measured were above the described reference values for owner-sound Warmblood horses measured in-hand in trot on a straight line (31), with 69% of the participating young Friesian horses having all parameters on all SETs above the reference range.

Gait parameters acquired by objective gait analysis systems can be compared using repeated measurements of the same horses over time (14). In the present study, young Friesian horses were measured twice on 1 day (before and after SET), and on three different days (SET-I, II and III) over time. Hardeman et al. (31, 32) showed that

TABLE 8 Results of HD_{min}, HD_{max}, WD_{min}, WD_{max}, PD_{min} and PD_{max} in trot (in-hand) in young Friesian stallions before and after repeated submaximal ridden standardized exercise test, related to high (>4 mmol/L) or low plasma lactate concentration.

		Main effects				Interaction effects		
Parameter	Intercept	SET-II	SET-III	After SET	Finishers	SET-II x after SET	SET-III x after SET	After SET x LA high
HD_{min} (mm)	**20.98**	2.14	11.09*	n.s.	n.s.	n.s.	n.s.	n.s.
95% CI	13.48; 28.40	−1.21; 5.49	5.90; 16.28					
% Change		10.2	52.9					
HD_{max} (mm)	**17.43**	1.04	7.95*	−4.03	4.31	7.526*	1.58	5.70*
95% CI	12.63; 22.49	−2.95; 5.04	1.49; 14.45	−8.39; 0.33	−1.69; 10.13	1.89; 13.16	−6.73; 9.90	0.064; 11.03
% change		6.0	45.6	−23.1	24.7	43.2	9.1	32.7
WD_{min} (mm)	**5.37**	3.42	1.61	0.93	n.s.	−1.82*	−1.23	0.3
95% CI	3.41; 7.25	2.17; 4.66	−0.38; 3.60	−0.43; 2.27		−3.57; −0.08	−3.79; 1.33	−1.56; 2.18
% Change		63.6	30.0	17.1		−33.9	−22.9	5.5
WD_{max} (mm)	**11.68**	2.18*	1.087	−0.11	n.s.	n.s.	n.s.	−2.45*
95% CI	8.79; 15.12	1.06; 3.30	−0.81; 2.99	−1.47; 1.25				−5.52; −0.12
% Change		18.6	9.3	−0.9				−20.9
PD_{min} (mm)	**9.14**	−1.78*	0.57	−0.60	n.s.	n.s.	n.s.	0.67*
95% CI	6.51; 11.64	−2.86; −0.70	−1.27; 2.41	−1.94; 0.74				1.26; 5.77
% Change		−19.5	6.2	−6.6				7.4
PD_{max}(mm)	**8.76**	3.79*	0.20	−0.0024	2.12	n.s.	n.s.	−2.20
95% CI	6.37; 11.09	2.63; 4.87	−1.67; 2.09	−1.39; 1.39	−0.94; 5.13			−4.47;0.046
% Change		42.8	2.3	−0.03	24.2			−25.2

SET, standardized exercise test; LA, plasma lactate concentration (mmol/L); HD_{min}, head minimal vertical displacement difference; HD_{max}, head maximal vertical displacement difference; WD_{min}, withers minimal vertical displacement difference; WD_{max}, withers maximal vertical displacement difference; PD_{min}, pelvis minimal vertical displacement difference; PD_{max}, pelvis maximal vertical displacement difference. *Indicating important difference from SET-I/before SET or LA low. n.s. indicating that this factor was excluded from the statistical model. Number of horses: SET-I *n* = 15, SET-II *n* = 11, SET-III *n* = 4.

between-horse measurements have substantially larger variation than within-horse measurements on different measurement moments, demonstrating the consistency in the locomotion pattern of individual horses. This enables repeated gait evaluation to compare the horse with itself. The larger variation in head parameters compared to withers and pelvis measurements has been shown previously (23, 31, 33). Excitement and environmental stimuli, as well as the handler of the horse can cause larger and perhaps more asymmetric head movements in horses, especially in our young stallion population. In this study, we tried to reduce these effects as much as possible by trotting the horses in a quiet surrounding where the horses were accustomed to and by using the same handler for all horses. However, some excitement could not be prevented, and results for ROM_{head} and asymmetry of the head should be interpreted cautiously.

The Friesian horses participating in the present study were pre-selected by the studbook to become an approved studbook/breeding stallion. Gait quality is one of the features in the selection process of these stallions (27), but is a subjective parameter and consists of elements such as ground cover and self-carriage which are judged by studbook judges. Stride length is seen as the gold standard to quantify ground coverage and is explained by kinematic measures of the limbs such as stance duration, swing duration and forelimb retraction and hind limb protraction angles (34–36). A larger stride length in Warmblood horses in trot is scored positively by judges (34, 37, 38). Friesian stallions completing the entire study had slightly longer strides durations (4,4%), ROM_{head} and ROM_{pelvis} compared to non-finishers. This can be explained by more expressive gaits resulting in more positive judge scores for these horses, and thus longer stay in the studbook approval program. Remarkably, over all measurements, the protraction angle of forelimbs and ROM_{protF} were lower in finishers. Thus the longer stride duration in finishers was not obtained by more protraction of the forelimbs. No other stride duration parameter was significantly different in finishing horses.

To the authors knowledge, no studies have been published yet on gait analysis in Friesian horses compared to other breeds. However, subjectively, Friesian horses have a different movement pattern compared to Warmblood or Thoroughbred horses. Rhodin et al. (39) described gait parameters of a group of 19 sound adult Warmblood riding horses and 23 healthy adult Iberian horses. Hardeman et al. (32) described gait parameters in 12 healthy adult Warmblood performance horses. When comparing stride kinematics of the Friesian stallions in the present study with the results of Hardeman et al. (32) and Rhodin et al. (39), mean stride duration (0.77 s) is comparable to reported mean of Warmblood horses (0.78 and 0.76 s), but higher than in Iberian horses (0.74 s). The expressive gait of the selected Friesian stallions seems to be represented by a longer swing duration (0.46 s fore, 0.48 hind) compared to Warmbloods (0.39 and 0.30 s fore and 0.46 s hind) and Iberian horses (0.30 s fore and hind) (39, 40). The ROM of the upper body kinematics of the young stallions were higher than published results of other breeds using the same IMU sensors. The estimate of ROM_{head} of the young Friesian horses

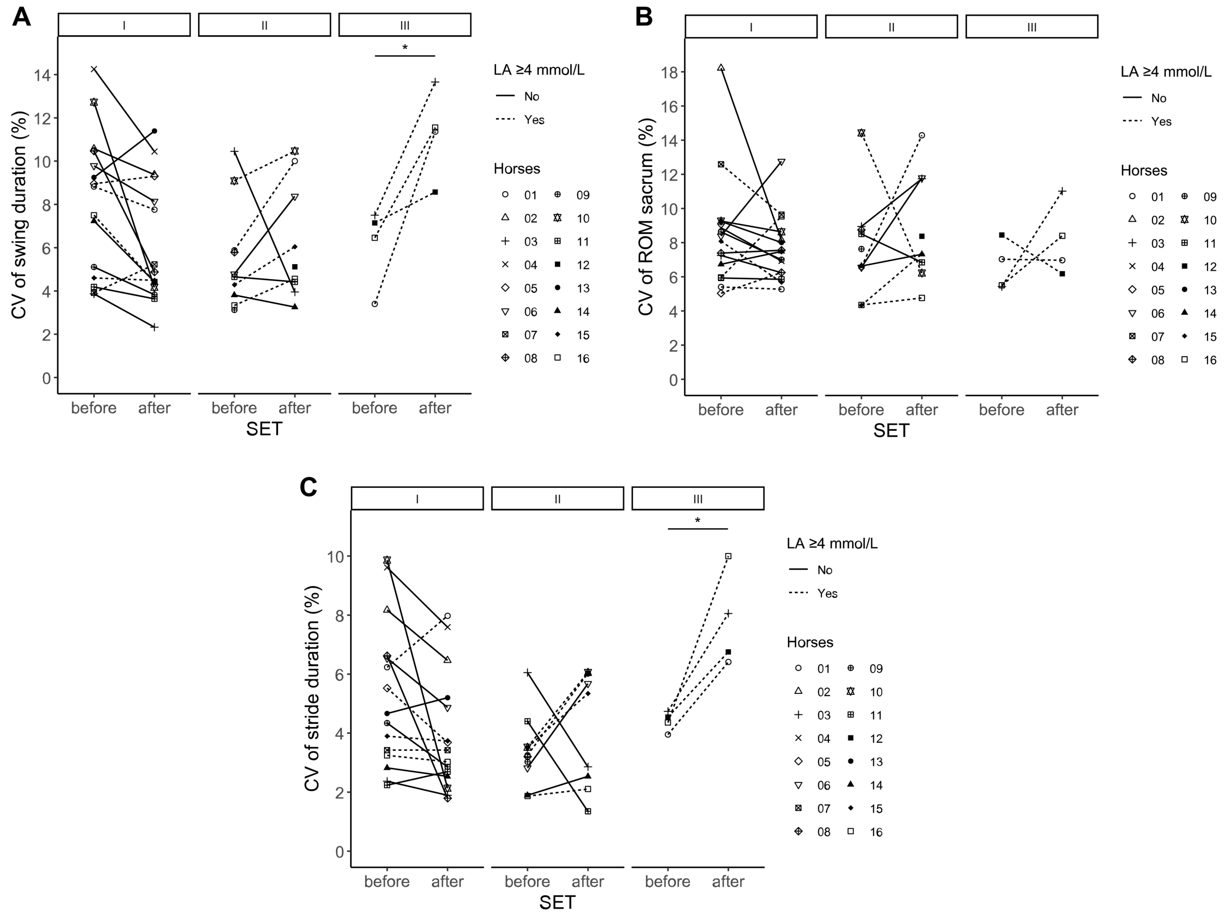

FIGURE 2
(A–C) results of mean coefficient of variation of swing duration of the hind limbs **(A)**, ROM of the plevis/sacrum **(B)** and stride duration **(C)** in trot (in-hand) in young Friesian stallions before and after repeated submaximal ridden standardized exercise test during a 10-week training program related to high (≥4 mmol/L, dotted line) or low (<4 mmol/L, solid line) plasma lactate concentration. Each marker represents the mean results per individual horse.* indicating important difference from before SET. SET, standardized exercise test; ROM, range of motion; CV, coefficient of variation (%).

was 80.73 mm (95% CI 74.98; 86.40), compared to a median of 67.21 mm (95% CI 51.99; 87.75) (32) and estimated marginal mean of 78.1 mm (95% CI 70.5–85.7) in mature Warmblood horses, and 63.7 mm (95% CI 56.8; 70.6) in mature Iberian horses (39). The $ROM_{withers}$ in the Friesian horses was even more different from Warmbloods and Iberian horses. The $ROM_{withers}$ was 29 mm and 35 mm larger in the young Friesian stallions than in Warmbloods and 55 mm larger than in Iberian horses. The ROM_{pelvis} was 3 mm and 2 mm larger in the Friesian stallions than in Warmblood horses and 28 mm larger than in Iberian horses (32, 39). The Friesian horses used in this study are an elite group as being in the selection of becoming an approved breeding stallion, and thus may not be fully representable for the entire breed. Taking that into account, the results do indicate different movements compared to adult Warmblood and Iberian horses. In the present study, ROM_{head} and ROM_{pelvis} were higher in finishers. The higher upper-body vertical ROM might be related to more expressive and higher vertical impulsion movement of the horse, and thus higher judge scores.

A ridden submaximal exercise test was used in the present study. The workload of this test is highly representative for the normal exercise for the young Friesian stallions and induced a relevant level of fatigue as measured by lactate concentrations exceeding the anaerobic threshold of 4 mmol/L during the SET in many of the participating horses (29, 30). Typically, cardiorespiratory fitness improves after several weeks of training (6, 41–43). However, HR and LA response to the SET in the present study increased markedly, implying a fatiguing training program. The workload during SETs was standardized, and the negative physiologic response suggests overreaching/overtraining of these young horses (44–46). In people, the effects of fatigue on gait parameters varies among individual athletes and is influenced by training status, running technique and field or treadmill exercise (47, 48). Back et al. (49) demonstrated individual different changes in gait kinematics in unexperienced 2.5 year-old Warmblood horses after a 10-week training protocol. Similarly, in the present study, no consistent changes in gait kinematics were found. Horses showed individually different responses in gait kinematics after SETs and in repeated SETs during a fatiguing training period. For example stride duration increased in six and decreased in nine out of 15 horses after SET-I compared to before SET-I. This implies that gait response to moderate fatigue does not follow a single pattern in young Friesian horses.

Speed could not be measured during gait analysis or SETs because the tests were performed indoors where satellite (GPS) signal was poor. In order to keep speed as constant as possible, one handler trotted all horses. Additionally, riders were asked to keep their speed as constant as possible during the SETs. In the present study, in SET-II stride duration, StanceF, StanceH, SwingF and SwingH were all lower compared to SET-I. It can therefore be suggested that speed during gait data collection in SET-II was lower compared to SET-I and SET-III. However, according to Hardeman et al. (31, 32) differences in trotting speed do not affect upper body ROM and asymmetry outcomes. Therefore, the ROM results of SET-II can be considered as valid.

The effect of acute fatigue was studied by gait analysis before and after SETs but is also influenced by medium to long term fatigue due to the fatiguing training program. Horses with LA $\geq$ 4 mmol/L reduced their stride duration after SETs by reducing swing duration, but maintaining stance duration constant. After SET-III, where all horses had LA $\geq$ 4 mmol/L, StanceF and SwingH were reduced. Darbandi et al. (23) collected gait analysis data before and after a SET from 60 horses to develop a model to detect fatigue using machine learning techniques. The 16 Friesian stallions from the current study were part of this dataset. In those 60 horses, swing duration decreased after SETs as well and was related to fatigue (23). In field conditions, Takahashi et al. (26) showed decreased speed, stride frequency and stride length in Thoroughbreds comparing the first and the second lap in a race using a stationary high-speed camera system. Horses did not lengthen their body as much as they did at the start of the race (26). It has been shown that muscle fatigue can lead to a decreased power output (9) and thus result in a shorter swing phase in horses. There are several studies in sport horses, using IMU's, evaluating the effect of fatigue on stride duration, but studies used all different methodologies leading to conflicting results among studies and making comparisons difficult (24, 50, 51). In the present study, $ROM_{withers}$ increased after SET-II and SET-III, but $ROM_{withers}$ and ROM_{pelvis} was lower after SETs in horses with LA $\geq$ 4 mmol/L. A lower SwingH could be the result of less push from the hind limbs due to muscle fatigue and can be directly associated with less motion of the withers and pelvis, but when stride length characteristics remained constant, dorsoventral motion of the withers was increased after SETs. Studies in human and equine athletes also show increased movement of the trunk: in human runners vertical lift increases in fatigued individuals (47), especially in less experienced runners (52). Additionally, peak forward trunk lean increases (48) leading to a less economical running technique. In a small study by Colborne et al. (53), dorsoventral displacement was larger under fatigued conditions than under non-fatigue conditions in Thoroughbreds on a treadmill. However, this was a small pilot study with vertical wither distance results for only 2 horses (53). A possible explanation of the increased vertical motion of the withers of the Friesian stallions in acute fatigue could be a diminished trunk stability leading to more movement of the withers.

In the parameters $ROM_{F,}$ ROM_{H}, protraction and retraction angles of fore and hind limbs and in asymmetry parameters, no consistent effect of fatigue or training was found. This is in contrast to the findings of other studies (23, 25, 26, 53), where ROM_{protF}, ROM_{protH}, and hind limb protraction/retraction angles decreased in more fatigued horses. However, three of these studies cannot be directly compared to the young Friesian stallions, since they used galloping racehorses, either on the treadmill or during a race (25, 26, 53). It is possible that horses in in-hand trot after ridden exercise respond differently to fatigue compared to racehorses measured in gallop during the fatiguing exercise.

Beside changes in absolute measurements, stride by stride variation can be related to fatigue due to adaptations of the neuromotor system (54). In a fatigued state, the nervous system lacks ability to recruit motor units adequately. This leads to a lower muscular power output, but also increased variability in movement and altered movement coordination (11). Hardeman et al. (31, 32) showed that variation reduced in repeated gait analysis measurements in horses, and individual variation was highest on the first measurement day compared to a second and third measurement day in healthy horses. In the present study, the coefficient of variation was not significantly higher during measurements before SET-I compared to SET-II and SET-III. However, the CV in stride duration increased by 54% after SETs in horses with LA $\geq$ 4 mmol/L, showing that more fatigued horses have less consistent stride durations. Upper body kinematics and limb ROM did not show a change in variation in the study, but asymmetry parameters did show more variation in more fatigued conditions. Similarly, in human athletes it has been shown that for some parameters, such as knee kinematics, variability increases, while in other parameters, for example ground reaction force, variability decreases in fatigued individuals (54, 55).

The horses in the present study showed marked upper-body asymmetries during all measurements. Reference values have been reported to evaluate what can be considered as 'normal'; however these are population and measurement system-specific and there is overlap between "owner sound" horses and horses with induced lameness (16, 33). Eleven out of 16 young Friesian (69%) stallions in the present study had no measurements within the reference values as determined for adult Warmblood horses by Hardeman et al. (31), using the same sensor system. None of the horses had results within this reference range for HD_{max}, WD_{max}, PD_{min} and PD_{max} before SETs. After SETs, none of the young Friesian horses had results within reference range for the same parameters and WD_{min}. The majority of these measurements exceeded the reference range for between day variation by more than 10% (326 out of 342 measurements) or 50% (259 out of 342 measurements) and had values associated with lameness that is visible for the human eye. This can be a potentially concerning result in a group of young and presumably healthy horses. All horses were evaluated by an experienced veterinarian before entering the study, and all horses were judged as "fit to compete." Also, horses were excluded from the study if lameness was evident as determined by trainer, rider, judge or veterinarian. Other studies in presumably healthy horses report similar high number of horses with motion asymmetries of up to 70% (16, 31, 50, 56–58). It is questionable at what threshold asymmetry becomes clinically relevant. It is unlikely that all these horses are lame due to a painful (orthopedic) condition, therefore better understanding of the relationship between pain and measured asymmetries is needed. Pfau et al. (33) suggested higher reference values for asymmetry of the head (14.5 mm) and pelvis (7.5 mm) in Thoroughbred horses in trot in-hand on a straight line. Most measurements in the young Friesian stallions still exceed these limits, however the trot of a Friesian might not be comparable to a Thoroughbred. The horses in the present study were young, have had limited training before the study and have high upper body ROM compared to other breeds. The large upper body ROM demonstrated in our study compared to other breeds might lead to higher absolute asymmetry values, underlining the importance of breed-specific reference and possibly even age-or training level-specific values for asymmetry and lameness.

In the Friesian stallions, elimination from the study was related to WD_{min}, but not to asymmetry parameters of head and pelvis. In the statistical analysis, horses with forelimb and hind limb asymmetries were combined in one group, therefore it was logical that only a relation with withers asymmetry was seen in the statistical model. We hypothesize that that more symmetric horses might have received better scores from the judges than the horses with a more asymmetric gait.

Limitations of the study include the small number of horses involved, mainly due to the selection process for Friesian stallions. Only four horses remained in the study until SET-III and became an approved studbook stallion for the Friesian Studbook. The factor that only a few horses completed the 10-week training program was statistically accommodated for with the use of the mixed effects model. Gait assessment was not conducted during the submaximal exercise tests (SETs) but before and after the SETs, and horses had a 10-min cool-down before the post-SET gait analysis, potentially affecting results. More uniform results might be obtained in the Friesian horses when gait kinematic data was collected at the peak of their fatigue. However, this would mean that horses should be measured during the ridden exercise tests and riders might influence the gait kinematics as well leading to factor contributing to the variation in the data. More intense saddle-based or treadmill tests could yield clearer results in future studies regarding investigating the effect of fatigue on locomotion parameters.

Overall, the findings in this study support the first hypothesis that stated that the fatigued young Friesian stallions would increase their step-by-step variability and gait kinematics. However, the second hypothesis, that stated that the young stallions would reduce stride length, ROM of limbs and upper body kinematics as a result of medium to long-term fatigue, could not be confirmed.

5 Conclusion

The young Friesian stallions showed individual changes in gait parameters after moderate intense exercise and during a fatiguing training program. An increased range of motion of the withers after exercise was a consistent finding. In more fatigued conditions, horses showed more variation in stride characteristics and asymmetry parameters. However, variation in ROM of limbs and upper body remained constant. Additionally, 69% of the young Friesian stallions showed higher asymmetry values in in-hand trot on a straight line compared to the published reference values for Warmblood sport horses. These high number of asymmetric horses have been described in other breeds and groups of presumably healthy horses. Thus, there is a necessity of breed-and possibly age-specific asymmetry reference values, and further evaluation of factors contributing to upper body asymmetries in healthy horses.

Data availability statement

The raw data supporting the conclusions of this article can be requested from the corresponding author.

Ethics statement

The animal studies were approved by Animal Welfare Body Utrecht, Utrecht University, The Netherlands. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

ES: Data curation, Formal analysis, Investigation, Methodology, Project administration, Validation, Visualization, Writing – original draft, Writing – review & editing. JP: Formal analysis, Investigation, Project administration, Writing – review & editing. MSO-O: Supervision, Writing – review & editing. CM: Conceptualization, Funding acquisition, Investigation, Supervision, Writing – review & editing. FS: Investigation, Software, Supervision, Validation, Writing – review & editing.

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Conflict of interest

CM was employed by Equine Integration.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2025.1456424/full#supplementary-material

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Acute whole-body vibration as a recovery strategy did not alter the content of gluteus medius monocarboxylate-transporters, lactatemia, and acidosis induced by intense exercise in horses

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Introduction: Several studies have explored alternatives to enhance the performance, health, and safety of sports horses. One promising method involves the use of vibrating platforms (VP), which offer passive exercise stimulation via mechanical oscillations distributed throughout the body. This type of exercise is referred to as whole-body vibration (WBV) and is an emerging strategy for accelerating muscle recovery. This study examined the dynamics of proteins responsible for transporting monocarboxylates (MCT1 and MCT4), and their relationship with lactatemia and acid-base balance in connection with WBV recovery following intense treadmill exercise in horses.

Methods: Eight crossbred horses underwent the standardized exercise test on the treadmill to determine the velocity corresponding to the lactate threshold. This velocity was used to prescribe the external load of the acute intense exercise bout (AIEB), which was performed to recruit rapidly fatigable type II muscle fibers and induce hyperlactatemia and metabolic acidosis. The horses were assigned to three experimental groups in a crossover design, with a 7-day washout period. The treadmill group (TG) actively recovered through low-intensity treadmill walking. The WBV group (WBVG) followed a stepwise recovery protocol on VP, with each step lasting 2 min and the frequencies decreasing in a specific order: 76, 66, 55, 46, and 32 Hz. The sham group (SG) was designated for horses with the VP turned off. All groups experienced a uniform recovery strategy duration of 10 min. Heart rate (HR), rectal temperature (RT), lactatemia, glycemia, acid-base status and electrolytes, strong ion difference (SID), and muscle monocarboxylate transporters (MCT1 and MCT4), were assessed.

Results: AIEB induced positive chronotropic effects, hyperlactatemia and moderate metabolic acidosis in all experimental groups. All groups also showed transitory hyperthermia, hyperglycemia, hypernatremia, hyperchloremia, hyperkalemia and SID reduction. HR was higher in TG than in the WBVG and SG immediately after the recovery procedures. Between the groups, there was no change in RT, lactatemia, glycemia and MCT1 and MCT4 content. Regardless of groups, the MCT4 content decreased 3 and 6 h after recovery strategies.

Discussion: It was concluded that a single whole-body vibration session did not enhance recovery of lactatemia or acid-base balance in horses after intense treadmill exercise.

KEYWORDS

acid-base balance, cool-down, exercise, heart rate, lactate, MCT1, MCT4, whole-body vibration

1 Introduction

Improving the quality and safety of athletic performance is an important scientific topic for humans and other animal species. Equestrian associations aim to enhance the athletic ability, health and wellbeing of horses involved in sports. This field encompasses various research areas, including whole-body vibration (WBV). WBV is considered a non-invasive and non-pharmacological rehabilitation and recovery strategy, which uses vibrating platforms that produce mechanical vibrations, exposing the organism to a mechanotransduction process, and has been studied in humans (1–10) and in rodents (11–15) as a training device, to improve involuntary neuromuscular activation, biomechanics, and physiological variables related to performance (16–18).

It is vital to understand that improper use of vibration therapy can lead to adverse effects in men, including skin erythema, edema, pain (19), hematuria (20), knee discomfort (21, 22), and fatigue (21). Furthermore, research has shown progressive intervertebral disc degeneration in mice (23) and worsened chronic lameness in horses after extended WBV sessions (24) and intensified thoracolumbar pain with short-term use (25). Customizing vibration parameters such as amplitude, frequency, and oscillation magnitude is essential, as individual responses vary (16).

Recent studies demonstrate the benefits of WBV in addressing obesity and enhancing physical performance. One study found that WBV decreased lactate, ammonia, and serum creatine kinase activity, while increasing blood glucose in rats subjected to swimming and WBV, indicating its potential to alleviate fatigue (11). Another study on middle-aged mice revealed that WBV improved aerobic fitness and reduced fatigue (12). Research on combining dehydroepiandrosterone supplementation with WBV in mice showed remarkable results, including enhanced exercise performance, increased serum testosterone and blood glucose, and higher liver and muscle glycogen (14). These findings further emphasize WBV's role in boosting fitness and overall wellbeing.

Isometric squat training for healthy active males on a vibrating platform effectively increases time to exhaustion and reduces neuromuscular fatigue in knee extensor muscles (8). Research indicates that combining physical exercise with whole-body vibration (WBV) lowers lactate levels, heart rate (HR), and blood pressure (2, 4, 5). However, a study on recreational runners using WBV and drop jumps as warm-ups found no significant changes in ankle dorsiflexion or running times (10). Similarly, a study on horses assessed the effects of WBV as a warm-up strategy on various physiological variables and found no significant results (26), underscoring the complexity of WBV's effectiveness in different contexts.

Methods to restore homeostasis after intense exercise, particularly in terms of body temperature recovery, hyperlactatemia, and metabolic acidosis, are gaining attention in equine studies (27–33). In the horse industry, WBV is proposed as a technique to accelerate recovery post-exercise. While manufacturers endorse this method (34), its use in equestrian centers often lacks scientific evidence, at least to our knowledge. We hypothesize that WBV enhances lactate clearance through monocarboxylate transporters, facilitating the transport of L-lactate across the sarcolemma and restoring acid-base balance after vigorous exertion. It should be highlighted that this paper seeks to improve the abilities of veterinarians, researchers, and horse owners in maintaining horses' health and wellbeing by bridging the gap between experimental research on WBV and real-world applications.

2 Materials and methods

The study was approved by the Ethics Committee on the Use of Animals (CEUA) of the School of Agricultural and Veterinarian Sciences UNESP-Jaboticabal Campus (protocol no. 08373/19) and carried out during October and November 2022 at GPS coordinates-21.245408 latitude and−48.299415 longitude.

Abbreviations: VP, vibrating platform; WBV, whole-body vibration; MCT1, monocarboxylate transporter 1; MCT4, monocarboxylate transporter 4; AIEB, acute intense exercise bout; TG, treadmill group; WBVG, whole-body vibration group; SG, sham group; HR, heart rate; RT, rectal temperature; SID, strong ion difference; CEUA, Ethics Committee on the Use of Animals; VLT, velocity corresponding to the lactate threshold; SET, standardized exercise test; VLT_V, velocity corresponding to the visual lactate threshold; VLT_{BI}, velocity corresponding to the bi-segmented lactate threshold; A, after acute intense exercise bout; BR, before recovery; AR, after recovery; EDTA, Ethylenediaminetetraacetic Acid; P_VCO_2, pressure of carbon dioxide; tCO_2, total carbon dioxide; BE_{ecf}, base excess/deficit; AnGap, anion gap; HCO_3^-, bicarbonate; Na^+, sodium ion; K^+, potassium ion; Cl^-, chloride ion; Hb, total hemoglobin; Hct, hematocrit; Ure, urea nitrogen; bpm, beats per minute; AUC, area under the curve.

2.1 Horses

Eight crossbreed horses were used (one gelding and seven females), with an average body mass of 406 ± 33 kg and aged between 7 and 19 years. The horses belonged to the didactic herd of the Equine Exercise Physiology and Pharmacology Laboratory (LAFEQ), Department of Animal Morphology and Physiology, School of Agricultural and Veterinarian Sciences, São Paulo State University (FCAV/UNESP), Jaboticabal, São Paulo, Brazil. The horses were included in the study after ∼2 months of adaptation to the experimental conditions, such as handling and feeding, treadmill (Galloper G 5500, Sahinco, Palmital, SP) and vibrating platform (TheraPlate®, Weatherford, TX, USA). The horses were kept in paddocks and fed with Tifton 85 hay, in addition to 0.2% of body mass in concentrate once a day. Mineralized salt and water were provided *ad libitum*. They were regularly hoofed, and, during the experimental period, they did not use horseshoes. Before the start of the experimental stages, the horses underwent clinical and hematological examinations to determine their healthiness and were previously vaccinated and treated with anthelmintics.

2.2 Determination of the velocity corresponding to the lactate threshold (VLT)

A standardized exercise test (SET) was conducted on all horses to determine the velocity corresponding to the lactate threshold (VLT), which was used to prescribe the external load for an acute intense exercise bout (AIEB). The SET protocol was adapted from Lamprecht and Williams (35) and performed on a treadmill. The warming-up consisted of 3 min of walking at a speed of 1.5 m/s with a surface inclination of 0%, followed by 2 min of trotting at 2.5 m/s and 5% positive slope. The SET started at 4.0 m/s, with the speed increasing by 1.0 m/s at 2-min intervals. The horses underwent a 2-min active recovery period at 1.5 m/s between each speed step. A surface inclination of 5% was maintained throughout the incremental phase. To ensure the horses' safety, the SET concluded when the horses reached a heart rate of 200 bpm or at the end of the speed stage of 9 m/s. At the end of the SET, the horses cooled down by walking at a comfortable speed for everyone, with an inclination of 0% (Supplementary Table S1).

Two complementary methods were used to determine the velocities corresponding to the lactate threshold: visual (VLT_V) and bi-segmented (VLT_{BI}). During the SET, the inflection point of the lactate-velocity curve was determined by analyzing the plasma lactate concentrations of each horse, indicating a non-linear increase. Four exercise physiology experts established this point. They then associated this point with the corresponding speed (x-*axis*) to establish the velocity corresponding to the visual lactate threshold (VLT_V). This method was refined by applying bi-segmented linear regression (VLT_{BI}). Two regression lines from the ordinary least squares regression were used, and the VLT_V obtained was considered. By equating the values of the two equations related to the y-values, the speed (x) was obtained. To ensure that the external load of AIEB would recruit rapidly fatigable type II muscle fibers and induce physiologic hyperlactatemia and metabolic acidosis, the highest speed obtained between VLT_V and VLT_{BI} for everyone was used, and the values are presented in Supplementary Table S2.

2.3 Acute intense exercise bout (AIEB)

All horses performed AIEB with an external load above the VLT. Initially, the horses were subjected to a warming-up period of 1 min at a speed of 1.5 m/s and 0% inclination, followed by 2 min at a speed of 3.5 m/s and 2 min at VLT, both with 5% inclination. To intensify the external load, aiming to induce hyperlactatemia and metabolic acidosis, the AIEB was completed in the gallop, with 2 min at 110% of the VLT and 3 min at 130% of the VLT, with an inclination of 5%. Once the AIEB was finished, the recovery phase commenced to assess the suggested strategies for recovery.

2.4 Experimental groups

Before the experiment began, the horses were randomly assigned into three experimental groups, in a crossover design, each specifically designed to evaluate a different type of recovery after AIEB. All horses participated in all three experimental groups, carried out in three blocks. The randomization process involved numbering the animals from 1 to 8, followed by a drawing to create the experimental blocks (1, 2, and 3) using an Excel spreadsheet with the formula =RANDBETWEEN(1;8). All strategies for recovery were meticulously planned and executed, each lasting 10 min. The treadmill group (TG) performed the active recovery period on the treadmill at a comfortable speed for each horse (between 1.2 and 1.6 m/s). The horses in the sham group (SG) remained on the turned-off vibrating platform, providing a control group for the experiment. The whole-body vibration group (WBVG) performed one WBV session during recovery. The horses in the SG and WBVG groups began their recovery roughly 5 min after the end of AIEB. For the TG group, the horses waited 5 min on the treadmill before the active recovery period began, further ensuring the scientific rigor of the experiment.

2.5 Acute whole-body vibration (WBV) session

The horses in the WBVG group underwent a WBV session after AIEB, using the Theraplate® (Original Equine Unit Model K21, Theraplate, Weatherford, TX, USA). This VP, equipped with a unique "proprietary technology" called vortex wave stimulation, utilizes centrifugal force and internal oscillating movement to provide zero-impact therapy. The horses were maintained in a quadrupedal position on the VP. The WBV recovery protocol, developed according to the manufacturer's guidelines from TheraPlate (TPR, LLC), was implemented for horse recovery following AIEB. This protocol lasted 10 min and was designed to gradually decrease the frequency levels. It included 2 min at each load corresponding to 100%, 80%, 60%, 40%, and 20% of the equipment's maximum motor capacity, which correspond to

76 Hz [peak displacement (Dpeak) = 0.09 mm; peak acceleration (Apeak) = 10.36 m/s^2], 66 Hz (Dpeak = 0.12 mm; Apeak = 9.96 m/s^2), 55 Hz (Dpeak = 0.16 mm; Apeak = 9.46 m/s^2), 46 Hz (Dpeak = 0.22 mm; Apeak = 9.12 m/s^2), and 32 Hz (Dpeak = 0.51 mm; Apeak = 10.35 m/s^2), respectively. The frequency and peak acceleration were measured using an accelerometer (App Accelerometer meter). The peak displacement was calculated using the formula:

$$Dpeak = \left[\frac{Apeak}{2 \times \pi^2 \times frequency^2}\right] \times 1000. \quad (1)$$

2.6 Assessment methods

2.6.1 Heart rate (HR)

HR was measured using a Polar Equine Heart Rate Monitor for Trotters (Polar Electro, Kempele, Finland). The peak HR for each stage was extracted from the Polar Flow® app (https://flow.polar.com/). The data were collected before (baseline) and after (A) AIEB, before (BR) and after (AR) recovery, and 10 min at the end of the recovery period (10 min).

2.6.2 Rectal temperature (RT)

A digital predictive thermometer (Clean View RM-TD0403A, Relaxmedic, Vargem Grande Paulista, Brazil) was used to measure RT. Temperature readings were taken at three time points: baseline, A, and AR.

2.6.3 Lactatemia and glycemia

Blood samples were collected through jugular vein catheterization in tubes containing sodium fluoride and EDTA to determine the plasma concentration of lactate and glucose. The samples were centrifuged, and the plasma was separated and immediately analyzed. The analyses were carried out using the electroenzymatic method with an automatic bioanalyzer (YSI 2300 Stat Plus®, Ohio-USA). Samples were obtained at the following time points: baseline, A, BR, AR, 10 min, and 1 h after the end of the recovery period.

2.6.4 Blood gases and electrolytes

Blood was collected through jugular vein catheterization to analyze blood gases and electrolytes, avoiding contact with environmental oxygen and carbon dioxide, in 3.0 mL blood gas syringes. Samples were collected at three-time points: baseline, A, and AR. Analyses were performed immediately after obtaining the samples using a portable analyzer (i-STAT Analyzer, Abbott Laboratories, Libertyville Township, IL, USA), cartridge EC8+, which contains tests for electrolytes, chemistries, blood gases, hematocrit, and hemoglobin. The variables measured were pH, venous partial pressure of carbon dioxide (P_VCO_2), total carbon dioxide (tCO_2), base excess/deficit (BE_{ecf}), anion gap (AnGap), bicarbonate (HCO_3^-), sodium ion (Na^+), potassium ion (K^+), chloride ion (Cl^-), total hemoglobin (Hb), hematocrit (Hct) and urea nitrogen (Ure). pH and P_VCO_2 values were adjusted for rectal temperature (36, 37). The strong ion difference (SID) is calculated as the difference between the total concentration of strong cations and the total concentration of strong anions, using the formula:

$$SID = \left(\left[Na^+\right] + \left[K^+\right]\right) - \left(\left[Cl^-\right] + \left[Lac^-\right]\right). \quad (2)$$

2.6.5 Muscle samples

The horses were subjected to muscle biopsies at the following time points: baseline (around 18 h before AIEB), AR, 3 and 6 h after recovery, as described previously (38). The horses were restrained in a quadrupedal position on a horse stock before biopsy sampling. The needle insertion site in the gluteus medius (gluteal) was in the middle third between the coxal tuberosity and the tailhead. Collections were alternated between the right and left sides of the gluteus medius muscles. The sample collection site was shaved and cleaned with chlorhexidine before being rinsed with 70% ethanol. A local anesthetic block was then applied with subcutaneous infiltration of 3 mL of 2% lidocaine hydrochloride without vasoconstrictor. After 5 min, an incision was made in the skin, subcutaneous tissue and gluteal fascia at the needle insertion site using a sterile disposable #24 scalpel blade. Next, the sterile 6.0-mm Bergström-type needle was introduced into the previously made incision to a depth of 60 mm at a 90° angle to obtain the muscle fragments. The muscle samples were immediately snap-frozen in liquid nitrogen and later stored in a −80°C freezer until the analysis.

2.6.5.1 Biomolecular analyzes of monocarboxylate transporters 1 (MCT1) and 4 (MCT4)

The analyses were performed based on a previous study in mice (39).

2.6.5.1.1 Extraction and quantification of total proteins

The samples (25 mg) were homogenized, through maceration, in 300 µL of RIPA buffer with the following components: 1% Tris HCl (50 mM), NaCl (150 mM), EDTA (1 mM), IGEPAL® CA-630 (1%), Deoxycholate (0.5%), SDS (0.1%), 1% protease inhibitor (Protease and Phosphatase Inhibitor Cocktail, cat# P8340, Sigma Aldrich®) and 1% phosphatase inhibitor (Phosphatase II Inhibitor Cocktail Set, cat# US1524625-1SET, Calbiochem®, San Diego, CA, USA), ensuring the highest quality of protein preservation. Then, the samples were placed in a sonicator (Q55 Sonicator, Qsonica®, Newtown, CT, USA) twice for 5 s (60%). Subsequently, the lysates were centrifuged at 12,000 rpm for 10 min at 4°C (Centrifuge 5424 R, Eppendorf AG, Hamburg, Germany). Soon after, the supernatant was separated (total protein extract), and the pellet was discarded. The Bradford colorimetric method was used to quantify total proteins. The absorbance was read at a wavelength of 595 nm using a spectrophotometer.

2.6.5.1.2 Electrophoresis

The samples (40 µg of total protein) were mixed with LDS buffer (lithium dodecyl sulfate and 1% mercaptoethanol) and taken to a dry bath at 94°C for 10 min. Then, the samples were placed on ice for 5 min. Next, 9 µL of each sample was pipetted into the gel wells (Mini Protean TGX Precast Protein Gels, 10% 15-well, Bio-Rad®), and then 7.5 μL of weight molecular marker was pipetted in the first well of the gel. Electrophoresis was performed using specific equipment (PowerPac 300 Electrophoresis Power Supply—Bio Rad, São Paulo, SP, Brazil) at a constant 130 V for ~60 min. An iBlot™ 2 Gel Dry Transfer Device (20 V for 7 min cat# IB21001, Waltham, MA, USA) was used to transfer proteins (PVDF) to the membrane (Invitrogen™, iBlot™ 2 Transfer Stacks cat# IB # IB24002, Waltham, MA, USA).

2.6.5.1.3 Western blotting of MCT1 and MCT4 proteins

Membranes were subjected to fluorescence staining (RevertTM total protein stain, cat # 926-11010) and scanned using the 700 nm channel of the LI-COR Odyssey Fc imaging system. Afterwards, blocking was performed in 1% milk (skimmed milk powder, cat# 9999, Cell Signaling Technology®, Danvers, MA, USA) in PBS (10 mL of PBS, 0.1 g of NFDM milk) for 1 h on an orbital shaker. Then, the membranes were immediately incubated with 5% milk in PBS-Tween for 1 h at room temperature (for MCT4 antibody, cat #BS-2698R, Bioss, Woburn, MA, USA) or overnight at 4°C (for MCT1 antibody, cat #20139-I-AP, Proteintech, Rosemont, IL, USA). Antibody binding was detected by preabsorbed goat anti-rabbit IgG H&L (cat. #ab216773) (IRDye 800CW) at a dilution of 1:20,000 for 1 h at room temperature in the dark. Fluorescence was detected at 800 nm using the same imaging system (Odyssey Fc, LI-COR Biosciences, Lincoln, NE, USA). For accurate results, protein expression was normalized by dividing the antibody signal by the band normalization factor.

2.7 Statistical analysis

The experiment was designed with a 3 × 4 factorial design, with 3 treatments (TG, WBVG, SG) and 4 timepoints (baseline, AR, 3 h, 6 h). It included three paired replications and a split-plot scheme. We used a paired randomized crossover design, allowing horses to experience three recovery strategies, each followed by a vital 7-day washout period. Muscle biopsy assessments were used for the proposed model. Statistical analysis was conducted using RStudio software for Windows (version 2023.06.0+421 "Mountain Hydrangea"), which uses the format "function {package}" to present functions and packages. Normality of residuals was assessed using the Shapiro-Wilk test (shapiro_test {rstatix}) and QQ graphs (qqplot {stats}). The Levene test (leveneTest {car}), considered to be more robust against potential deviations from normality, was applied to evaluate the homoscedasticity of variances. A two way ANOVA for repeated measures (anova_test {rstatix}) and paired *t*-tests with Bonferroni correction (pairwise_*t*_test {rstatix} with argument "p.adjust.method = bonferroni") were used for statistical analysis. To evaluate potential improvements in aerobic fitness throughout the experiment, we assessed aerobic fitness between blocks (periods) using one way ANOVA for repeated measures (anova_test {rstatix}), followed by *post-hoc* paired *t*-tests. We applied the Bonferroni correction (pairwise_*t*_test {rstatix} with argument "p.adjust.method = bonferroni") to reduce the risk of false-positive results (type I error) in our heart rate and plasma lactate measurements. The use of Bonferroni correction is warranted due to the large number of comparisons made after each ANOVA. This method ensures that the significance level remains consistent across all comparisons (40). The area under the curve (AUC) (auc {MESS}) for plasma lactate, MCT1 and MCT4 was determined using all samples obtained by the trapezoidal method for numerical integration (41). The presence of linear relationships between the variables was evaluated by calculating the Pearson correlation coefficient (r) (cor {stats}). Based on the value obtained, the following degrees of correlation were defined as $r = 0$, absence of correlation; $r < \pm 0.29$, weak correlation; $\pm 0.3 < r < \pm 0.49$, moderate correlation; $\pm 0.5 < r < \pm 0.79$, strong correlation; $r > \pm 0.8$, very strong correlation. We used a significant level of 5% for all analysis.

3 Results

3.1 Aerobic conditioning between experimental blocks

Aiming to verify a possible increase in aerobic fitness throughout the experiment, the variables heart rate and plasma lactate were checked, right after the end of AIEB, and there was no difference between blocks ($P = 0.114$, for HR and $P = 0.36$, for lactate), indicating that there was no gain in physical conditioning throughout the experimental trial course (Figure 1; Supplementary Table S3). It is worth noting that all horses used in the current study were previously conditioned. No horses were interrupted during the test attributable to fatigue, as indicated by their ability to maintain speed on the treadmill and preserve motor coordination.

3.2 Heart rate

The HR average values are shown in Supplementary Table S4. As expected, AIEB induced positive chronotropic responses in all experimental groups. After implementing recovery strategies, the HR was higher ($P = 0.001$) in the TG compared with the other groups (Figure 2).

3.3 Rectal temperature

The RT of the animals in all experimental groups increased after AIEB ($P = 0.037$; Supplementary Table S5). There was no difference between groups at any time point ($P = 0.876$; Figure 3; Supplementary Table S5).

3.4 Lactatemia and glycemia

Although there was a trend toward an increase in lactatemia ($P = 0.08$) for SG, there was clearly physiological hyperlactatemia in all groups immediately after AIEB, which decreased over time, regardless of recovery strategy (Figure 4A; Supplementary Table S6). It should be emphasized that there is no difference between groups at any time for glycemia ($P = 0.488$) and

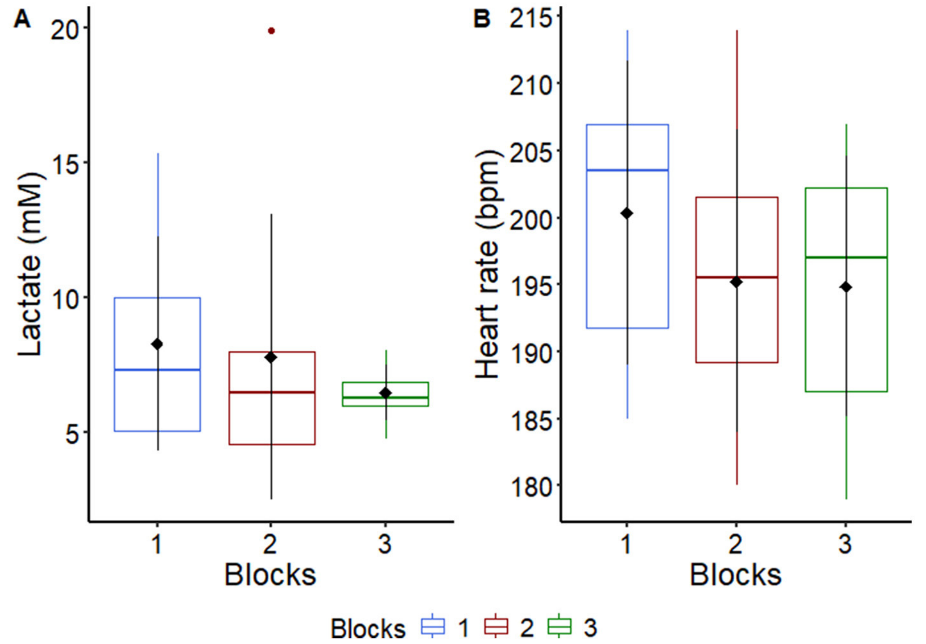

FIGURE 1
Graphical representation of median, interquartile range and means ± standard deviation (pointrange) of **(A)** plasma lactate and **(B)** heart rate of horses ($n = 8$) after an acute intense exercise bout in each experimental block. For more details see Supplementary Table S3.

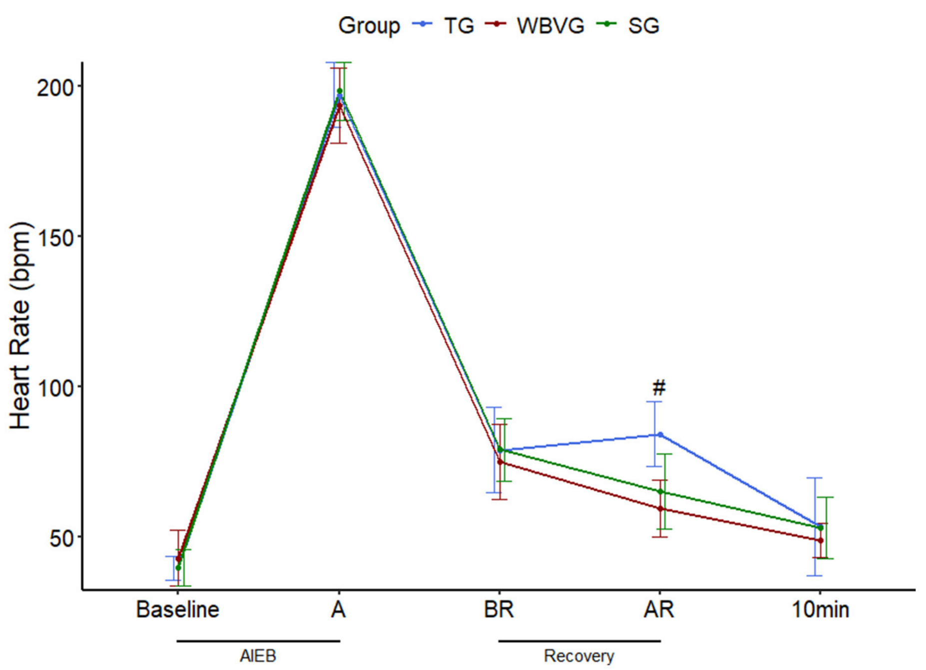

FIGURE 2
Graphical representation of means ± standard deviation of heart rate of horses ($n = 8$) submitted to an acute intense exercise bout (AIEB) and a 10-min recovery period on the treadmill (TG), on a whole-body vibration session (WBVG), or the vibrating platform off (SG). # Indicates that HR was higher in TG concerning SG and WBVG at a significance level of $P < 0.05$. A, after AIEB; BR, before recovery; AR, after recovery; 10 min, 10 min after the end of the recovery period. For more details see Supplementary Table S4.

lactatemia ($P = 0.352$; Figure 4; Supplementary Table S6). Plasma glucose peaked five (BR) to 15 min (AR) after AIEB, returning to baseline values 1h after all recovery strategies (Figure 4B; Supplementary Table S6).

3.5 Blood gases and electrolytes

Except for $PvCO_2$ and Ure, all blood gas variables showed significant changes after AIEB for all experimental groups,

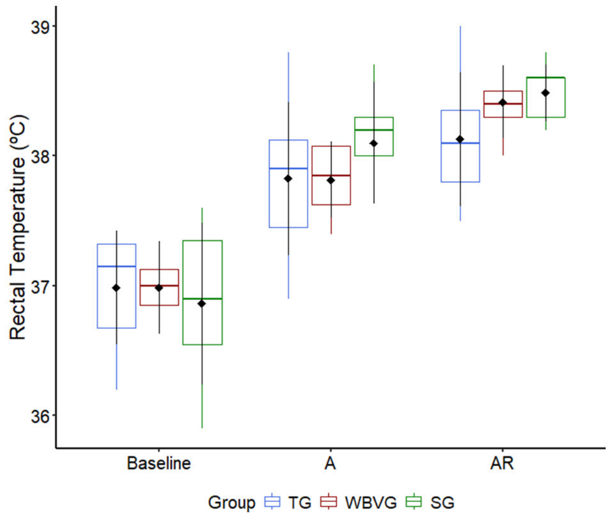

FIGURE 3
Graphical representation of median, interquartile range and means ± standard deviation (pointrange) of the rectal temperature of horses (*n* = 8) submitted to an acute intense exercise bout (AIEB) and a 10-min recovery period on the treadmill (TG), on a whole-body vibration session (WBVG), or the vibrating platform off (SG). A, after AIEB; BR, before recovery; AR, after recovery. For more details see Supplementary Table S5.

indicating metabolic acidosis (Table 1). Between the groups, a higher Ure was identified in the WBVG in relation to the TG at baseline ($P = 0.01$). It is worth noting that the average values of Ure remained within the reference range for the equine species (42).

3.6 Biomolecular analyzes of monocarboxylate transporters 1 (MCT1) and 4 (MCT4)

There was no difference between groups ($P = 0.705$) or overtime ($P = 0.485$) for MCT1 (Figure 5A; Supplementary Table S7). No difference between groups at any time ($P = 0.298$) was detected for MCT4. There was a decrease in MCT4 protein content 3 and 6 h after recovery for all experimental groups ($P = 0.007$; Figure 5B; Supplementary Table S7).

3.7 Area under the curve and correlation of lactate, MCT1 and MCT4

None of the AUCs showed any differences between groups for any variable (Figure 6; Supplementary Table S8). Likewise, no correlation was significant (Figure 7; Supplementary Table S8).

4 Discussion

The importance of researching WBV's role in aiding horses to regain internal homeostasis should be emphasized. The current study is paving the way to identify rational therapeutic targets for WBV in horses. Contrary to our hypothesis, the results obtained regarding recovery from exercise-induced hyperlactatemia and acidosis using the WBV were not different from those horses walking on the treadmill or those of the horses placed on the VP turned off. Also, this research sheds light on the relationship between WBV, horses, and muscle lactate recovery after intense exercise. It is also important to highlight that the application of WBV was considered safe, and no adverse effects were observed throughout the current study.

The existing literature on WBV in horses has primarily focused on the effects of WBV on clinical and blood parameters after a single session (43–45), neuromuscular activation (26), use as a warming-up exercise (26), its impact on lameness (24, 44), symmetry and muscle area (25, 46), hoof growth (47), stride length (44, 45), bone mineral content (45), postural stability (25), and thoracolumbar pain (25). No studies in horses have delved into the effects of an acute WBV session as a method to stimulate the transfer of lactate between white, glycolytic muscle fibers, which produce lactate, and red, oxidative muscle fibers, which consume lactate, within the working muscle (48) and a decrease in its concentration from the bloodstream after a high-intensity exercise session, which could help improve the body's acid-base balance (49).

The exercise bout was performed above the individual lactate threshold intensity, leading to increased muscular lactate production, inducing hyperlactatemia. One important performance-related marker is the lactate threshold, which correlates strongly with endurance performance and has proven sensitive to different acute exercise prescriptions. It characterizes the boundary between the heavy and severe exercise intensity domains, representing the threshold at which fast-twitch type II muscle fibers are more activated and the glycolytic pathway hyperactivate lactate production (50). This approach may provide a standard design for further studies focusing on the relationship between whole-body vibration and the acceleration of lactatemia and acid-base status recovery in exercising horses.

The use of WBV has increased in the equestrian industry to improve the performance and health of horses. However, little published research supports its use, and it is unclear what vibration magnitude is required for noticeable changes. The studies have shown mixed results, with some studies showing beneficial effects, others showing no effect and others showing adverse effects. The varying results are caused by differences in study protocols and designs and a lack of standardization in the physical aspects of the device, such as frequency, amplitude, and oscillation magnitude (18, 26, 51). In most studies involving horses, crucial details about vibratory intervention are often missing, such as the type and direction of vibration, the actual frequency, and the extent of vibration (displacement). This lack of information makes it difficult to compare data. However, these parameters are essential for evaluating vibratory intervention (51, 52).

During intense exercise, increased sympathetic activity in the nucleus tractus solitaries leads to elevated secretion of adrenal hormones (53). From an integrative perspective, the AIEB promoted transient adjustment in the homeostasis of the physiological variables studied herein, such as hyperlactatemia, mild acidosis, hyperglycemia, positive cardiac chronotropic, and elevated body temperature across the three recovery strategies

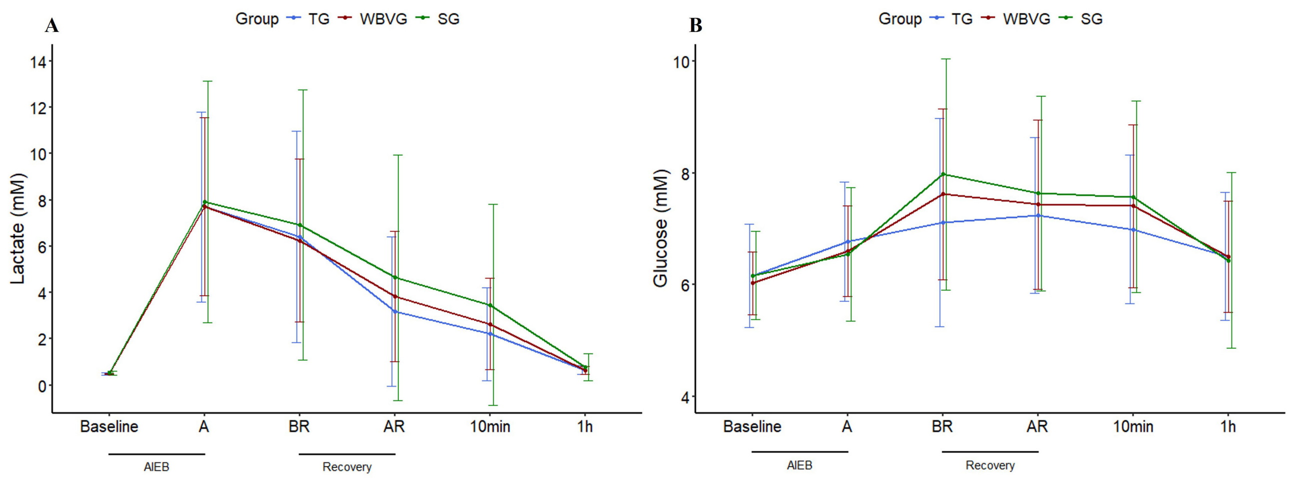

FIGURE 4
Graphical representation of means ± standard deviation of **(A)** plasma lactate and **(B)** plasma glucose of horses ($n = 8$) submitted to an acute intense exercise bout (AIEB) and a 10-min recovery period on the treadmill (TG), on a whole-body vibration session (WBVG), or the vibrating platform off (SG). A, after AIEB; BR, before recovery; AR, after recovery; 10 min, 10 min after the end of the recovery period. For more details see Supplementary Table S6.

TABLE 1 Means ± standard deviation of blood gases and electrolytes of horses submitted to an acute intense exercise bout (AIEB) and a 10-minute recovery period on the treadmill (TG), on a whole-body vibration session (WBVG), or the vibrating platform off (SG).

Variable	TG			WBVG			SG		
	Baseline	A	AR	Baseline	A	AR	Baseline	A	AR
pH	7.43 ± 0.02[a]	7.33 ± 0.06[b]	7.41 ± 0.04[c]	7.42 ± 0.02[a]	7.33 ± 0.07[b]	7.38 ± 0.04[c]	7.42 ± 0.02[a]	7.32 ± 0.08[b]	7.36 ± 0.08[c]
$PvCO_2$	43.1 ± 2.5	45.5 ± 4.4	39.1 ± 3.9	44.8 ± 2.8	44.2 ± 4.8	42.8 ± 4.1	42.4 ± 3.8	43.7 ± 3.9	42.8 ± 5.7
tCO_2	29.6 ± 2.1[a]	25.0 ± 2.4[b]	25.2 ± 4.5[b]	30.6 ± 1.9[a]	24.0 ± 3.1[b]	26.2 ± 3.5[b]	29.0 ± 3.0[a]	23.8 ± 4.2[b]	25.4 ± 5.5[b]
BE_{ecf}	3.88 ± 2.03[a]	− 1.88 ± 3.27[b]	− 0.38 ± 5.15[c]	5.12 ± 1.88[a]	− 2.62 ± 3.66[b]	0.63 ± 3.78[c]	3.25 ± 2.82[a]	− 3.00 ± 5.18[b]	− 1.12 ± 6.49[c]
AnGap	12.1 ± 1.5[a]	17.1 ± 2.6[b]	16.0 ± 3.3[c]	11.4 ± 1.2[a]	17.9 ± 3.6[b]	14.5 ± 2.8[c]	11.3 ± 1.9[a]	16.5 ± 2.3[b]	15.6 ± 4.5[c]
HCO_3^-	28.4 ± 1.9[a]	23.8 ± 2.5[b]	24.2 ± 4.6[c]	29.4 ± 1.8[a]	22.9 ± 3.0[b]	25.2 ± 3.3[c]	27.8 ± 2.8[a]	22.6 ± 4.1[b]	24.1 ± 5.3[c]
Na^+	139 ± 1[a]	141 ± 2[b]	139 ± 3[a]	138 ± 1[a]	140 ± 2[b]	137 ± 1[a]	138 ± 1[a]	140 ± 2[b]	138 ± 1[a]
K^+	3.59 ± 0.23[a]	4.90 ± 0.67[b]	3.80 ± 0.43[c]	3.58 ± 0.21[a]	5.05 ± 0.48[b]	3.69 ± 0.17[c]	3.62 ± 0.14[a]	5.09 ± 0.32[b]	3.76 ± 0.18[c]
Cl^-	102 ± 1[a]	105 ± 2[b]	103 ± 4[a]	101 ± 2[a]	105 ± 2[b]	101 ± 2[a]	102 ± 2[a]	106 ± 3[b]	102 ± 2[a]
Hb	11.6 ± 0.8[a]	16.9 ± 1.5[b]	12.6 ± 1.3[c]	11.6 ± 0.6[a]	17.1 ± 1.7[b]	12.9 ± 1.3[c]	11.5 ± 0.9[a]	17.4 ± 1.3[b]	13.2 ± 1.3[c]
Hct	34.1 ± 2.2[a]	49.6 ± 4.2[b]	37.1 ± 3.8[c]	34.2 ± 1.8[a]	50.2 ± 5.1[b]	38.0 ± 3.8[c]	34.1 ± 2.5[a]	51.2 ± 3.8[b]	39.0 ± 3.9[c]
Ure	17.6 ± 2.6[Aa]	18.5 ± 2.7[b]	17.2 ± 2.7[ab]	19.6 ± 1.7[B]	19.9 ± 2.7	19.1 ± 2.6	17.8 ± 2.6[AB]	18.8 ± 2.3	18.1 ± 2.4
SID	40.3 ± 1.8[a]	33.3 ± 4.3[b]	37.1 ± 4.5[c]	40.3 ± 1.7[a]	32.8 ± 3.5[b]	35.9 ± 3.5[c]	39.2 ± 1.2[a]	31.9 ± 5.1[b]	34.9 ± 6.0[c]

A, after AIEB. AR, after recovery. $PvCO_2$, venous partial pressure of carbon dioxide, in mmHg. tCO_2, total carbon dioxide, in mM. BE_{ecf}, base excess/deficit, in mM. AnGap, Anion gap, in mM. HCO_3^-, bicarbonate, in mM. Na^+, sodium ion, in mEq/L. K^+, potassium ion, in mEq/L. Cl^-, chloride ion, in mM. Hb, total hemoglobin, in g/dL. Hct, hematocrit, in %. Ure, blood urea nitrogen, in mg/dL. SID, strong ion difference, in mM. A, after AIEB; AR, after recovery.
Different lowercase letters indicate differences in the intragroup comparison at $P < 0.05$.
Different capital letters indicate differences in the intergroup comparison at $P < 0.05$.

trialed. Studies in humans have shown lower lactate concentrations in individuals who cooled down with WBV (2, 4, 5), indicating that WBV can lead to more efficient recovery from exercise attributable to continuous stimulation of blood vessels in the muscle. This stimulus could increase the use of lactate as energy for activated cardiac muscle and skeletal muscles and cause faster distribution of lactate to the liver (2). The findings indicated no difference between recovery strategies, demonstrating that the WBV protocol was ineffective in enhancing muscle and plasma lactate clearance.

Another way to accelerate lactate clearance would be via lactate transporters (MCTs). This mechanism would assist in a reduction of the plasma lactate concentration, which has been speculated to help the lactate outflow from type II muscle fibers (54). WBV also did not modify the MCT1 and MCT4 protein gluteus medius content, mainly expressed in oxidative and glycolytic fibers, respectively. It should be noted that this mechanism could help lactate exchanges between white-glycolytic and red-oxidative fibers and extracellular spaces (48, 55). Although muscle contractile

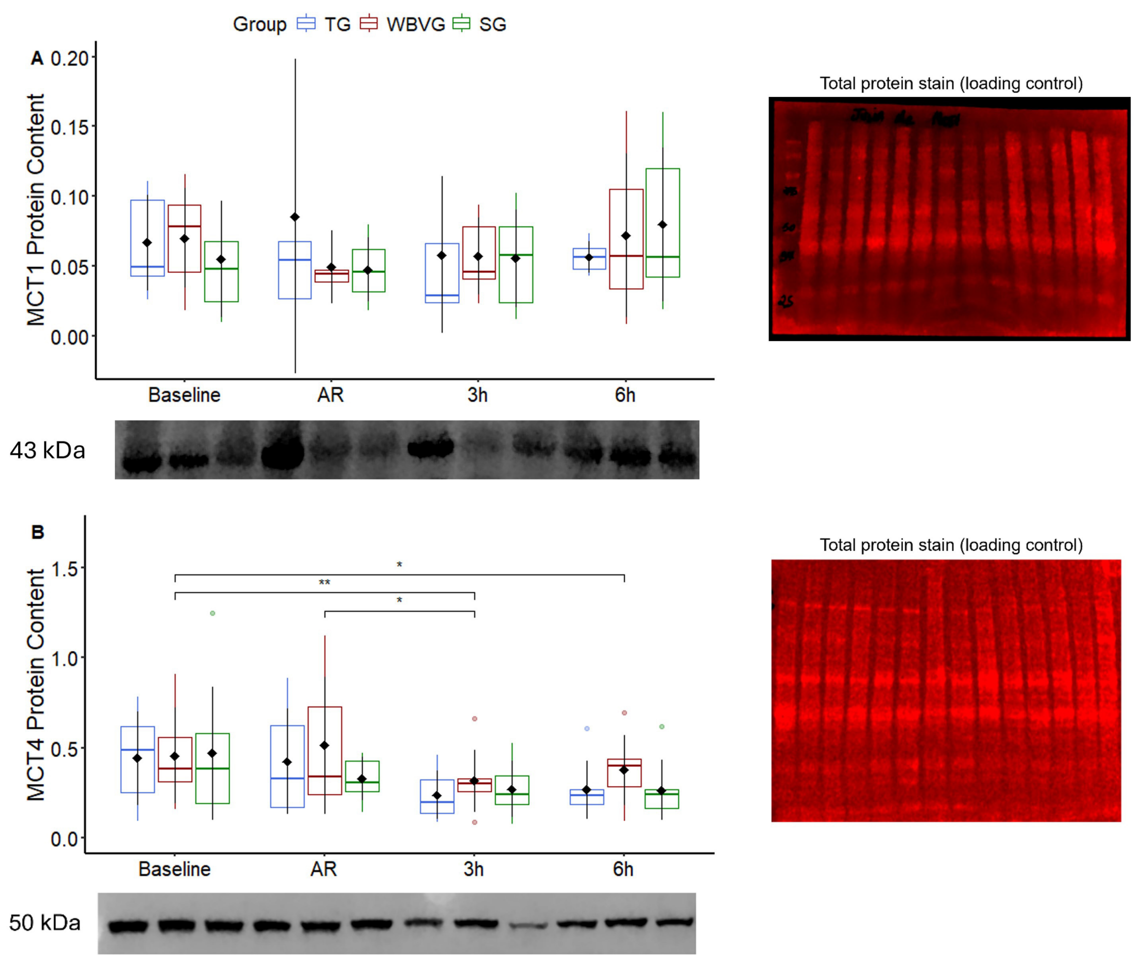

FIGURE 5
Graphical representation of median, interquartile range and means ± standard deviation (pointrange) of the **(A)** MCT1 protein content and **(B)** MCT4 protein content in the gluteal muscle of horses ($n = 8$) submitted to an acute intense exercise bout (AIEB) and a recovery period on the treadmill (TG), on a whole-body vibration session (WBVG), or on the vibrating platform off (SG). Representative images of the membranes with protein bands are shown. All membranes were stained for total protein to normalize differences between protein labeling bands. *Indicates difference between moments ($P < 0.05$). **Indicates difference between moments ($P < 0.01$). A, after AIEB; BR, before recovery; AR, after recovery; 3 h, 3 h after the end of recovery; 6 h, 6 h after the end of recovery.

activities can stimulate the gene and protein expression of MCTs in horses (56), a few studies have examined the effects of a single aerobic/anaerobic burst on MCTs (38). As traditionally reported, these authors found that both MCT1 and MCT4 increased following a single bout of maximal incremental exercise test. Aside from horses, this finding has already been described in rodents (57, 58) and humans (59). WBV did not alter lactate shutting through monocarboxylate transporters. Unexpectedly, MCT4 content decreased after AIEB in all recovery scenarios. Similarly, a human study reported that high-intensity exercise acutely decreased the amount of MCT4 protein in the sarcoplasm (60). These fortuitous findings may be caused by differences in exercise intensity, duration, testability, or sample preparation methods (61). Furthermore, it has been demonstrated that the expression of MCT is influenced by the breed of the horse (62, 63).

The results obtained showed the interference of exercise on blood gases and electrolytes, being that the AIEB protocol was of high intensity, characterized by the mobilization of the glycolytic pathway with the development of hyperlactatemia and metabolic acidosis, considering the findings of plasma lactate, pH, BE_{ecf}, and SID. High-intensity exercise leads to sharp increases in $[H^+]$, which leads to decreased pH and reduced HCO_3^- and tCO_2 (64). Racehorses that underwent 2 min of high-intensity trotting showed decreased SID on jugular venous blood, mainly responsible for the observed plasma acidosis. The reduction in SID resulted from increased lactate, Na^+, K^+, and Cl^- (65, 66). The decrease in SID observed in the present study was mainly induced by the increase in lactate production caused by intense exercise.

Our study found no changes in $PvCO_2$, consistent with a former study on intense treadmill exercise that suggested compensatory hyperventilation (67). In this study, HCO_3^- values after exercise were lower than baseline values, like what Miranda et al. (67) observed, compatible with buffering mechanisms (68). The increase in Hct and Hb after AIEB is attributable to the release of the splenic reserve of red blood cells, mediated by the action of catecholamines in response to intense exercise (6),

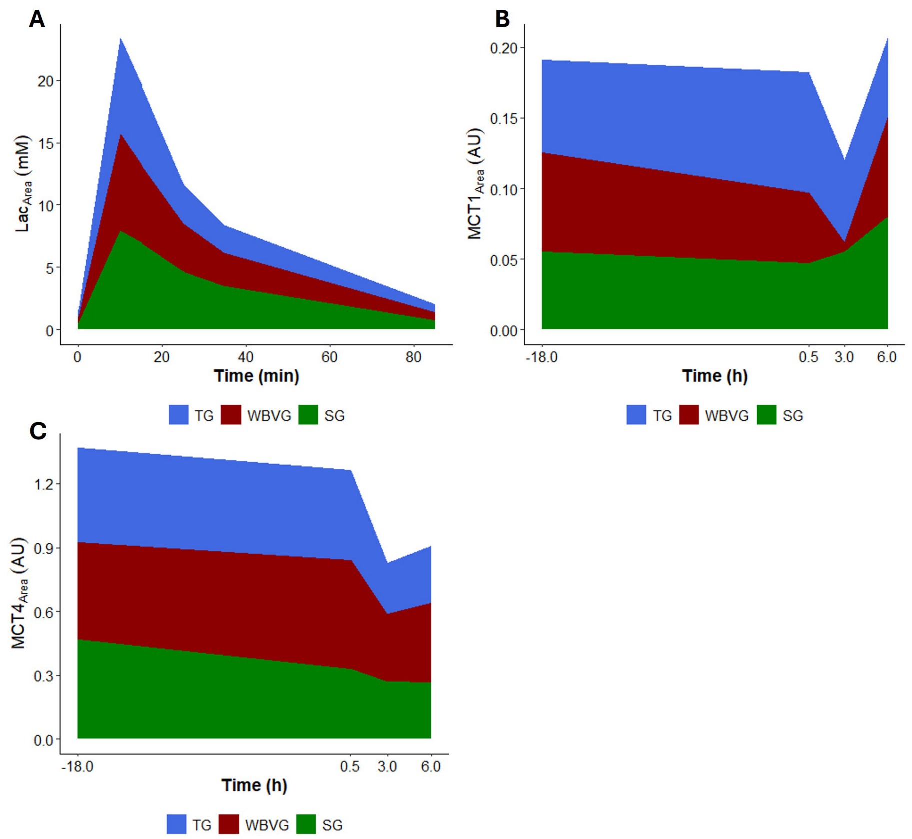

FIGURE 6
Graphical representation of area under the curve (AUC) of **(A)** plasma lactate, **(B)** MCT1 protein content and **(C)** MCT4 protein content of horses (n = 8) submitted to an acute intense exercise bout (AIEB) and a 10-min recovery period on the treadmill (TG), on a whole-body vibration session (WBVG), or the vibrating platform off (SG).

despite the changes observed after exercise. The results showed no differences in recovery strategies for blood gases and electrolytes, indicating that the recovery methods used did not affect the body's compensatory response to moderate metabolic acidosis caused by exercise.

Some studies have indicated that WBV can control glycaemia in humans (69, 70) and rodents (11, 71). The current study did not indicate that WBV could favor such an effect. The only thing that influenced plasma glucose was AIEB, which increased the bioavailability of this energy substrate for skeletal muscles. This finding can be explained by the increased activity of hormones that regulate energy metabolism, such as catecholamines and glucagon. These, when released, promote hepatic glycogenolysis and neoglycogenesis, an essential mechanism for maintaining plasma glucose concentrations during exercise (66).

After the recovery methods, the WBVG and SG showed lower HR compared to the TG, which can be explained by the fact that WBV is considered a form of passive recovery. However, there was no difference between the WBVG and SG, indicating that the VP turned off had the same effect as the equipment turned on. In adult men subjected to maximal exercise, a more pronounced reduction in HR was observed when recovering with an association of light exercise and WBV, compared to light exercise alone (4) and recovery with squats in VP on, compared to passive rest or only squats (5). These findings indicate that WBV led to a quicker heart rate recovery, a result not seen with the protocol used in the current study.

In the present study, it was possible to observe an increase in rectal temperature after AIEB. This variable increased even more after all interventions, around 15 min after the end of AIEB, in all groups. The increase in rectal temperature after exercise is significantly correlated with exercise (37). Corroborating these results, a study that evaluated different types of cooling with water after medium-intensity exercise observed an increase in rectal temperature, which remained elevated for up to 30 min after exercise, and the use of cooling with water as a form of recovery did not influence the decrease in post-exercise rectal temperature (31). Therefore, WBV did not accelerate the cooling down of this cohort of horses.

The use of mechanical vibration as an alternative to active exercise is of increasing interest. The effect of WBV on vital parameters, muscles and bones is investigated and it is widely suggested that WBV may be an alternative to resistance training for stimulation of the musculoskeletal system (43), which could make the technique an important training option, aiming to reduce the time of animals on the track, especially those with previous injuries and which would have a greater chance of recurrence. With the current search for alternatives to improve athletic capacity

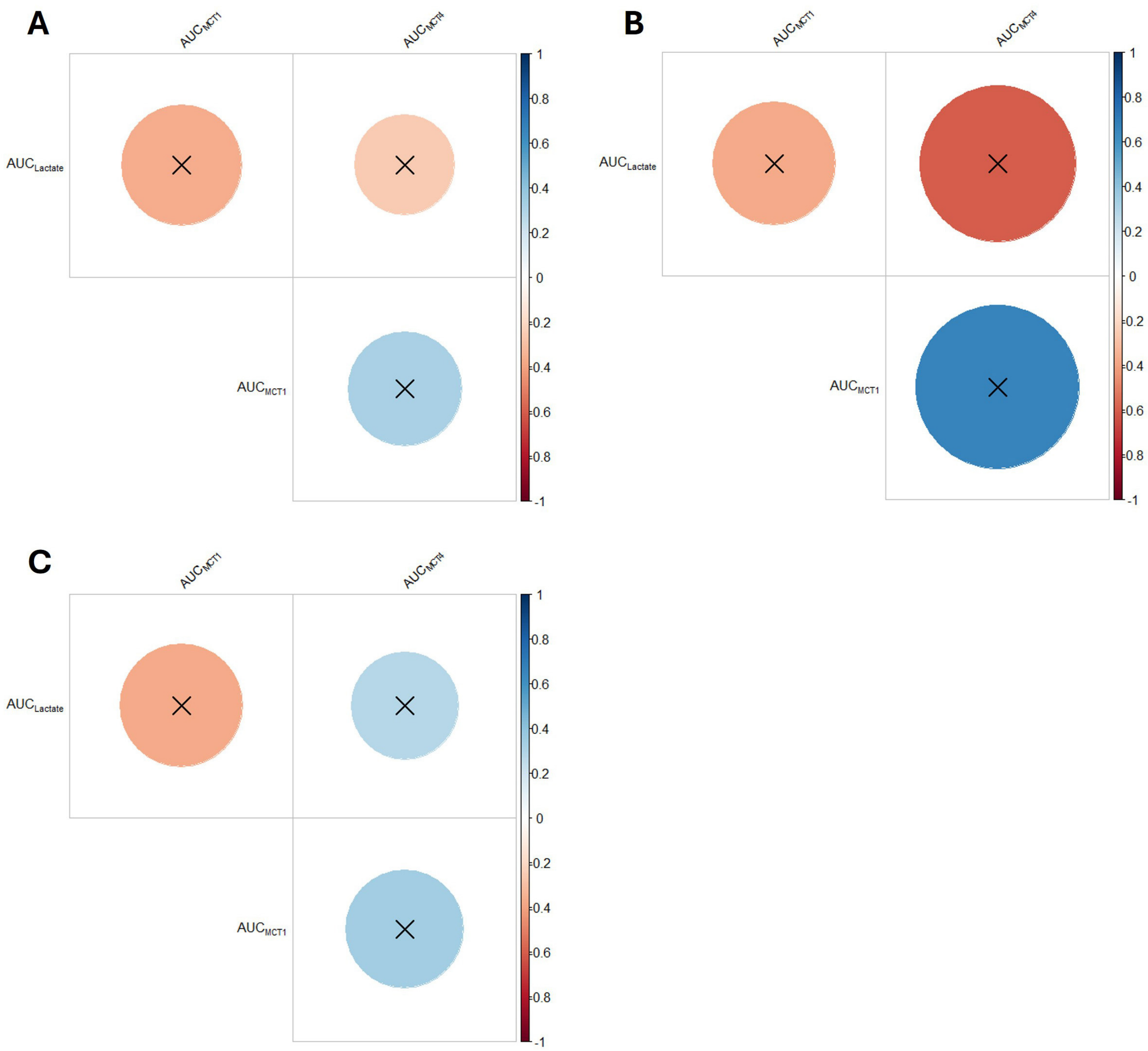

FIGURE 7
Correlogram of plasma lactate, MCT1 protein content and MCT4 protein content of horses ($n = 8$) submitted to an acute intense exercise bout (AIEB) and a 10-min recovery period on the **(A)** vibrating platform off (SG), **(B)** treadmill (TG), or **(C)** on a whole-body vibration session (WBVG).

in the horse industry, it is not uncommon for participants to be mesmerized by the miraculous effects of therapies on the market. Companies that produce vibrating stimulation plates claim results such as increase/maintenance of muscle mass, reduction of injuries, faster healing, improved balance, and increased circulation, among others. The effectiveness of WBV as a training method, however, is quite controversial, and the results presented here did not indicate any effect on the physiological variables analyzed. In a study that investigated the effects of warming up with WBV in horses, the results showed that WBV can be seen as a passive movement of the limbs and trunk, as a type of relaxation of the locomotor system, without any active involvement (26).

More studies are needed in horses, especially regarding different vibration patterns, as the transmission of vibration through the equine body is poorly understood and is a fundamental consideration in interpreting the effects of WBV. A reduction in amplitude observed in the dorsal compared to the extremities of horses' bodies denotes that vibration transmission is greatly attenuated and may not be effective in provoking a physiological response, such as increased lactate removal from muscles, in the upper part of the body (72). To date, the use of WBV in horses is primarily based on results reported in human or rodent literature. However, horses are the largest animals on which WBV has been tested, and this size difference may impact vibration transmission. Furthermore, a possibly significant difference between WBV in men and horses is body posture during vibration training (24, 26). Moreover, most studies on WBV in humans involve performing other exercises on the VP (4, 5, 8), which, to date, is not possible to be carried out in the equine species.

While the study provides valuable insights, it is important to acknowledge that it has some limitations. For instance, there were a small number of horses involved, although this was enough for the statistical analysis. Additionally, using a single WBV session in the protocol may not have been enough to induce significant changes. It is possible that different results could be observed with longer periods of training using WBV. Also, the way vibrational transmission occurs throughout the horse's body is poorly understood; therefore, electromyography would be necessary to estimate how muscle activation occurs using WBV. Furthermore, although the gluteus medius muscle is the largest muscle in the horse and of great importance in its locomotion, showing great propulsive activity in the gaits during the exercise (73–75), it may be beneficial to assess other muscles in the distal thoracic or pelvic limbs, such as the triceps or semitendinosus, as the effects of vibration may diminish when moving dorsally (72). Hence, it is important to study different protocols for sport horse training to better evaluate the effects of WBV as a recovery method after intense exercise.

5 Conclusion

The research demonstrated that a single session of whole-body vibration (WBV) does not improve recovery from hyperlactatemia or acid-base balance in horses after intense exercise. This insight offers veterinarians, researchers and riding instructors the opportunity to develop better recovery protocols for horses. Future studies should focus on other protocols and the potential benefits of chronic WBV applications for enhancing recovery after exercise in horses.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by Ethics Committee on the Use of Animals. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

JC: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Writing – original draft, Writing – review & editing. NS: Formal analysis, Investigation, Methodology, Software, Writing – review & editing. TL: Formal analysis, Investigation, Methodology, Software, Writing – review & editing. GC: Formal analysis, Investigation, Methodology, Software, Writing – review & editing. CC: Formal analysis, Investigation, Software, Writing – review & editing. EP: Data curation, Software, Writing – review & editing. JO: Data curation, Software, Writing – review & editing. GR: Data curation, Software, Writing – original draft, Writing – review & editing. IS: Data curation, Formal analysis, Writing – review & editing, Software. CG: Resources, Supervision, Writing – review & editing. FM-G: Resources, Supervision, Writing – review & editing. GF: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Gen AI was used in the creation of this manuscript.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2025.1538195/full#supplementary-material

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Exploring the impact of housing routine on lying behavior in horses measured with triaxial accelerometer

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Introduction: Methods to assess the positive behavior of horses in relation to their environment can be used to provide information to enhance animal welfare. One of the most important experiences that can be observed in mammals is sleep, a universal behavior relevant for the welfare of all species. To achieve paradoxical sleep, horses must lie down in lateral recumbency for a sufficient time, but they only do so when feeling safe and comfortable. Recently, technological tools like accelerometers have opened the possibility of non-invasive continuous monitoring of lying behavior, thus implementing the way we assess equine behavior in relation to their management and environment.

Methods: The aim of this study was to investigate whether a sudden change in housing routine affects lying behavior in horses. In 10 riding school horses, lying behavior was continuously monitored using triaxial accelerometers for two separate 5-day periods, each under a different housing routine (i.e., ordinary: in a paddock in small groups; modified: in single boxes).

Results: The results show no statistical differences in the total daily duration of lying behavior between ordinary (25.19 ± 21.81 min) and modified (23.16 ± 20.05 min) housing routines. However, in the ordinary housing routine, when horses were kept outdoors in groups of varying sizes, larger groups exhibited synchronized lying behavior, with longer lying bouts, while smaller groups lay down more frequently throughout the day.

Discussion: The results show that sudden change in housing routine does not have a significant effect on lying behavior, while group size appears to be an important factor for behavioral synchronization. However, the small sample size, the single location, and mixed-age and sex population may have influenced the findings. Accelerometers were shown to be beneficial for monitoring natural behaviors such as lying and thus inferring information about equine behavior in relation to daily routine management.

KEYWORDS

horse, lying, accelerometer, positive welfare, animal-based measure

Introduction

Horse (*Equus caballus*) is a versatile species that can be kept as companion animals, for sport, leisure, and for production. The adaptability of this species to different contexts worldwide has led to the growth of the equine industry, with approximately 58.6 million horses registered by 2021 (1). This growth has simultaneously raised the public concern for their welfare (2), with housing and management playing a crucial role (3), as they can influence physical exercise and the ability to lie down due to reduced space, social interaction and perceived safety through group vigilance due to isolation; as well as lead to a lack of comfort (presence of artificial light, bedding type and depth, temperature and humidity); and hypo stimulation, which lead to lethargy and mental fatigue (3). In this sense, resilience is another important concept related to welfare which refers to the degree to which an animal's behavior and physiology are affected by stressors or challenges (e.g., change of housing conditions). Positive experiences can instead enhance resilience, enabling individuals to better cope with challenging situations (3). Over the past decade, there has been an increasing interest in finding methods to assess the positive experiences (i.e., condition or interaction that promotes positive states, enhances survival and fitness, or serves as a reward an animal will work for) and welfare of horses in response to their management and environment. Several studies have shown that healthy horses living in a natural environment dedicate a large part of their time to all the behaviors intended to keep the animal alive and healthy, also defined as "maintenance behaviors" (4). Among these, sleep is particularly important as it is essential for both physiological (e.g., high caloric ingestion without weight gain, reduction in anabolic hormones) and cognitive functions (e.g., regulation of neuronal functioning during memory storage and consolidation and cerebral metabolism within the prefrontal cortex, responsible for judgment and decision making) (5–7).

Sleep is defined as a maintained state of quiescence characterized by relative inactivity, loss of consciousness, and/or increased threshold of arousal to environmental stimuli. It is regulated by circadian rhythms, which organize timing over 24 h, and by homeostatic mechanisms, which determine the amount of sleep required for each species (5). Horses exhibit a polyphasic sleep pattern, distributed across 5–7 shorter episodes, with most sleep occurring at night, particularly between 12 a.m. and 4 a.m. (8). To achieve paradoxical sleep, horses need to lie down for sufficient time in recumbency (9). However, as prey animals, horses only lie down when they feel comfortable and safe (10, 11). Consequently, measuring lying behavior can be a valuable starting point for monitoring natural maintenance behavior, thus inferring about positive welfare (11).

Variations in management can significantly influence the amount of time a horse spends in decubitus, which in turn affects its time in Rapid Eye Movement (REM) sleep. Studies have shown that lying behavior of the horse depends on biological characteristics, such as age (i.e., increased in young animals) (12), sex (12, 13) and breed (11). The latter is affected as well by social factors, such as herd size (14, 15), hierarchical position (12, 16), health conditions such as joint damage and chronic orthopedic disease (11, 17), body condition score (11), seasonal changes (12), and housing factors, such as light conditions (10), auditory stimuli (e.g., presence of music) (8), litter height and type (10, 18, 19), and dimensions of the resting area (12, 16, 20, 21). Examining the duration of lying behavior is a valuable tool for assessing positive experiences, particularly, the continuous monitoring of subjects over several days. However, both continuous real-time monitoring and analysis of 24-h video recordings are challenging due to time constraints and accuracy (22). For this reason, precision tools for monitoring and tracking lying behavior can provide greater objectivity in assessing an individual's behavior by facilitating accurate analysis and quantification of the time spent in different activities (22, 23). Accelerometers have been reported to accurately measure the amount of time the horse spends in decubitus, taking advantage of the principle of the absence of gravitational force in the horizontal position of the instrument itself (22, 23).

One of the most common phenomena in horses that can disrupt sleep is a sudden change in the housing condition. Horse housing can be often modified to accommodate individual needs (e.g., box housing due to injuries), for welfare improvements (e.g., paddock/group housing during the dry season), and work needs (e.g., competitions and training schedules). This can negatively affect horse welfare by disrupting established routines and causing stress, especially if the basic needs in the changed housing are not met (e.g., transition from spacious group pasture to restrictive individual boxes). The aim of this study was to investigate whether a sudden change in housing routine affects animal-based measures and lying behavior in school riding horses. Additionally, since horses were housed in groups of varying sizes, we examined the impact of social groups on lying behavior and synchronization.

Materials and methods

Ethical approval

All procedures were approved by the Committee for the Welfare of Animals for Experimental Purposes of the Veterinary Faculty of the University of Ljubljana (033-5/2024-5).

Horses, management and housing routines

The study was conducted at the Educational Research Center for Horse Breeding Krumperk in Gorjuša, Slovenia, during the summer season, from June to September 2024. The experimental period was characterized by stable climatic conditions. The study involved 10 healthy Lipizzan horses (6 mares and 4 geldings) aged 10 to 20 years (mean age 14.8 ± 3.7 years). All horses were trained and ridden using classical English riding methods and were regularly used in a children's riding school. The horses were housed in standard individual boxes (3.0 × 3.5 m²) with wood shavings as bedding and visual access to other conspecifics. By assessing the horses' body size, we determined that the boxes were adequately sized to allow all horses to lie down in lateral recumbency and get up without restriction. They had *ad libitum* access to hay and fresh water, and they were additionally fed a barley-oat mixture. The horses also had access to four outdoor grazing paddocks, each measuring 0.5 hectares. These paddocks provided water and grazing opportunities and did not include a specific resting area. One paddock housed four horses, another housed three, the third housed two, and the last held a single horse (see Supplementary Table S2). The group compositions were the same in both treatments, and they had been constant for many years prior to the study and were deliberately structured to include

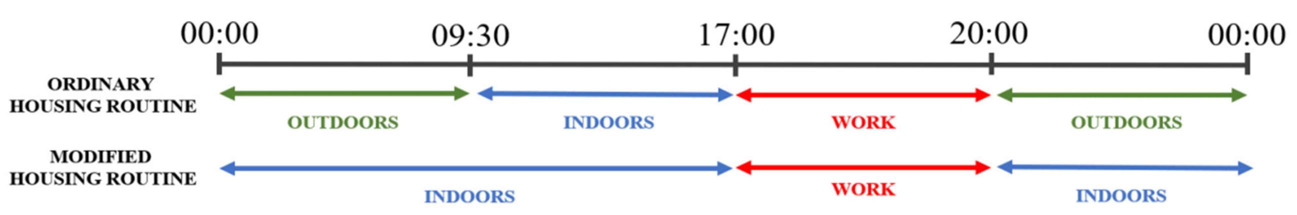

FIGURE 1
Different housing routines of horses during the working days.

horses that exhibited mutual social compatibility. Horses had visual access to at least one other paddock. During weekdays, the selected horses participated in riding school activities between 17:00 and 20:00, with each horse engaged in ridden work for a maximum of 2 h per day.

Data were collected twice from the same horses under two different housing routines: ordinary housing routine and modified housing routine (Figure 1). During ordinary housing routine, all horses were housed in individual boxes during the daytime hours of higher temperatures (09:30–17:00) and allowed access to outdoor confined paddocks (either individually or in groups) overnight. On non-working days, they remained in single boxes from 09:30 to 20:00 and were turned out overnight (20:00–09:30). Under the modified housing routine, horses were kept stabled in individual boxes without pasture access and were let outside the box only during riding sessions. On non-working days, they remained continuously housed in single boxes without outdoor access. Due to the summer season, the horses followed their ordinary housing routine. Next, to create a sudden change, they transitioned to a modified housing routine for 1 week. After 1 month, all horses were re-evaluated under the ordinary housing conditions.

Data collection of lying behavior and data processing

Lying behavior, defined as a posture in which a horse adopts either lateral or sternal recumbency (12), was continuously recorded for 5 days (120 h per horse) using MSR145 triaxial accelerometers (MSR Electronics GmbH, Seuzach, Switzerland). The MSR145 was set at a sampling frequency of 1 Hz. According to the literature, this sensor has been validated for estimating lying behavior in horses (12). For each horse under each housing routine, data was collected over 3 working days and 2 non-working days. The accelerometer was attached to the metacarpal bone of the left forelimb using a Velcro strap (Figure 2). To prevent pressure on the bone and minimize the risk of abrasions, foam padding was placed beneath the device (Figure 2A). Additionally, the sensor was secured with an elastic band to protect it from damage and ensure stability (Figure 2B).

At the end of each monitoring period the accelerometer data was downloaded from the MSR software to a.csv file. Then, the raw data time series were analyzed using MATLAB R2024b. The calculation of the lying position has been estimated starting from the assumption that the accelerometer was positioned by orienting the Y-axis parallel to the limb (vertical), the X-axis in the cranial direction and the Z axis (lateral) perpendicular to the limb. Before processing the data, preliminary numerical check and data-cleaning were carried out. A first check was carried out with the aim of evaluating the correct positioning of the accelerometer on the horse's limb during the monitoring period. Then, from the analyses of the modification of the acceleration values along the Y-axis it has been possible to identify the moment associated with vertical or horizontal position of the limb. When the limb was identified in horizontal position it was possible to assume the horse in lying position (12). Then, for each horse, estimates of hourly and daily lying time were obtained by summing the decubitus measurements per hour and per day, respectively. Counts and duration of lying bouts were also assessed. In the count of the number of lying bouts, a threshold of minimum duration equal to 2 min has been applied (12).

Animal-based measures assessment

On the final day of recordings for each housing routine, animal-based measures (ABMs) from the first level of the AWIN Welfare assessment protocol for horses (24) were collected by a trained observer. For a detailed description of the assessment methodology of each ABM refer to the AWIN welfare assessment protocol for horses (24). Each ABM was considered as binary variable (appropriate/non-appropriate): the scoring system used for each evaluated ABM is presented in Supplementary Table S1.

Statistical analysis

All statistical analyses were conducted using SPSS 28 (SPSS Inc., Chicago, USA). Differences were considered statistically significant if $p < 0.05$. A Generalized Linear Mixed Model (GLMM) was used to evaluate the effects of housing condition, day of the week (working and non-working days), group size (≤ 2 horses or >2 horses), and the interactions, housing condition × day of the week and housing condition × group size, on mean lying duration. To assess lying bouts frequency and lying bouts duration, the housing condition was included as the main fixed effect. Horse identity was included as a random effect in all models to account for repeated measures. Mean lying duration and lying bouts duration (continuous data) were modeled using a Gamma distribution with a log link, while lying bouts frequency (count data) was modeled

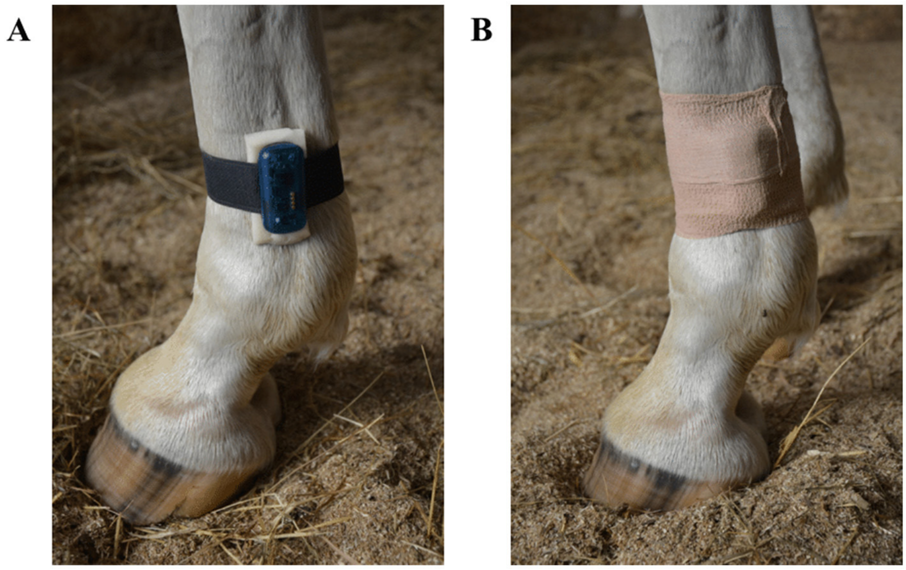

FIGURE 2
Positioning and application of a triaxial accelerometer during data collection of lying behavior. **(A)** Foam padding placed beneath the device to prevent pressure on the bone; **(B)** Wrapping of the device with an elastic band to keep it safely and firmly in place.

using a Poisson distribution with a log link. Model selection was guided by biological relevance and comparison of model fit using the Akaike Information Criterion (AIC). Model stability and estimate reliability were assessed by inspecting standard errors and confidence intervals of the fixed effects. Due to the unbalanced sample size across group size categories ($n = 3$ for $\leq$2 horses; n = 6 for >2 horses), interaction effects involving group size are interpreted with caution. Results are reported as estimated marginal means ± standard error of the mean (SEM).

For animal-based measures analysis, the proportion of appropriate scores for each welfare indicator (e.g., percentage of horses with absence of lesions or showing no avoidance when approached by an unknown human) was calculated in each housing routine. A Chi-square test was used to investigate possible differences in the percentage of each ABM between the two housing routines.

Results

Animal-based measures (ABMs)

The percentage of horses with appropriate scores of different animal-based measures in the two housing routines is reported in Figure 3. No differences were found between the two housing routines (Chi-square test, $p > 0.05$).

Lying behavior

One of the horses never showed lying behavior during the data collection and was therefore excluded from the analysis. Although not statistically significant, the mean daily lying duration in the ordinary housing routine was longer than in the modified housing routine ($p = 0.809$; $\beta = -0.165$, $t = -0.426$), with 25.19 ± 21.81 min and 23.16 ± 20.05 min, respectively (Figure 4A). Considering lying bouts (Figure 4B), no statistically significant differences were found between housing conditions ($p = 0.788$): in the ordinary routine, horses lie down 2.67 ± 1.69 times per day, and the mean duration for each lying bout was 10.14 ± 6.24 min. While in modified routine, horses lie down 1.83 ± 1.56 times for a mean lying bout duration of 10.85 ± 6.68 minutes. Comparing weekdays, lying duration was slightly higher on working days (12.42 ± 4.97 min) than on non-working days (10.95 ± 4.39 min), though this difference was not statistically significant ($p = 0.278$; $\beta = 0.001$, $t = 0.006$). Group size comparison results showed that horses in smaller groups ($\leq$2 horses) had a mean lying duration of 15.78 ± 14.11 min, compared to 36.99 ± 31.76 min in larger groups (>2 horses), with no statistically significant difference ($p = 0.318$; $\beta = -0.933$, $t = -1.702$). No significant interaction was found between housing condition and day of the week ($p = 0.223$; $\beta = 0.250$, $t = 1.218$), with the modified routine having a non-significantly longer daily lying duration ($p = 0.162$) during the working days (13.51 ± 5.50 min) than during non-working days (10.52 ± 4.28 min). Similarly, no statistical differences were found between working (11.41 ± 4.65 min) and non-working (11.40 ± 4.68 min) days during the ordinary routine ($p = 0.995$; Figure 4C). In addition, the interaction between housing condition and group size was not significant ($p = 0.813$; $\beta = 0.161$, $t = 0.241$), with horses housed in modified housing routine, having a lying duration of 15.75 ± 14.73 min in smaller groups ($\leq$2 horses) and 34.06 ± 29.98 min in larger groups (>2 horses; $p = 0.371$). As in ordinary housing routine, lying duration was 15.80 ± 14.78 min in smaller groups and 40.17 ± 35.35 min in larger groups ($p = 0.341$).

The assessment of lying behavior synchronization showed that when kept in groups of 3 and 4 horses, animals seem to lie down

FIGURE 3
Bar plot reporting the prevalence (%) of horses with no alterations in ABMs in the two housing routines.

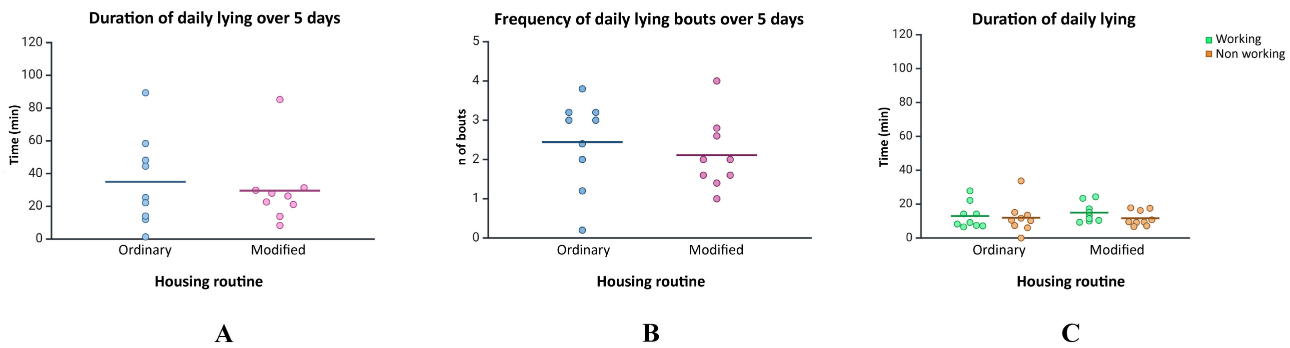

FIGURE 4
Graphs reporting the lying behavior in ordinary and modified housing routines where each dot represents a subject, while the line is the mean value. **(A)** Mean daily lying duration (minutes) over 5 days; **(B)** Mean daily frequency of lying bouts over 5 days; **(C)** Mean daily lying duration (minutes) in the working days and in non-working days.

at the same hours of the day (Figure 5). In the ordinary routine horses in the same group tended to lie down at the same hours of the night and day (between 22:00 and 6:00; Figure 5A). On both working and non-working days, horses from paired and individual housing frequently lay down during the day, while, in the modified routine housing, all the horses preferred to lie down between 23:00 and 5:00 on both working and non-working days (Figure 5B).

Discussion

Our results showed no significant differences between the two housing routines in the percentage of appropriate scores of ABMs, confirming that horses were healthy and in an overall good welfare condition in both routines. In terms of lying behavior, horses in the ordinary housing routine appeared to have a longer daily lying duration compared to those in the modified routine, although this difference was not statistically significant. During the ordinary housing routine, when horses were kept outdoors in groups of varying sizes, larger groups exhibited synchronized lying behavior, with longer lying bouts, while smaller groups lay down more frequently throughout the day. While horses seem to express natural behavior in both housing routines for what concerns lying quality and quantity, it appears that group size particularly influences how safe the horse feels.

Welfare assessments revealed all horses enjoyed good health and welfare condition during both housing routines and highlights that the ABMs included in the AWIN welfare assessment protocol are not meaningful when a small stressor is applied in the everyday routine of horses. Despite horses spent a significant amount of time in the paddock together with other horses as part of their ordinary routine, where they could possibly be injured by other subjects (25), such injuries were not observed in our study. It appears that the main causes of these types of injuries, such as lack of space and sub-optimal group composition that leads to fights (26) were not present among our groups. One horse did not lay down at all during the observational period. Although adult

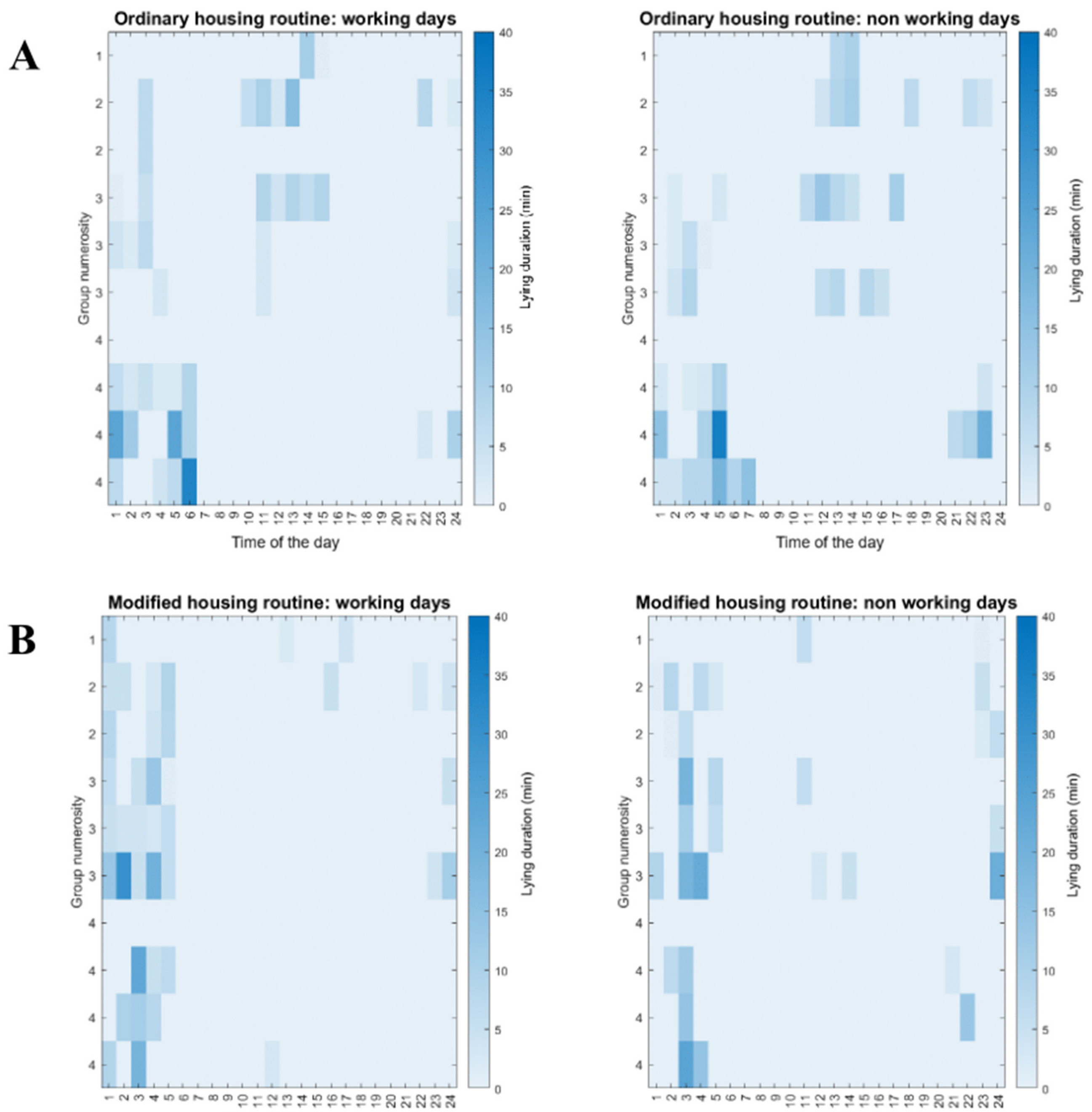

FIGURE 5
Heatmaps reporting the lying behavior synchronization within each horse group in the ordinary and modified housing routines during working and non-working days. The color represents the mean duration in minutes (0–40 minutes per hour) of lying behavior over the 24 h (time of the day) in working and non-working days and for groups of different numerosity (from 1 to 4 animals). **(A)** Ordinary housing routine; **(B)** Modified housing routine.

horses require at least 30 min of lying down per day to achieve 3.5–4.5 min of REM sleep and avoid sleep deprivation, which can result in excessive secondary sleepiness, collapse (11), and severe physiological effects (10), it is not uncommon for individual horses to forgo lying down entirely for a few days. Other studies have also documented instances where horses did not lie down during the observed periods (21, 27).

In this case, ABMs collected twice during our study did not indicate that horses would show any specific health-related issue that could explain the reluctance to lie down (e.g., lameness or back pain). The box size was assessed as appropriate, and the horse could easily lie down and get up. It is then possible that the presence of the accelerometer itself may influence the behavior of the horse, however the horse did not show any signs of discomfort in wearing the accelerometer. To test this hypothesis, it could be suggested to monitor the subject who did not exhibit lying behavior for the same period of time without the accelerometer, or increase the monitoring period with the accelerometer, to see if they actually show reluctance to lie down.

Looking at the lying results, the small differences between the duration of lying in the paddock compared to the indoor box may be attributed to several factors. One possible explanation is that the larger space available in the paddock could promote greater freedom of movement and comfort, potentially encouraging lying behavior (5, 12, 28). Furthermore, the open view provided by the paddock, as well as social companions present, may have created a greater sense of security for the horses, reducing stress and enabling them to rest more comfortably (5). Another possible explanation is the variation in ground materials, as previous studies highlight the importance of bedding for lying behavior. Horses generally prefer to rest and sleep on softer surfaces that provide greater comfort (12, 29). Specifically, wood shavings, the bedding material used in our study, have been reported to be less preferred compared to other options, such as straw, with bedding depth also playing a significant role (29, 30). Furthermore, considering that equine sleep appears to be closely associated with environmental seasonal fluctuations with higher temperature lowering the time spent sleeping, we might think that being in an enclosed environment with the high temperatures recorded at the time of data collection (June 2024) may have affected the duration of sleep itself. Our results are in line with what was found by Chaplin and Gretgrix (21) who also reported that changing the housing conditions (i.e., paddock, fully stabled, partly stabled, and yard) did not significantly affect the time horses spent lying, as well as the number or the duration of lying bouts. All horses were subjected to the same feeding management in both routines, which may have contributed to the lack of differences observed.

While comparing working and non-working days, spending the night in the paddock after work appears to promote increased lying behavior in horses, likely due to the larger space, natural ground surfaces, and greater environmental enrichment compared to confined indoor stalls (5, 12, 28). Also, access to a more natural environment (31) may help reduce stress and fatigue accumulated during work, further supporting the need for adequate recovery and facilitating restorative rest. Grouping horses in paddock during ordinary housing routine revealed the importance of the social group for fostering lying behavior. Among horses, it is widely recognized that living in social groups promotes positive psychological and physical welfare, with social support (i.e., the benefits provided by social partners) enhancing an individual's ability to cope with challenges (32). Additionally, animals that live in groups often present some type of behavioral synchronization (33). In prey animals, behavioral synchronization helps reduce predation risk, while group living also decreases everyone's likelihood of being caught (33). That is why in horses, complete synchronicity of the group is almost never observed while lying or standing resting (34). To ensure safety, individuals often alternate between lying down and standing, creating a rotational vigilance system that helps protect the group (14, 35). This allows individuals to spend more time lying down, sleeping, and resting. Such patterns were clear in our group of four horses, where three horses synchronized their lying behavior, while the fourth horse did not lie down. In contrast, this behavior was less clear in pair of individuals, suggesting that differences in group sizes can probably play a significant role in both synchronization and the duration of lying. However, it is important to note that the observed increase in lying behavior was confounded by several factors, including outdoor access, limited number of groups of the same size, a different substrate for lying, and the presence of social company, therefore drawing definitive conclusions about the specific role of a particular parameter alone is not warranted.

Regarding the hour of the day at which horses lay down most frequently, the results are mainly consistent with reports from previous studies (13, 21, 36). Despite the time of the day when horses decided to lying down seem to differ for every subject between the two routines, considering the group numerosity it has been found that, interestingly, on both working and non-working days, horses from groups of 3 subjects frequently lay down during the day, contradicting previous reports that horses rarely do so outside their usual resting periods (13, 36). Additionally, since ordinary housing required staying in the box during the day, it is remarkable that some horses often lay down indoors (36).

Conclusions

It appears that, as long as an adequate space for both lying (sternal and lateral) and getting up is provided and sociality is granted thanks to at least visual access to conspecifics, a sudden change in housing routine does not significantly affect lying behavior or animal-based welfare measures. This shows that, for horses, it is possible to show natural behavior such as lying in both housing routines if some species-specific needs are respected.

The use of non-invasive devices such as triaxial accelerometers for continuous monitoring over extended periods has proven to be an effective method for observing behaviors such as lying, including its frequency and duration at the individual level. These devices are also well-suited for use in outdoor environments, making them valuable tools for research aimed at enhancing the welfare assessment of horses, including positive behaviors. Further studies are suggested to better understand the role that group size and social relationships can play for behavioral synchronization of lying in horses as well as acquire more knowledge about the role of restorative rest after a period of work.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal studies were approved by Committee for the Welfare of Animals for Experimental Purposes of the Veterinary Faculty of the University of Ljubljana (033-5/2024-5). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

EG: Conceptualization, Funding acquisition, Investigation, Methodology, Writing – original draft, Writing – review & editing. CM: Writing – original draft, Writing – review & editing. MZ: Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing. MB: Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. EA: Data curation, Formal analysis, Writing – original draft, Writing – review & editing. MM: Writing – review & editing. ED: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Task-specific morphological and kinematic differences in Lipizzan horses

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Introduction: Equine locomotion emerges from a dynamic interplay between morphology, biomechanics, and functional demands. This study examines the relationship between morphological measurements and gait kinematics in Lipizzan horses, a breed renowned for its diverse work tasks and standardized environmental conditions. These horses offer a unique opportunity to explore task-specific adaptations in biomechanics, with significant implications for breeding strategies and welfare practices.

Materials and methods: The study involved 71 healthy Lipizzan horses that were housed at the Lipica stud farm and performed various work tasks. Morphological measurements were taken with the help of a sartorial meter and an equine measuring stick to determine head and body measurements. Both the left and right sides of the body were measured to ensure consistency. Kinematic data, including regularity, symmetry, cadence, dorsoventral power, propulsion power, stride length and speed, were recorded using the Equimetrix accelerometer at a sampling rate of 100 Hz. The data was collected during several walks and trots where the horses were led over a 50-meter track.

Results: Task-based analysis revealed strong links between morphology and gait in four working groups, with distal limb measurements, especially hoof and pastern lengths, most consistently associated with stride and rhythm parameters. No significant associations were found at the whole-cohort level. Several morphological measurements showed contrasting effects across working groups, and half of the bilaterally measured traits revealed side-specific correlations. The clearest patterns emerged in horses used for general training and riding school. In horses in general training, strong associations were found between distal limb measurements and stride length or cadence, particularly during walk. In riding school horses, broader body measurements were linked to kinematic parameters including propulsion power, dorsoventral power, and symmetry.

Discussion: This study highlights the dynamic interplay between conformation and functional demands in clinically sound horses. Rather than exerting fixed effects, morphological measurements interacted with work type to shape gait expression, even in the absence of pathology. These findings underscore the need to consider both structure and task when evaluating locomotion. Integrating morphometric assessment into training and selection strategies may support performance, soundness, and welfare in healthy working horses.

KEYWORDS

equine, locomotion, non-pathological, morphology, positive experiences

1 Introduction

The animal kingdom exhibits a remarkable diversity of locomotion (1), with each species using unique movement patterns tailored to its environment and needs (2). Quadrupedal mammals such as horses, for example, have fascinating and highly specialized locomotion, with the most common gaits being walk, trot, canter, and gallop (3). Each gait involves a distinctive patterns of leg movements and speeds that enable horses to move efficiently over different terrains. During walk, at least one front and one hind foot are always in contact with the ground, resulting in a consistent four-beat rhythm. In contrast, the trot is a two-beat gait in which all four feet lift off the ground, a characteristic that is also present in faster gaits such as the canter and gallop (4).

Gait is directly influenced by morphology, which serves as a structural foundation that both enables and limits the range of movement (5). Together, these two aspects show how the physical form of an organism influences the efficiency and effectiveness of its locomotion. Variations in bone length, joint structure, and muscle arrangement all play a crucial role in determining locomotor capabilities. Studies on mammals show that the length of the hind limbs and the ratio of the metatarsal to femur correlate with running speed (6). In humans, larger body size has been shown to correlate with higher optimal walking speed (7). Adaptations for speed are important in mammalian evolution, suggesting that animals optimize their morphology for speed and to reduce the cost of locomotion (8). In quadrupeds, the limbs are important to support the body mass, with the morphology of the forelimbs fulfilling various functional roles (9).

The locomotor system is particularly important in working animals, such as horses. In practice, selective breeding produced different types of horses - heavier draught horses were bred to pull heavy carts, while leaner and faster horses were selected for their speed and endurance (10). Also, parameters such as quality of gait, which reflects the way horses move according to functional and esthetic principles, are thought to predict future performance, making it an important breeding goal for European sport horses (11). Kinematic studies in horses have primarily focused on the limbs, as they are of immediate importance for performance, locomotor efficiency and the diagnosis of lameness. However, this does not mean that other regions of the body are irrelevant. For example, although gait speed does not differ significantly between breeds such as Andalusian, Arabian and Anglo-Arabian horses, marked differences have been found in terms of propulsion, timing of hoof contact, percentage of deceleration during stance and maximum limb retraction (12). These kinematic characteristics reflect different biomechanical strategies that influence how effectively horses generate thrust, absorb impact and coordinate their movement patterns, characteristics that are not only performance-related, but may also be relevant to breeding decisions and training programs (13–15). However, it remains unclear whether kinematics differ within a breed when horses perform different tasks and whether kinematic gait analysis can be reliably used to select horses for specific performances (13). However, some kinematic measurements are promising for breeding. For example, jump duration is a heritable variable that can be used as a breeding criterion in jumping horses (14), and trotter racehorses have a higher stride frequency and longer stance and propulsion durations at maximum speed (15), suggesting that targeted selection based on movement characteristics for specific disciplines is possible.

Kinematic data can be collected using marker-based video systems (16) or wearable inertial measurement units, such as EquiMoves (17) or Equimetrix (15, 18, 19), which incorporate accelerometers and gyroscopes to capture detailed motion parameters; Equimetrix was the system used in this study. Kinematic analysis has traditionally focused on pathological changes that limit locomotion, such as lameness and injury, primarily to prevent suffering and address negative welfare outcomes in horses (20–24). However, it is increasingly applied to investigate locomotion in clinically healthy horses across various disciplines. By observing the gait of healthy horses (without clinically observable locomotor abnormalities or other signs of disease), this study also fills a gap in the understanding of how non-pathological differences in kinematic parameters relate to morphology and type of work within a single breed. This within-breed focus allows us to explore both potentially inherited conformational traits that may have influenced selection for specific type of work, and physiological adaptations that may have developed over time through habitual training and exercise.

Accordingly, the aim of this study was to investigate how morphological and kinematic characteristics vary in Lipizzan horses performing different types of work under standardized environmental conditions to decipher possible selection- and experience-related influences on locomotion. The standardized breeding and housing conditions of Lipizzan horses at Lipica Stud Farm in Slovenia significantly reduce environmental variability. Their locomotion is fascinating due to their remarkable physical strength and speed (25, 26).

In recent years, the focus has shifted from simply managing negative experiences in farm animals to promoting environments in which animals can thrive (27, 28). Selecting horses with certain morphological and kinematic characteristics for appropriate types of work can provide animals with "a good life" by promoting skills such as competence (29) and resilience (30) and, thus, positive experiences. The implementation of such practices supports the concept of positive animal welfare, which is increasingly recognized as a more ethical approach to animal care. We hypothesize that certain morphological and kinematic characteristics can be identified depending on the type of work performed by the horse. These findings may help to develop future strategies for task selection, training and breeding, ultimately improving the welfare of working horses.

2 Materials and methods

2.1 Ethics statement

The procedures were approved by the Committee for the welfare of animals for experimental purposes of the Veterinary faculty of the University of Ljubljana under reference number 033-5/2024-5 as a part of a larger project. The designated authority in Lipica Stud Farm agreed on the procedures, as well as on the use of pictures, videos, and data for scientific purposes. The horse handlers/trainers were also given the right to withdraw from the study at any time and to be present during testing.

2.2 Animals and housing

This study examined a group of 71 healthy adult Lipizzan horses, consisting of 7 mares, 17 geldings, and 47 stallions, with ages ranging from 5 to 25 years (mean age: 10.1 ± 4.7 years). Horses showed no signs of lameness or disease prior or during data collection. All horses were sourced from the Lipica Stud Farm in Slovenia and housed in conventional individual boxes. Until the age of four, they were trained and ridden under the same conditions. After that point, they were assigned to different roles. Some were used for classical dressage, carriage pulling, or riding school activities. Others had not yet been assigned a specific task and continued with general daily training, such as lunging exercises. A few horses were not involved in any regular work or training at the time of the study (Table 1).

During their off-duty hours and overnight, all tested horses were kept in their individual boxes. Occasionally they were allowed access to pastures during the day. The horses had *ad libitum* access to fresh water and hay, and their primary diet comprised of individually tailored barley-oat mixture.

2.3 Morphological measurements

Based on the Slovenian breeding program for Lipizzan horses, descriptions of morphological measurements (31) and previous study assessing morphology and other parameters in this breed (32), 95 different morphological measurements of the head and body were collected. Using a sartorial meter (Figure 1A), 29 measurements were taken from the front (Figure 2A), and both side profiles of the head (Figure 2B). Using the same approach, 63 measurements were taken from the front (Figure 3A) and both side profiles of the body (Figure 3B). The heights at the withers, back, and croup (Figure 3B) were taken from the left side of the horse using a specialized equine measuring stick (Figure 1B).

TABLE 1 The descriptions of working tasks and within-group demographics of horses.

Working task	Description	*N*	Mean age	Sex
Carriage pulling	Pulling the tourist carriages around Lipica premises	22	9.5 ± 3.0	6 mares, 7 geldings, 9 stallions
Classical dressage	Participation in classical dressage exhibitions and competitions	18	12.1 ± 5.1	2 geldings, 16 stallions
Riding school	Participation in touristic riding school lessons	8	11.5 ± 3.7	1 mare, 5 geldings, 2 stallions
General training	Involved in general everyday training, no specific working task	11	7.3 ± 1.8	1 gelding, 10 stallions
No working task	Not actively involved in everyday training or work	12	9.8 ± 7.6	2 geldings, 12 stallions

All measurements were performed with the horse standing on a flat surface (e.g., in front of the box) by a team of three experimenters - one responsible for handling the horse, one for taking the measurements, and one for recording the data. Initially, on 24 horses, the measurements were performed twice across two separate days by the same experimenter. Once the intra-class correlation coefficient (ICC) was calculated to assess consistency between the two sets of measurements and demonstrated excellent reliability (ICC = 0.99), subsequent measurements for the remaining horses were conducted only once.

From the body measurements, the horse's weight was calculated using a formula: Weight = (girth^2 * length)/Y. Girth was defined as body circumference behind the elbow and just behind the highest point of the withers (in cm), while the length was defined as the distance from the greater tubercle of the humerus to the tuber ischia (in cm). The used value for Y (in cm^3/kg) was 11,877, since Carroll and Huntington (33) presented it as the most accurate value.

2.4 Kinematic recordings

The kinematic recordings were performed using Equimetrix®, a three-dimensional accelerometer known for its validity and reproducibility (15, 19). This device consisted of an acceleration sensor and a data logger enclosed in a small block (4 × 2.2 × 1.7 cm). The block was placed in a leather bag and secured with an elastic strap to the caudal part of the sternum at the level of the chest girth (Figure 4A). The data were recorded continuously at a sampling rate of 100 Hz. As previously proposed, the horse was led by the hand (20) and covered a distance of 50 meters at its own comfortable speed (34), first at a walk, then at a trot–each activity was repeated four times (Figure 4B). The surface (freshly leveled sandy ground) was the same for all horses. One experimenter was responsible for handling the horse, while the other monitored the time with a stopwatch (for manual calculation of speed). This process was repeated twice within a week, with the same experimenter positioning the device and leading the horse. The data were uploaded to the Equimetrix Centaure® software for processing. The kinematic parameters analyzed automatically [for description of calculations see Leleu et al. (15, 19)] are described in Table 2. The mean values of all walk and trot repetitions were used for statistical analysis.

2.5 Statistical design and analyses

We conducted a correlation analysis using the SAS software package (Statistical Analysis System, version 9.4) to explore the relationships among various phenotypic measurements in horses engaged in different working tasks. We employed Pearson's correlation coefficient, calculated using the CORR procedure. In the first step of the analysis, we reduced the dataset by excluding morphological measurements that were highly intercorrelated ($r \geq 0.8$). When measurementsfrom both sides of the body were strongly correlated, we retained the left-sided measurements and excluded the right-sided ones to avoid redundancy. Additionally, since all three height measurements and distances between the fetlocks and the carpi were strongly correlated, we retained only the height at the withers (FB38) and the distance between the carpi (FB08). This reduction resulted in a final set of 63 morphological measurements. In the second step, to assess the strength and direction of linear relationships between

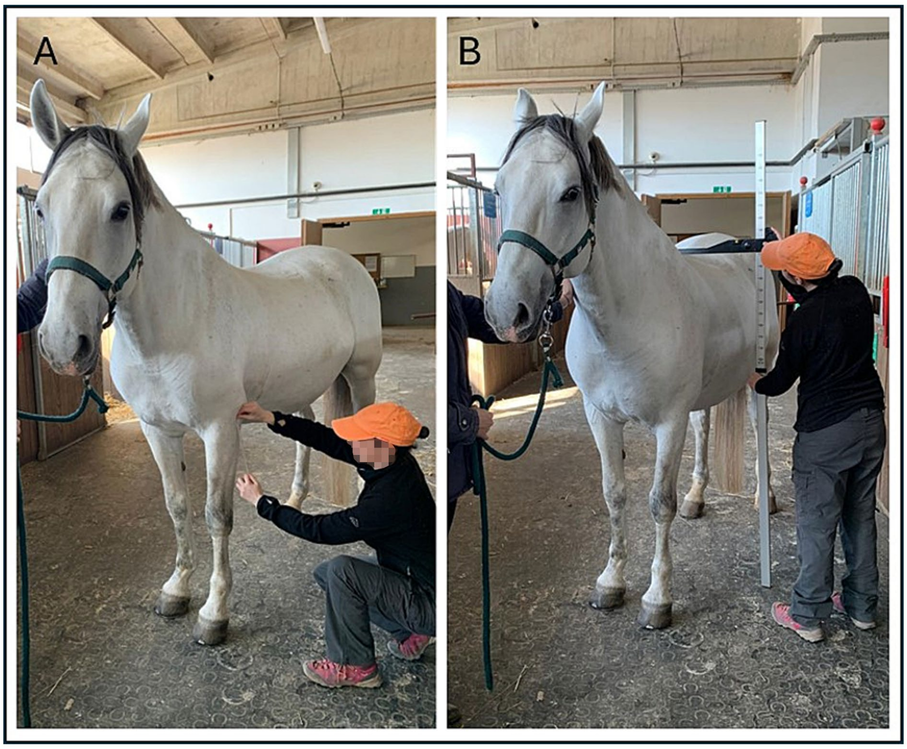

FIGURE 1
The use of a sartorial meter **(A)** and measuring stick **(B)** for morphological measurements.

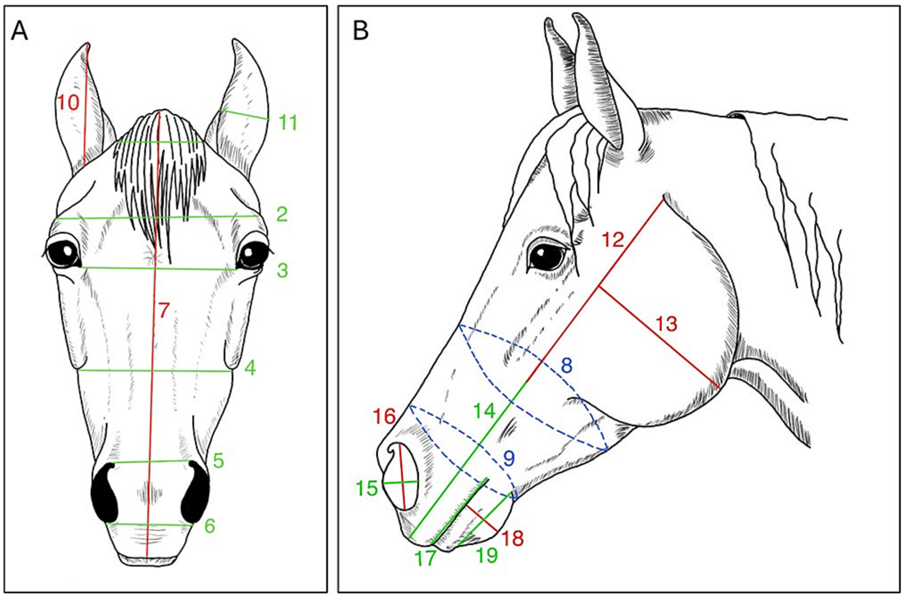

FIGURE 2
Head morphological measurements: **(A)** front view, **(B)** profile view.

variables, we used partial correlation with PARTIAL statement to control for speed and age. For clarity, only results that met the criteria of a strong ($r \geq 0.8$) or a very strong correlation ($r \geq 0.9$) and a significance level below 0.025, considering Bonferroni correction for multiple testing, were included in the results section. Our analysis uncovered several significant correlations, highlighting the importance of these variables and laying the groundwork for further exploration of their interdependencies. To quantify within-group variability and support interpretation of correlation results, group-specific means and standard deviations (SDs) were calculated. Additionally, thresholds at ± 2 SD and ± 3 SD from the

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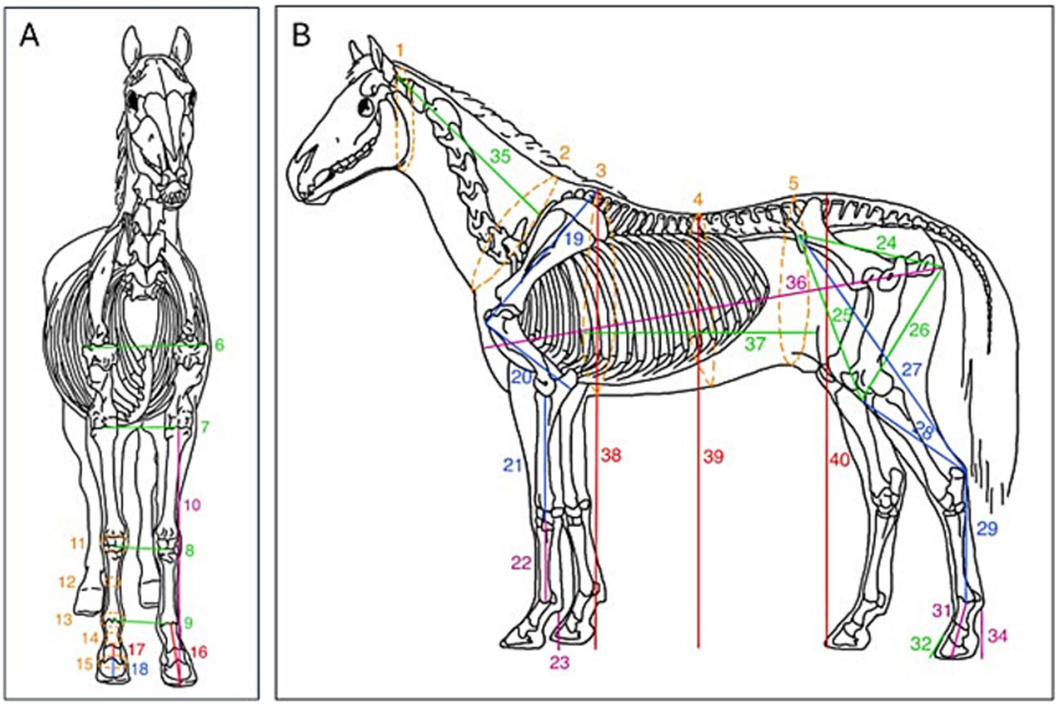

FIGURE 3
Body morphological measurements: **(A)** front view, **(B)** profile view.

FIGURE 4
The application of the device: **(A)** placement of the device, **(B)** example of kinematic assessment during walk.

group mean were used to identify potential outliers. Scatterplots were generated separately for each group to visualize the distribution of data points and assess the consistency of associations, as well as to identify potential outliers.

Recognizing that a horse's body weight could influence the observed correlations, we applied the General Linear Model (GLM) procedure to examine the effect of different work tasks on weight. To gain deeper insights into the differences among the levels of that variable, we performed pairwise comparisons using Tukey's adjustment method to control for Type I error, thus preserving confidence levels across multiple comparisons. Ultimately, we found no statistically significant effect. One-way analysis of variance was used to assess differences in weight, kinematic parameters and the most important morphological measurements among the five working groups of

TABLE 2 The descriptions of kinematics parameters.

Parameter	Unit	Description
Speed	m/s	The rate at which the horse covered the distance of 50 m
Stride length	m	Distance covered between successive placements of the same hoof
Cadence	strides/s	The number of strides taken in 1 s
Regularity	dimensionless	The consistency of successive stride acceleration patterns.
Symmetry	dimensionless	The similarity between the acceleration patterns of the right and left diagonals
Dorsoventral power	g^2/Hz	Loading activity and limb suspension.
Propulsion power	g^2/Hz	Acceleration and deceleration along the longitudinal axis.

horses. It was followed by Tukey's *post-hoc* test to identify pairwise group differences. Considering Bonferroni correction for multiple testing, values below 0.025 were considered significant.

3 Results

3.1 All horses

Table 3 presents the descriptive statistics of the kinematic parameters for a horse's walk and trot, highlighting the numerical differences due to the distinct nature of these gaits. The correlation analysis between gait kinematics and morphological characteristics for both walk and trot, conducted across all horses ($n = 71$), revealed only weak associations (correlation coefficients < 0. 44). Due to a large number of results ($n = 756$), these correlations are not included in the text.

3.2 Horses grouped by type of work

After grouping the horses based on five different working tasks, 3 head measurements and 15 body measurements (Table 4) showed very strong (≥ 0.8) and excellent (≥ 0.9) correlations with kinematic parameters in four groups. Of the 18 significantly associated measurements, 14 were measured bilaterally, and half of these showed side-specific correlations. There was no statistical difference between the type of work they performed and their weight ($p = 0.35$; $F = 1.13$).

3.2.1 General training

For horses in general training, three very strong and three excellent correlations were found during walk (Table 5). Lower part of the neck circumference was negatively correlated with regularity. Length of the pastern on forelegs was negatively correlated with cadence and positively with stride length. Forehoof length was positively correlated with symmetry. Hind hoof length was positively correlated with stride length and negatively with cadence, the latter showing the only correlation with a coefficient exceeding 0.9 and the lowest *p*-value observed across all measurements, indicating an exceptionally strong and statistically significant relationship. During trot, there were no correlations found.

3.2.2 No working task

For horses with no working tasks, two very strong correlations were found during walk (Table 5). Distance between the left and right carpus on forelegs was positively correlated with cadence and negatively with stride length. During trot, four very strong correlations were found (Table 5). Length of the pastern on forelegs was positively correlated with regularity. Forehoof length was positively correlated with cadence and negatively with stride length. Distance between the endpoints of facial crest bones was negatively correlated with propulsion power.

3.2.3 Classical dressage

For horses in classical dressage, one very strong correlation was found during walk (Table 5). Body length was negatively correlated with stride length. During trot there were no correlations found.

3.2.4 Riding school

For horses used in riding school, six very strong, and five excellent correlations were found during walk (Table 5). Lower part of the neck and body circumferences were positively correlated with dorsoventral power. Distance between the elbows was positively correlated with stride length. Pastern circumference on forelegs was negatively correlated with symmetry and positively with propulsion power. Length of the fore pastern and forehoof and distance between the tuber coxae and the point of hock were positively correlated with propulsion power, while the distance between the stifle joint and the point of the hock was negatively correlated. Distance between the tuber coxae and the tuber ishii was positively correlated with dorsoventral power. Body length was negatively correlated with regularity.

During trot, three very strong, and three excellent correlations were found (Table 5). Upper part of the neck circumference was negatively correlated with symmetry. Distance between the elbows was positively correlated with stride length. Cannon bone and pastern circumference on forelegs were positively correlated with dorsoventral power. Distance between the fetlock joint and the end forehoof was positively correlated with propulsion power. Distance between the endpoints of nostrils was negatively correlated with regularity.

3.2.5 Carriage pulling

For horses used for carriage pulling, there were no correlations found during walk or trot.

3.2.6 Overlap between working groups

Several morphological measurements were found to be correlated with kinematic measurements in two or more working groups (Table 4). For example, the lower neck circumference showed opposite relationships in different groups—it was negatively correlated with regularity in group 1 but positively correlated with dorsoventral power in group 4. Body length was negatively associated with stride length in group 3 and with regularity in group 4. The length of the foreleg pastern displayed multiple correlations: in group 1, it was negatively correlated with cadence and positively with stride length; in group 2, it was positively associated with regularity; and in group 4, with propulsion power. The length of the fore hoof was positively correlated

TABLE 3 Mean values (± standard deviations) of kinematic parameters.

Horses	Gait	Regularity (dimensionless)	Symmetry (dimensionless)	Cadence (stride/s)	Dorso-ventral power (g^2/Hz)	Propulsion power (g^2/Hz)	Stride length (m)	Speed (m/s)
All horses	Walk	155.70 ± 37.81	192.72 ± 40.55	0.94 ± 3.24	0.52 ± 0.23	0.97 ± 0.44	1.78 ± 0.15	1.42 ± 0.11
	Trot	308.51 ± 44.17	259.59 ± 42.59	1.29 ± 0.06	14.98 ± 3.45	4.87 ± 1.86	2.45 ± 0.26	3.18 ± 0.40
General training	Walk	145.70 ± 28.75	180.94 ± 39.42	0.79 ± 0.06	0.51 ± 0.19	0.85 ± 0.27	1.78 ± 0.07	1.42 ± 0.11
	Trot	307.80 ± 44.29	251.15 ± 35.42	1.29 ± 0.04	15.19 ± 2.76	4.05 ± 1.11	2.36 ± 0.19	3.03 ± 0.25
No work	Walk	146.13 ± 46.24	194.72 ± 42.78	0.84 ± 0.12	0.52 ± 0.25	0.88 ± 0.29	1.72 ± 0.21	1.44 ± 0.12
	Trot	308.78 ± 48.06	259.60 ± 38.49	1.32 ± 0.07	15.50 ± 3.38	4.22 ± 1.13	2.43 ± 0.26	3.21 ± 0.40
Classical dressage	Walk	167.41 ± 38.65	185.02 ± 36.59	0.83 ± 0.13	0.59 ± 0.22	1.11 ± 0.49	1.77 ± 0.15	1.44 ± 0.12
	Trot	316. 13 ± 40.52	252.74 ± 43.79	1.27 ± 0.05	14.04 ± 3.62	4.57 ± 1.64	2.40 ± 0.25	3.06 ± 0.36
Riding school	Walk	166.03 ± 36.46	195.56 ± 39.34	0.79 ± 0.05	0.55 ± 0.30	0.95 ± 0.39	1.77 ± 0.11	1.40 ± 0.15
	Trot	310.19 ± 41.72	273.48 ± 47.09	1.32 ± 0.05	14.26 ± 0.05	6.03 ± 2.02	2.41 ± 0.15	3.19 ± 0.23
Carriage pulling	Walk	152.57 ± 33.15	202.80 ± 41.04	0.77 ± 0.18	0.47 ± 0.18	0.98 ± 0.51	1.83 ± 0.13	1.39 ± 0.10
	Trot	301.86 ± 45.07	264.36 ± 43.78	1.29 ± 0.07	15.64 ± 3.68	5.47 ± 2.17	2.57 ± 0.30	3.34 ± 0.48

with symmetry in group 1 and with propulsion power in group 4. In group 2, it showed a positive correlation with cadence and a negative one with stride length.

3.2.7 Within working groups variability

A total of 14 individual data points were identified as outliers based on the ± 2 standard deviation (SD) criterion, whereas no outliers were detected using the ± 3 SD threshold (Supplementary File S1). Of these, six corresponded to kinematic parameters and eight to morphological measurements. The identified outliers originated from nine individual horses. Three of these, each representing a different work group, exhibited two or three outliers across different measurements. The highest number of outliers ($n = 4$) was identified in the riding school group, which represented the smallest working group in the study.

Scatterplots of the eight strongest correlations ($r > 0.9$, $p < 0.0001$) are presented in Figure 5, while additional scatterplots for important correlations are provided in Supplementary File S1. Among these strongest correlations, only a single outlier was identified and observed in the riding school group during walk, for body circumference at the withers (FB03; Figure 5).

3.3 Differences between working groups

No statistically significant differences between working groups were observed in kinematic parameters during walk (Supplementary Table S1). During trot, only one parameter, propulsion power, showed a statistically significant difference (Supplementary Table S1). *Post-hoc* analysis indicated a trend toward a difference between horses in general training and those used in riding schools, though this did not reach statistical significance ($p = 0.082$).

Among the 18 most important morphological measurements, statistically significant differences were found in two: lower part of the neck circumference (FB02) and right hind hoof length (FB32R) (Supplementary Table S2). For FB02, *post-hoc* analysis revealed a significant difference between carriage-pulling horses and those used in classical dressage ($p = 0.011$). For FB32R, *post-hoc* analysis revealed a significant difference between carriage-pulling horses and those with no working task ($p = 0.002$). There were no statistically significant differences in weight.

Using multiple criteria to assess the meaningfulness of the observed correlations, all of the most statistically significant associations were also determined to be biologically meaningful. The task-specific correlations appear particularly informative, as they were minimally affected by outliers.4 Discussion

In this study, we investigated the relationship between morphological measurements and gait kinematics in Lipizzan horses, a breed well suited for biomechanical analysis due to its involvement in a range of working tasks. This study focused exclusively on non-pathological, functionally sound gaits. It contributes to a broader understanding of equine locomotion in routine working environments, an area that remains underrepresented in a literature that largely focuses on lameness and injury detection. When the population was assessed as a whole, no significant correlations were detected between morphological measurements and kinematic parameters. This likely reflects the heterogeneity introduced by combining horses with different functional demands and movement patterns. Once horses were grouped by type of work, several clear and group-specific associations emerged, highlighting the task-dependent nature of conformation–locomotion relationships. These findings align with previous work demonstrating complex interactions between genetics, morphology, and kinematic measurements (35). Moreover, the near absence of statistically significant differences in both kinematic and morphological measurements between groups, as well as a small number of detected outliers, further supports the importance of task-specific associations between morphology and kinematics.

The clearest task-specific associations were observed in the general training group. Several notable correlations between morphological measurements and kinematic parameters appeared, particularly during walk. The strongest association was the

TABLE 4 The descriptions of all morphological measurements that correlate with kinematic parameters and working group they appear in.

Measurement name	Description	Working group	Figure
Head (FH)			
FH04	Distance between the endpoints of facial crest bones	2	Figure 2A
FH06	Distance between the endpoints of nostrils	4	Figure 2A
FH14	Distance between the end of facial crest bone and the end of the muzzle	4	Figure 2B
Body (FB)			
FB01	Upper part of the neck circumference	4	Figure 3B
FB02	Lower part of the neck circumference	1,4	Figure 3B
FB03	Body circumference at the wither	4	Figure 3B
FB07	Distance between the elbows	4	Figure 3A
FB08	Distance between the left and right carpus on forelegs	2	Figure 3A
FB12	Cannon bone circumference	4	Figure 3A
FB14	Pastern circumference on forelegs	4	Figure 3A
FB16	Distance between the fetlock joint and the end of the forehoof	4	Figure 3A
FB17	Fore pastern length	1,2,4	Figure 3A
FB18	Forehoof length	1,2,4	Figure 3A
FB24	Distance between the tuber coxae and tuber ishii	4	Figure 3B
FB27	Distance between the tuber coxae and the point of hock	4	Figure 3B
FB28	Distance between the stifle joint and the point of the hock	4	Figure 3B
FB32	Hind hoof length	1	Figure 3B
FB36	Body length	3,4	Figure 3B

Working group: 1 = general training, 2 = no working task, 3 = classical dressage, 4 = riding school.

negative relationship between hind hoof length and cadence, suggesting that horses with longer hind hooves take fewer steps. This may reflect a biomechanical trade-off, in which longer hooves prolong the break over phase and increase distal limb mass, resulting in reduced stride frequency (36, 37). Simultaneously, hind hoof length was positively associated with stride length, indicating a compensatory mechanism that may help maintain forward progression. A similar pattern emerged for fore pastern length, which was positively associated with stride length. While prior studies have focused on pastern angle (38), our linear measurements support the view that longer pasterns contribute to extended stride mechanics. Together, these findings suggest that distal limb conformation plays a meaningful role in shaping gait characteristics during general training activities.

In addition to the general training group, the riding school horses also showed numerous correlations, particularly during walk, where all measured kinematic parameters, except cadence, were affected. This extensive pattern may reflect the highly variable movement demands of riding school environments, where horses must frequently adapt to changes in rider ability, balance, and movement cues (39). Such variability may amplify the expression of morphology-dependent gait patterns. Interestingly, narrower nostril width was associated with lower regularity, suggesting a potential link between craniofacial measurements and coordination, although this warrants further investigation. However, the unexpectedly high number of correlations in this small group calls for cautious interpretation. Small sample sizes increase the influence of individual variability, potentially inflating correlation strength and limiting generalizability. Future studies with larger cohorts are needed to confirm the robustness of these associations.

In contrast to these findings, the classical dressage group exhibited only one significant correlation. Longer body length was associated with shorter stride length. Although limited, this finding is relevant for dressage, where extended, expressive strides are central to performance (40). It suggests that body length may influence a horse's capacity to achieve the desired movement profile in this discipline and should be considered during selection and training.

The group of horses without assigned working tasks showed a different set of correlations. A shorter distance between the carpal joints was associated with increased cadence and reduced stride length during walk. This finding aligns with previous observations linking narrower limb spacing to more vertical, collected movement patterns, which support increased cadence but limit protraction and stride length (41). In trot, longer forehoof length was associated with increased cadence and reduced stride length. This is the opposite of what was observed in the general training group, where longer hind hooves were linked to decreased cadence and increased stride length. These contrasting patterns may reflect anatomical differences between fore- and hind hooves. As noted by Tijssen et al. (42), hind hooves typically have a steeper dorsal wall angle and are narrower than forehooves. Such differences can influence the hoof unrollment pattern and, in turn, the dynamics of limb movement. This suggests that the absence of structured training may alter how morphological measurements are expressed in locomotion. Additionally, longer fore pasterns were associated with greater regularity, a finding not observed

TABLE 5 Correlations for horses from different working groups between body measurements and kinematics during walk and trot.

Working group	Gait	Body measurement	Regularity	Symmetry	Cadence	Dorsoventral power	Propulsion power	Stride length
General training	Walk	FB02	−0.85**					
		FB17R			−0.88**			0.91**
		FB18LR		0.91**				
		FB32R			−0.92***			0.82**
No working task	Walk	FB08			0.84**			−0.87**
	Trot	FB17R	0.87**					
		FB18LR			0.86**			−0.85**
		FH04					−0.83**	
Classical dressage	Walk	FB36LR						−0.81***
Riding school	Walk	FB02				0.87*		
		FB03				0.95**		
		FB07						0.87*
		FH14LR		−0.87*			0.92**	
		FB17R					0.89*	
		FB18LR					0.95**	
		FB24LR				0.89*		
		FB27LR					0.88*	
		FB28LR					−0.90*	
		FB36LR	−0.92**					
	Trot	FB01		−0.93**				
		FB07						0.88*
		FB12R				0.91*		
		FB14L				0.91*		
		FB16R					0.87*	
		FH06	0.89*					

*$p < 0.025$; **$p < 0.005$; ***$p < 0.0005$. L – left side of the body; R – right side of the body.

in other groups. Narrower facial crest width was also linked to increased propulsion power in this group, though the mechanism remains unclear and warrants further study.

The carriage-pulling group, despite being the largest, showed no significant correlations. The linear, repetitive nature of carriage work likely contributes to highly uniform gait patterns, reducing inter-individual variability and masking the effects of morphological variation. Furthermore, the mechanical restrictions of harness and carriage equipment may limit natural variation in limb movement, diminishing the expression of conformation-dependent gait differences.

Despite variation across working groups, several morphological measurements, particularly forelimb hoof and pastern lengths, emerged repeatedly as important predictors of gait kinematics. However, these associations differed by context. The same measurement was linked to different kinematic parameters depending on the horse's work. This reinforces the idea that morphological measurements do not exert a fixed influence, but rather interact dynamically with training, task demands, and environmental context. Such findings underscore the value of aligning selection, management, and conditioning strategies with both morphological structure and functional use.

Beyond work-specific patterns, the analysis revealed consistent bilateral symmetry in many morphological–kinematic associations. Among the measurements significantly correlated with movement parameters and measured on both sides of the body, half showed bilateral associations, while the rest were side-specific. This suggests that asymmetries may develop in response to specific work demands or individual adaptation. For example, dressage often involves lateralized, non-natural movements that can promote asymmetry (43), while variability in rider input or saddle fit may further contribute to uneven loading (44). In contrast, carriage-pulling typically involves linear, symmetrical movement with minimal lateral bias. These asymmetries may reflect biomechanical adaptations or training-induced preferences. Nonetheless, the predominance of bilateral correlations supports the established importance of coordinated limb function for maintaining gait stability and reducing injury risk (21, 34, 45).

Although no significant differences were observed between working groups, several strong correlations were detected within groups. This

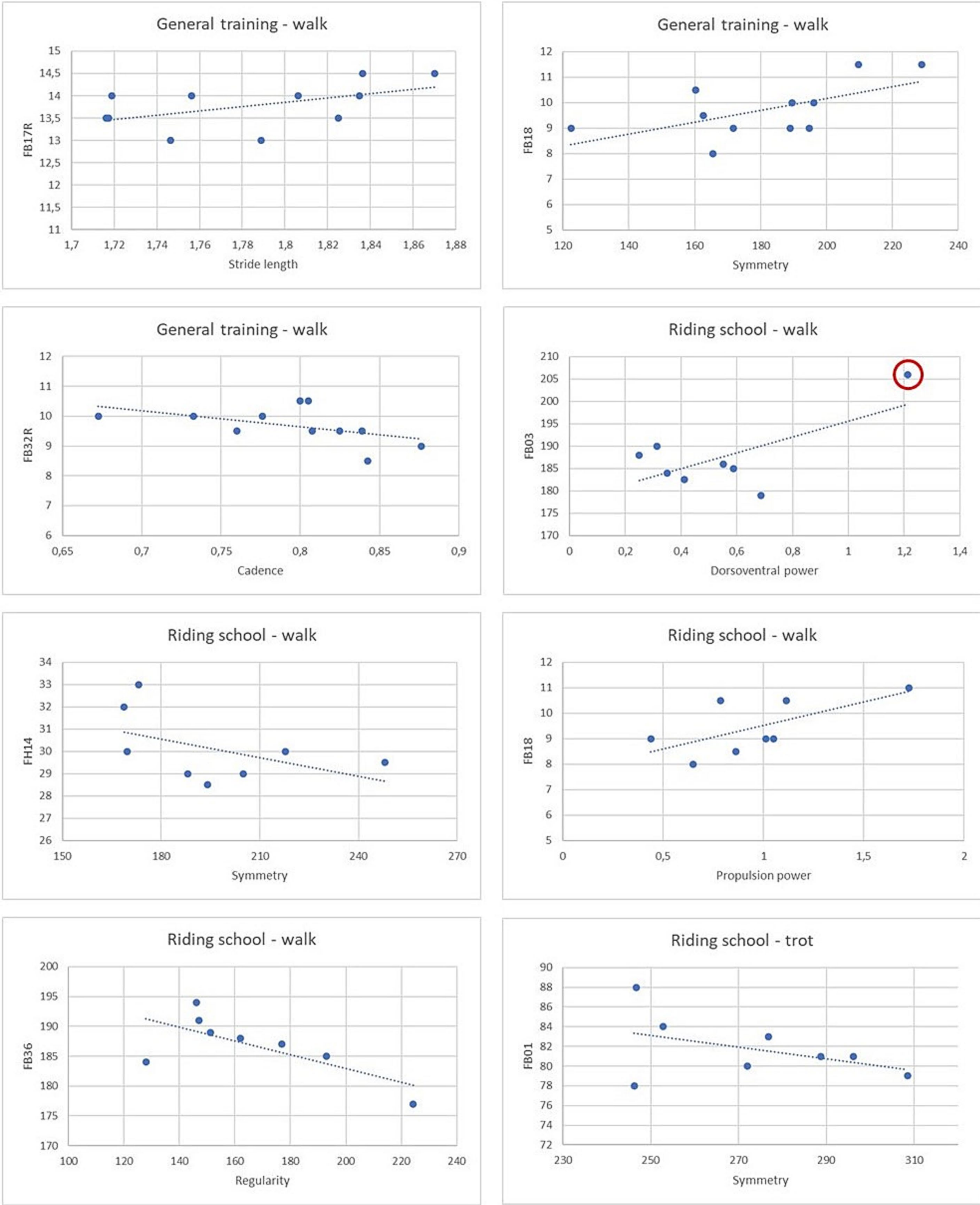

FIGURE 5
Scatterplots of the eight strongest correlations. A detected outlier is marked in red.

pattern suggests that individual-level variation, rather than group-level context, underlies the observed measurements relationships. The absence of outliers in these associations further supports the robustness of the findings and indicates that the correlations reflect biologically meaningful patterns rather than statistical artefacts. These within-group associations may point to stable, trait-level mechanisms that are not captured by comparing group means alone, highlighting the importance of individual differences in animal research.

One methodological limitation of this study was the inability to control for sex distribution across working groups. Although previous studies have reported sex-related differences in equine gait and performance traits, findings remain inconsistent (46–49).

Management practices often favor geldings for riding school tasks due to their predictability, and stallions for high-performance work, such as classical dressage, due to their strength and expression (49, 50). These trends were reflected in our sample, with mares underrepresented in physically demanding work. While sex may influence movement to some extent, the group-specific associations observed in our data appear consistent across sexes.

Despite this limitation, understanding how conformation relates to gait can inform individualized training intensity, conditioning programs, and the selection of horses for specific tasks, particularly when supported by biomechanical analysis of horse–rider interaction (51). For example, prioritizing horses with favorable morphometric profiles may enhance movement efficiency, reduce strain on vulnerable structures, and support long-term soundness. Aligning morphology with functional demands not only promotes biomechanical efficiency and resilience but, in line with the principles of positive animal welfare (52), by supporting horses that are physically capable, adaptable, and confident in their roles.5 Conclusion

This study demonstrated that the relationship between morphological measurements and gait kinematics in Lipizzan horses is strongly influenced by the type of work performed. While no significant associations were detected across the entire cohort, task-specific analyses revealed clear and meaningful correlations within individual working groups. Furthermore, the minimal statistically significant differences between groups, combined with the small number of outliers, highlight the importance of assessing these associations within specific work contexts rather than across a heterogeneous population. The most pronounced associations involved distal limb measurements, particularly hoof and pastern lengths, which were linked to stride length, cadence, and regularity. The observed effects were most evident in horses engaged in general training and riding school activities. Importantly, the same morphological measurements influenced different kinematic parameters depending on the work context, underscoring the dynamic interaction between conformation and functional demands. These findings highlight the practical value of morphometric assessment in supporting informed training decisions, improving performance and soundness, and aligning horses more effectively with the physical demands of their intended work.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author/s.

Ethics statement

The animal study was approved by the Committee for the Welfare of Animals for experimental purposes of the Veterinary Faculty of the University of Ljubljana under reference number 033-5/2024-5. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

MZŠ: Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Visualization, Writing – original draft, Writing – review & editing, Formal analysis. LP: Visualization, Writing – review & editing. EG: Conceptualization, Data curation, Investigation, Methodology, Visualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2025.1569067/full#supplementary-material

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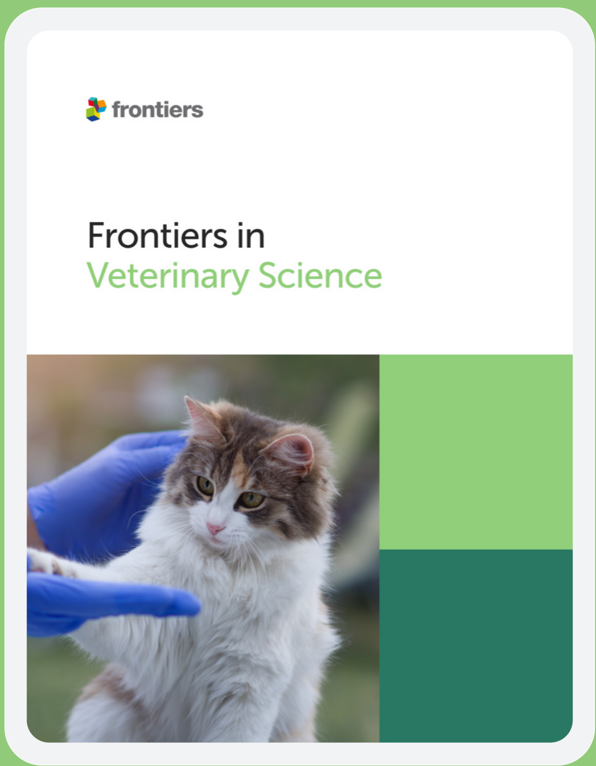

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